EPISODIC-LIKE MEMORY IN PUREBRED AND CROSSBRED YUCATAN MINIPIGS (Sus scrofa)

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Episodic-like memory in purebred and crossbred Yucatan minipigs (*Sus scrofa*)

by

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ABSTRACT

Episodic memory is a human ability that involves mental re-creation of a personally experienced past event. Its existence is controversial in animals because it requires demonstration of self-consciousness and mental time travel. Episodic-*like* memory (simultaneous recall of what, where, when aspects of a past event) has been demonstrated in non-humans and appears to depend on the hippocampus. We explored the potential for episodic-like memory in pigs, a previously un-studied species reported to have high mental capacity. As was done for rats, we adopted a definition of episodic-like memory that equates recall of time ("when") with recall of context ("which"). We tested pigs' ability to remember what (object), where (location) and *which* (context). Through novel object recognition, pigs identified the less familiar of two object/location/context configurations. Since configuration familiarity differed only if all aspects were remembered simultaneously, we concluded that pigs were able to recall what/where/which, providing evidence of episodic-like memory.

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Chapter 1: GENERAL INTRODUCTION

Episodic memory is characterized by Tulving (1983; 2002) as a discrete form of memory that involves mentally re-enacting previously experienced events. Specifically, this type of memory requires the integrated recall of the "what, where and which" circumstances of an event, ability to recognize subjective time, and autonoetic consciousness (knowledge of self; Tulving, 1983; 2002). Episodic memory has been well documented in humans, but its presence in non-humans is controversial. However, through studying food caching, food finding and novelty exploration, researchers have been able to demonstrate episodic-*like* memory in several species. Initially, episodic-like memory encompassed the same definition as human episodic memory, except that it did not require demonstration of a sense of self and/or autonoetic consciousness (Clayton and Dickinson, 1998). Recently, studies of non-human episodic memory on different species have led to alternate definitions, based on the "what/where/which" aspects of an event (Eacott and Norman, 2004). Regardless of varying definitions, non-human episodic memory research has helped to illuminate the functions of particular brain structures, such as the hippocampus (Fortin *et al.*, 2002; Suzuki & Clayton, 2000).

Human Episodic Memory

As mentioned, episodic memory requires the retrieval of "what, where and when" circumstances of an event and appears to depend on the integrity of the hippocampus (Fortin *et al.*, 2002; Suzuki & Clayton, 2000). Arguably, this link to the hippocampus distinguishes episodic memory as a separate system from semantic memory, since semantic memory remains intact despite hippocampal damage (Vargha-Khadem *et al.*, 1997). A second major distinction is that semantic memory involves the retrieval of discrete facts (e.g., Marconi received a wireless transmission at Signal Hill in 1901), while episodic memory involves mental re-creation of a personally experienced event (e.g., I was on Signal Hill yesterday and read a sign about Marconi). This personal element of episodic memory suggests that it requires self-consciousness and the ability to mentally travel forward and backward in time (Tulving, 1983; 2002), both of which are extremely difficult to demonstrate without the use of complex verbal language. Therefore, despite the fact that episodic memory has been well documented in humans, its presence in non-humans is controversial.

To date, it has not been possible to demonstrate that non-humans possess episodic memory ability that is equivalent to humans. However, by studying food caching, food finding and novelty exploration, researchers claim to have demonstrated a form of episodic memory in scrub jays (Clayton & Dickinson, 1998), pigeons (Zentall *et al.*, 2001), mice (Dere *et al.*, 2005), rats (Eacott & Norman, 2004), gorillas (Schwartz & Evans, 2001), and chimpanzees (Menzel, 1999). These studies, particularly those that compare the performance of hippocampally-damaged subjects with healthy subjects (e.g., Eacott & Norman, 2004; Fortin *et al.*, 2002), indicate the presence of a memory system in animals that is different from the semantic memory system. However, the interpretation of such studies is controversial because there is no agreed definition of non-human episodic memory (Hampton & Schwartz, 2004). As outlined by Schwartz, Hoffman and Evans (2005), five operational definitions of non-human episodic memory include: (1) demonstration of what/where/when memory (Clayton & Dickinson, 1998; Babb &

Crystal, 2006), (2) demonstration of what/where/which memory (Eacott and Norman, 2004), (3) demonstration of spontaneous recall (Menzel, 1999), (4) ability to recall an event when not expecting a test (Zentall *et al.*, 2001), and (5) ability to report on past events over a long term (Schwartz & Evans, 2001). These definitions form the foundations of several streams of non-human episodic memory research, each of which tends to focus on a particular species. The definitions tend to be species-specific because methods to demonstrate them require the animals to exhibit particular behavioural traits. For example, the original methods for Clayton and Dickinson's (1998) what/where/when definition rely on the natural food-caching behaviour of Western scrub jays. Since these methods are not easily adapted to non-caching species, alternative methods and definitions have been developed for rodents, primates, and non-food-caching birds.

What/Where/When Memory in Western Scrub Jays

Clayton and Dickinson (1998) have been largely responsible for introducing and developing the concept of episodic memory in non-humans. Through their studies of the food caching behaviour of Western scrub jays, they claim to have demonstrated that these birds form integrated memories of what, where and when in the context of caching and recovering food (Clayton & Dickinson, 1998). Furthermore, they suggest that the types of caching behaviour shown by the scrub jays requires them to mentally travel forward and backward in time, which is a component of human episodic memory (Clayton, Yu, & Dickinson, 2003). However, since Clayton, Dickinson and their colleagues have not been able to demonstrate autonoetic consciousness (a sense of self) in scrub jays, they have stopped short of declaring that scrub jays have human-equivalent episodic memory.

Instead, they have opted to conclude that scrub jays possess “episodic-like memory.” This type of memory shares some characteristics with the definition of human episodic memory (i.e., according to Tulving, 1983), but avoids the currently impossible task of demonstrating consciousness without the use of verbal language (Clayton, Yu, & Dickinson, 2003).

Clayton and Dickinson’s (1998) publication on episodic-like memory in scrub jays focussed on demonstrating that jays form an integrated memory of the type of food (what), location the food was cached (where), and how long ago it was cached (when). This study took advantage of the scrub jays’ natural food-storing behaviours and allowed each bird to cache both perishable (wax moth larvae or “worms”) and non-perishable (peanuts) food items in opposite sides of an ice-cube tray filled with sand. In addition to a difference in perishability, the foods were also differentially preferable, with scrub jays distinctly favouring the worms. Initially, the scrub jays demonstrated the ability to recall the location (“where”) in which they cached each type of food (“what”), and consequently retrieved the preferred food, worms, before peanuts. In subsequent trials, researchers replaced freshly cached worms with decayed worms if worms were cached first (124 h before retrieval) and peanuts cached second (4 h before retrieval). In contrast, fresh worms were left in their cached locations if peanuts were cached first (124 h before retrieval) and worms cached second (4 h before retrieval). Remarkably, the scrub jays quickly learned to retrieve peanuts if worms were cached first (since decayed worms are unpalatable) and to retrieve worms if peanuts were cached first. A similar result, although less compelling, was found when jays were taught that worms were removed (pilfered) if they were cached 124 h before retrieval. Retrieval, in both cases, was measured in terms of which food cache was visited first and the number of inspections of each food cache.

The conclusion that birds showed integrated what/where/when recall is supported by results for control birds that were never presented with decayed worms regardless of the time interval before retrieval. These birds always chose to retrieve worms before peanuts, which rules out the explanation that jays in the experimental group preferred to retrieve peanuts based on differential forgetting of the worm caches (Clayton & Dickinson, 1998). Additionally, the control results show that differential retrieval of worms based on caching time is not genetically based. If the behaviour was innate, birds in the control group should have shown differential retrieval of worms despite never having been exposed to decayed worms (Griffiths & Clayton, 2001). Clayton and Dickinson (1998; 1999a) also showed that olfactory cues were not responsible for the differential retrieval of caches because the jays showed this behaviour even when cache trays were emptied of their contents and filled with only fresh sand before test trials. Also, the use of two sides of a single tray for caching scrub jays means that the jays could not base their retrieval choices on the relative familiarity of objects (i.e., trays) experienced during caching (Griffiths & Clayton, 2001).

In numerous studies following their 1998 study, Clayton and Dickinson have further developed and refined their case for what/where/when memory in scrub jays. Specifically, through allowing jays to cache peanuts and dog kibble and then recover these items on successive trials, they demonstrated that scrub jays update their memories about which cache sites contain food (Clayton and Dickinson, 1999b). Furthermore, by making one food less preferable than another through pre-feeding, they found that jays successfully identified food caches that were both non-recovered and contained preferable food. Clayton and Dickinson (1999b) argue that this ability indicates that scrub jays form episodic-like memories that integrate the type of food in a cache, the location of that cache, the last activity at that cache (recovery or caching) and how long ago food was stored. Clayton, Yu, and Dickinson (2003) have also shown that scrub jays use novel information about the decay of a food source to reverse their strategies for recovery, since jays cache more non-perishable food items if their caches are consistently degraded on recovery (Clayton *et al.,.* 2005). Finally, Emery and Clayton (2001) found that scrub jays who have previously raided the food cache of a conspecific will re-cache food if they are observed during their own caching process. Taken together, these findings provide preliminary evidence that scrub jays make decisions based on past episodes and anticipated future needs. Since these results suggest that episodic-like memory includes aspects of the mental time travel involved in human episodic memory, further study in this area is suggested (Clayton *et al.*, 2003).

Criticisms of What/Where/When Episodic-like Memory Definition

Despite the presence of a large amount of data on the food caching behaviour of scrub jays, the inferences about episodic memory that have arisen from this data have faced much scrutiny, largely due to the non-standard methods for measuring episodic memory in non-humans. In a review of the scrub jay research, Suddendorf and Busby (2003) argue that the what/where/when memory demonstrated by scrub jays does not constitute episodic-like memory. They agree that the scrub jays show memories of what/where/when, but that this definition does not account for the full complexity inherent in episodic-like memory. Specifically, Suddendorf and Busby (2003) cite the ability to explicitly declare the details of a remembered episode (through behavioural expression), ability to reconstruct and alter a memory at the time of retrieval, ability to meta-represent memory content, and ability to plan for the future as essential criteria for the presence of an episodic-like memory. In response, Clayton *et al.* (2003) argue that scrub jays show the ability to declare the contents of remembered events through the integrative and flexible nature of their memories. Secondly, they disagree that reconstruction of memories is necessary for episodic-like memory because this reconstruction is not required in current explanations of human episodic memory retrieval (Tulving, 1983). Thirdly, Clayton *et al.* (2003) disagree that meta-representation is required for episodic-like memory. Regardless, they argue that scrub jays appear to represent the content of their minds when they alter their re-caching and recovery strategies based on presence or absence of conspecifics during caching. Finally, Clayton *et al.* (2003) concede that requiring evidence for future planning and mental time travel would improve their definition of episodic-like memory. Although they maintain that the type of memory shown by scrub jays extends beyond semantic memory, they identify the study of mental time travel as a priority in future scrub jay research. Furthermore, in light of criticisms by Suddendorf and Busby (2003), Clayton and Dickinson (2003) have recognized that their basic what/where/when criteria no longer adequately define the evolving concept of episodic-like memory. In response, they have refined their definition of episodic-like memory to include three particular behavioural criteria. Specifically, they state that a solid demonstration of episodic-like memory requires *content* (what/where/when details of a specific past event), *structure* (integration of the what/where/when details into a consolidated memory), and *flexibility* (ability to change how information gained from an episodic-like memory is used) (Clayton, Bussey & Dickinson, 2003).

In their refined definition of episodic-like memory, Clayton *et al.* (2003) stop short of requiring demonstration of mental time travel, instead opting for demonstration of flexibility. This omission illustrates the disconcerting fact that the definition for episodic-like memory is merely a description of what has been found in scrub jays rather than an unbiased definition of a discrete type of memory. Although it is expected that the definition of such an evolving concept (episodic-like memory) will undergo changes, particularly if the definition of its parent concept (i.e., human episodic memory) undergoes similar modifications, these changes would be more robust if they were established more generally.

What/Where/When Memory in Other Species

Griffiths & Clayton (2001) have recognized the importance of studying what/where/when memory in species other than food-caching birds. Many researchers have used the basic what/where/when definition proposed by Clayton and Dickinson (1998) in their attempts to demonstrate episodic-like memory in species such as pigeons (Skov-Raquette, Miller & Shettleworth 2006), mice (Dere *et al.*, 2005), and rodents (Babb & Crystal, 2006; Fortin *et al.*, 2002; Kart-Teke *et al.*, 2006). The majority of these studies have been done on mice and rats, which has led to several different testing paradigms for these species. Specifically, Babb and Crystal (2006) developed a radial maze task that required rats to remember the type of food contained in different maze arms at different times. They showed that rats were able to integrate what/where/when memories to obtain preferred foods, and that rats changed their preferences if these preferred foods were devalued.

Fortin *et al.* (2002) took a different approach to studying episodic memory by developing a task in which rats were required to remember a series of odour cues to obtain food from sand-filled cups. The rats were able to remember the type of odour and whether it occurred before or after another odour in the sequence. Furthermore, rats with hippocampal lesions were found to be impaired in their ability to judge the sequence of the odours, which suggests that rats did not base their choices on relative familiarity of the odours. However, Clayton, Bussey, Dickinson (2003) argue that rats may have solved the task using internal interval timing, and that this task does not demonstrate integrated memory for "where."

Kart-Teke *et al.* (2006) employed a novel object recognition task that required rats to discriminate more novel objects based on a combination of the objects' locations and the order in which they were presented. Since rats spent more time exploring a less recently presented object compared to a more recently presented object, Kart-Teke *et al.* (2006) argue that the rats integrated "what and when" memory. They further claim that "what and when" memory was integrated with "where" because rats responded differently to displacement of more recent and less recent objects. When presented with two more recently experienced objects, the rats spent more time with the object that had been displaced to an unfamiliar location as compared the object in a familiar location. In contrast, when presented with two less recent objects, the rats spent more time with the object in the familiar location than with the object in an unfamiliar location. Kart-Teke *et al.* (2006) conclude that these findings provide evidence for integration of what/where/when memories because they show that these three dimensions are not encoded, stored and retrieved independently. As well, they argue that rats could not use relative memory strengths to discriminate whether an object was displaced because spatial information was obtained on a single trial.

Although the above descriptions do not include all episodic-like memory studies carried out on non-food caching species, they illustrate the main testing strategies that have been used demonstrate what/where/when memory. Furthermore, they identify some of the challenges and confounds that accompany each of these strategies. The absence of caching behaviour in species like rats is a serious hindrance to replicating the results found in scrub jays. Although numerous clever methods for testing what/where/when dimensions have been developed, none of these appears to avoid alternate, more parsimonious explanations for results. This is particularly true for the "when" aspect of episodic-like memory. Even studies that have gone so far as to show that memories are flexible (i.e., rat's change in food preference shown by Babb & Crystal 2006) are confounded by the possibility of relative memory strengths and internal time intervals experienced by subjects.

What/Where/Which Episodic-like Memory Definition

In an attempt to avoid some of the confounds and problems involved in demonstrating "when" memory, Eacott and Norman (2004) developed an altered version of Clayton and Dickinson's (1998) what/where/when definition. Eacott and Norman (2004) use *context* to replace time as the "when" aspect of episodic-like memory, which broadens the definition of episodic-like memory to include integration of the "what, when, and *which*" details of an event. They argue that the function of the "when" aspect of episodic memory is simply to mark an event as being unique. Therefore, requiring animals to remember the discrete time at which an event occurred (e.g., 1 hour ago or 24 hours ago) is the same as having animals discriminate the context in which an event occurred (e.g., white-walled room vs. black-walled room; Eacott & Norman 2004; Eacott & Gaffan 2005). Either chronological time or context can serve as the reference point that identifies a specific event and allows it to be recalled. This idea is further supported by the fact that time does not appear to be an essential part of human episodic memory. Rather than time, humans tend to use background cues that are present during an event to distinguish it from other similar events (Friedman, 1993). Using background cues as reference points for memories is termed "scene memory" (Gaffan & Harrison, 1989) and it appears to depend on the hippocampus (Gaffan, 1994). Similarly, memory for context appears to be impaired in rats with hippocampal lesions (Eacott & Norman, 2004). This dependence on the hippocampus suggests a similarity between what/where/*which* memory and what/where/*when* memory, since the latter also depends on the integrity of the hippocampus (Fortin *et al.,* 2002; Suzuki & Clayton, 2000).

Novel Object Recognition Task

In addition to the innovative what/where/which definition, Eacott and Norman's (2004) unique method of testing episodic-like memory meets requirements of spontaneous recall (Menzel, 1999) and recall during an unexpected test (Zentall *et al.,* 2001). Specifically, using a novel object recognition task (Ennaceur & Delacour, 1988), Eacott & Norman (2004) found that rats can integrate memories of a specific object (what), its spatial location (where) and the context in which it occurs (which) to discriminate the more novel of two object/location/context combinations. Their methods involved two trials during which a rat explored the locations (left or right) of each of the objects (A or B) in each of the two contexts (1 or 2). After an inter-trial interval, the rat was placed in one of the contexts with two copies of the same object (e.g., A and A), and the amount of time the rat spent exploring each object was recorded. Since identifying the more novel of two configurations requires the simultaneous recall of what, where and which (object/location/context), Eacott and her colleagues argue that novel object recognition tasks are an acceptable means of testing episodic-like memory (Kart-Teke *et al.,* 2005; Eacott *et al.,* 2005). In fact, they argue that object recognition is superior to other methods because it requires very little training before subjects are tested, which reduces potential confounds caused by reinforced learning (Eacott & Norman 2004). Furthermore, since exploring novelty is a natural response for subjects, recall of the more novel object/location/context appears to be spontaneous, which meets Menzel's (1999) criterion for episodic-like memory. As well, explicit cues or rewards are not needed to prompt memories, which meets Zentall *et al.*'s (2001) criterion that episodic-like memory tests must be unexpected.

Episodic-like Memory in Pigs

Eacott and Norman's (2004) successful demonstration of what/where/which memory in rats has led to an interest in applying this definition and method to other species. Pigs (*Sus scrofa*) are a particularly attractive due to evidence that they have good spatial memory abilities and they are able to learn tasks quickly (e.g., Croney, 1999; Held *et al.,* 2002; Baldwin & Start, 1989; Held *et al.,* 2005; Puppe *et al.,* 2007). As well, wild and feral pigs have a life history in which memory is valuable; particularly because they live socially, have large foraging ranges, and have foraging habits/movement patterns/nesting sites that change with season and food availability (Graves, 1984). Since domestic pigs have retained many natural behaviours despite the domestication process, particularly in foraging (Gustafsson *et al.*, 1999), it is reasonable to speculate that domestic pigs retain the memory abilities possessed by their wild ancestors. These factors indicate that episodic-like memory in pigs may be more developed than that of other animals.

Another argument for the need to study pig memory is the physiological similarity between humans and pigs, which may allow for a more effective comparison between human episodic memory and episodic-like memory in animals (for review, see Lind *et al.*, 2007). Specifically, the pig brain is more similar to the human brain in structure (gyration), myelination and electrical activity than are the brains of rodents and other small laboratory animals (Dickerson & Dobbing, 1966; Pond *et al.*, 2000). Also similar to humans, the pig brain develops perinatally, with a growth spurt extending from midgestation to about 40 days after birth (Dickerson & Dobbing, 1966; Dobbing and Sands, 1973; Pond *et al.*, 2000). Such similar physiological brain development may be particularly valuable in studies of changes in memory with age.

To date, the existence of episodic-like memory in pigs remains virtually unexplored. The only existing study was based on the methods of a rat study done by Ergorul & Eichenbaum (2004) and required pigs to remember the locations and order of presentation of a series of odour cues (Mian 2006, unpublished). This study found that pigs were sensitive to temporal order and to location, but was unable to determine if pigs made choices using episodic-like memory or relative memory strength (Mian 2006, unpublished).

The current study was a variation of Experiment 1 of Eacott and Norman's (2004) novel object recognition method. This testing method is particularly suited to pigs, since pigs naturally tend to explore novel aspects of their environment (Wood-Gush & Vestergaard, 1991; Moustgaard *et al.,* 2002). However, several changes to the experimental procedure were required to accommodate species differences, such as anchoring objects and altering the definition of "exploration of objects." These changes are described in detail in the subsequent chapters.

CHAPTER 2

Experiment 1: Pilot Episodic-Like Memory Tests and Novel Object Test

Introduction

Since there is no existing test for episodic-like memory in pigs, we modified methods previously used for testing rat episodic-like memory that were developed by Eacott and Norman (2004). In their study, Eacott and Norman (2004) defined episodic-like memory as the integrated recall of what (object), where (location of the object) and which (the context in which the object and location occur). We adopted this definition and its associated testing method. Specifically, after a brief habituation period, each of our subject pigs was exposed to different objects in particular locations and contexts, and was then tested on its preference for the less familiar of two configurations. If remembered individually, all aspects (objects, locations and contexts) that occurred in the test configurations were equally familiar. Therefore, in order to identify the less familiar configuration, the pig had to remember each aspect of a configuration (object/location/context) simultaneously (i.e., an object was more or less familiar due to the location and context in which it occurred). Since pigs are known to explore novel situations preferentially (Wood-Gush & Vestergaard, 1991; Moustgaard *et al.,* 2002), comparing how long pigs spent with each object/location/context configuration was expected to give an indication of whether pigs exhibit episodic-like memory.

Although measuring episodic-like memory was the main purpose of Experiment 1, it also served the equally important role of providing data necessary to determine any preferences for particular test objects, to determine adequate pen habituation time, and to provide a quantitative measurement of pigs' response to the testing apparatus. Specifically, it allowed for comparison of durations spent with each object, and the characterization of pigs' movement around the test arena. Furthermore, it was expected that Experiment 1 would give some insight into an adequate sample size for statistical analysis, and that it would identify any sex differences among subjects.

In addition to the episodic-like memory test, Experiment 1 also involved a basic test to confirm previously reported findings on pigs' response to novelty (Moustgaard *et al.,* 2002). Each pig was exposed to a novel object and a familiar object, and time spent with each object was recorded.

Method

Subjects

Six Yucatan miniature pigs (3 males, 3 females) were used for Experiment 1. Pigs were born of two different sows at the Memorial University Vivarium pig breeding facility. Paternity was not recorded during breeding, so pigs may have been sired by different boars. Pigs were born between March 7 and March 9, 2007 and were 60-62 days of age (pre-puberty) at the start of Experiment 1. Pigs were vaccinated, injected with iron and ear-tagged within the first week of life. At this time, pigs also had their needle teeth clipped (to prevent injury to the sow's udder and littermates). Male pigs were not castrated. All procedures and daily husbandry practices were carried out according to guidelines set out by the Canadian Council of Animal Care.

Housing

Subject pigs were housed as a group along with six additional pigs of similar age that were not used in experiments. The pen was indoors and consisted of a 6.0m X 6.0m concrete floor surrounded by chain-link fencing. The floor of the pen was washed daily with a hose and feed was placed directly on the floor. Pigs were fed one meal of Co-op Pig Grower at approximately 11:30am every day. Pigs were maintained on a 14:10 light/dark schedule (lights on at 6am, off at 8pm).

Testing Arena

All tests were done in a large, minimally-insulated, high-ceilinged room lit with white fluorescent lights. The room was generally cool (~10°C), but became warmer (~15°C) as outdoor temperatures increased. For trials after the initial habituation trials and the preliminary episodic-like memory test, a radio (Durabrand™ CD player/radio) was played to provide uniform background noise in the test room. The test room was separated from the main Vivarium building by a brick wall. The test room contained six identical pens in a row, but only the first three pens were used as test arenas (Figure 1.1). The pens were, respectively, "Context 1," "holding pen," and "Context 2." Pigs entered the room through a single door into a short, narrow corridor and then turned into a second narrow corridor. The second corridor led to Context 1 and 2 and to the holding pen. The end of the second corridor was blocked by a large plastic storage container to prevent pigs from going to beyond Context 2.

Both contexts had the same dimensions (Figure 1.1). The back wall of each context was brick and the side walls were solid galvanized steel sheeting. These side walls were defined as left and right relative to the observer when facing the back wall of the context. The front of each context was chain-link with a swinging door at each end. The brick wall stretched from floor to ceiling, while the steel and chain-link walls were 2.43m high, with a gap of 0.05m between the floor and the base of the wall. A 0.12m high concrete ridge ran along the floor of the context from front to back, separating the context into two equal halves. Pigs had no difficulty crossing this ridge during trials. At the centre of the ridge was a tall steel pole with a circumference of 0.20m and a height of 2.43m (the same height as the chain link fence). The back wall of each context contained four hatchways leading into pens in adjacent rooms. The two hatchways on right side of each context were covered with plywood (0.61m wide by 0.83m long). The two left-side hatchways had been previously cemented shut, but remained as 0.46m wide by 0.68m long squares indented 0.27m into the wall.

Floor covering was the major distinguishing feature between the contexts. Context 1 had a bare concrete floor, while the floor on either side of the concrete ridge in Context 2 was covered by grooved, black rubber mats. Floors in both contexts were divided by chalk lines into four equal quadrants. Quadrants 1 and 4 were on either side of a low concrete ridge at the front of the context, while Quadrants 2 and 3 were on either side of the same ridge at the back of the context.

The holding pen was located in the test arena located between Contexts 1 and 2. It consisted of wooden chipboard left-side and back walls, a steel right-side wall, and a chain-link front wall with a door. The steel and chain-link walls were 2.43m high and the chipboard walls were 1.08m high.

Materials

Objects

For the pilot episodic-like memory tests, six different objects were used (Figure 1.2). These objects included: a black, plastic rake with no handle (0.55m wide by 0.42m long), an orange, plastic traffic cone (0.14m high and 0.24m wide at base), a rectangular metal basket made of crisscrossing metal wires (0.08m by 0.31m), a heavy metal horseshoe (0.17m by 0.24m), a rectangular wooden cutting board (0.13m by 0.24m by 0.01m), and a wooden coat hanger with no metal hook (0.43m by 0.04m by 0.01m). A black rubber bicycle tire (0.50m diameter) and a red hot-water bottle (0.19m by 0.38m) were used only for habituation trials carried out prior to the episodic-like memory tests. Green, plastic-covered electrical wire was used to attach objects to the steel pole in the centre of each context and to the lower left-hand side of the chain-link front wall of each context.

Event Recorders

Data for each experiment was recorded using two event-recording devices made from two standard PC towers, two keyboards, a monitor and a switchbox. The computer program developed for the devices recorded a timestamp for each keystroke made and this data was later summarized using an extraction program. The programs used to collect and extract the data were developed by Mr. Avery Earle. Both keyboards could be carried comfortably to both Context 1 and Context 2. All data recording equipment was located on a table to the left of the holding pen, out of sight from the pigs.

Data Analysis

Data were analyzed using repeated-measures Analyses of Variance (ANOVA). In all of these analyses, sex was included as a between-groups variable. We tested for sphericity and for most analyses could be assumed. When sphericity could not be assumed, Greenhouse-Geisser adjusted degrees of freedom were used. Where appropriate, Bonferroni post-hoc tests were done.

For some trials, two observers recorded data for each trial. Pearson's r was computed for the duplicate data to test for inter-observer reliability. Inter-observer reliability was found to range from $r=0.923$ ($p<0.01$) to $r=0.959$ ($p<0.01$), depending on the behavioural measure analyzed (see Appendix 1).

Experimental Design

Pig Movement and Scoring

For each trial, a pig was taken from the home pen and walked down the 25.6m corridor that led from the pigs' home pen to the test room. Adjacent corridors were blocked with corrugated cardboard (0.73m high) and two metal carts (0.87m and 0.61m high). Observers guided the pigs using a large piece of white, corrugated plastic (0.84m X 1.28m). When pigs refused to walk, they were encouraged with a gentle squeeze on their hindquarters or a light push with the board. Apart from the first or second time they were moved, pigs generally moved readily between the home pen and test room.

Upon entering the test room, each pig was placed into the holding pen for two minutes. The pig was then guided into either Context 1 or 2 and the event recorder was started. Duration spent in each quadrant of the context was defined by the location of the pig's front feet. A pig was considered to be in one of the marked quadrants in Contexts 1 or 2 when the pig's front feet were both on the ground in the given quadrant. Since the door of each context opened into Quadrant 1, starting the event recorder was equivalent to starting the timer for Quadrant 1. Duration spent exploring each object was defined as the pig manipulating the given object with its snout, or holding its snout within 0.10m of the object. For the duration that a pig spent exploring the object, the event recorder continued to record the pig as being in the same quadrant as it was in when object exploration began.

Observers sat in the corridor outside the chain-link front walls of the contexts on small, green, Chinook™ folding camp chairs. The base of these chairs was 0.53m from the front of the context, which prevented pigs from physically contacting observers through the chain-link. Pigs ignored the observers within the first day of habituation.

Cleaning and Odour Control

Urine and feces were cleaned up following every trial. Feces were removed using a plastic dustpan. The area was scrubbed with paper towel and sprayed with a 0.05% vinegar solution. Urine was soaked up with paper towels and the area was sprayed with 0.05% vinegar solution. On two occasions, both contexts and the holding pen were completely washed with water. Both times hosing was done at the end of a day and before a weekend to allow enough time for the test room to dry completely.

Before the start of each trial, objects used in each context were sprayed on both sides with a 0.05% vinegar solution using a 1L plastic spray bottle, in an attempt to mask odour cues on objects.

Procedure

Habituation Trials

The subject pigs underwent habituation trials consisting of 2min in the holding pen followed by 10min in Context 1 or 2, then 5min in the holding pen, then 10min in the same context, then 5min holding pen, and finally, 5min in same context. Pigs were randomly assigned Context 1 on one day and Context 2 on the other, so that pigs received habituation trials in both contexts. Each context contained a rubber hot water bottle (Object A) attached at the front left corner of the context (Location 1) and a rubber bicycle tire (Object B) attached on the pole at the centre of the context (Location 2). These rubber objects were used only in habituation trials. Each habituation trial was carried out simultaneously with another, so that two pigs were present in the testing room at once.

Episodic-like Memory Test

Episodic-like memory trials consisted of 2min in the holding pen, 10min in one context containing two objects (Exposure 1), 5min in the holding pen, 10min in other context containing the same to objects in opposite locations (Exposure 2), 5min holding pen, and 10min in one context or the other with two identical objects (Test Phase). For example, for Exposure 1, a pig was placed in Context 1 in which Object A was attached to a pole at the centre of the context (Location 1) and Object B was attached to the chain-link at the front left corner of the context (Location 2). The pig was allowed 10min to explore the objects and context and was then removed to the holding pen for 5min. Next, for Exposure 2, the pig was placed in Context 2 with the same objects placed in opposite locations. That is, Object A was attached to the left front corner of the context (Location 2) and Object B was attached to the pole (Location 1). The pig was allowed to explore the objects and the context before being removed to the holding pen for another 5min. For the Test Phase, the pig was placed in either Context 1 or Context 2 for 10min and allowed to explore identical objects present in Location 1 and Location 2 (e.g., Context 1 with Object A in both Location 1 and Location 2). By remembering the location and context in which each object occurred during the two exposure phases, it was possible for the pig to allocate its exploration time differentially, based on the familiarity of the object/location/context configurations during the test phase.

During both exposure and test phases, time spent with each object was recorded, as was time spent in each floor quadrant. Number of visits to each object and quadrant were also recorded. Two objects were assigned to each pig and objects were presented in pairs according to the material of which they were made: plastic cone (Object A) and plastic rake (Object B), metal horseshoe (Object A) and metal basket (Object B), wooden cutting board (Object A) and wooden coat hanger (Object B). During testing, objects were always placed in the same orientation on the floor to the right of their anchoring point (i.e., pole or wall). The trial (which consisted of two exposure phases and one test phase) was repeated three times for each pig. Objects, locations and context were counter-balanced across these trials. Each pig received only one trial per day. In addition, since it was found that pigs tended to vocalize more when another pig was in the test room during habituation trials, pigs underwent episodic-like memory tests individually.

Novel Object Test

The findings from the episodic memory test (described in results section) suggested that pigs' response to novel objects should be tested, and that modification of the habituation and handling protocol was required. Testing for preferential exploration of novelty was particularly important since novelty preference is a key requirement in Eacott and Norman's (2004) episodic-like memory testing method.

Prior to the novel object test, each pig received two habituation trials consisting of 2min in the holding pen, 20min in a randomly assigned context, 5min in the holding pen, and 20min in the other context. Contexts did not contain any objects, two pigs were present in the test room at once, and pigs received only one trial per day. After habituation to the contexts, each pig underwent five 27min exposure trials. Each pig was assigned a context (Context 1 or 2) containing two different objects, and was limited to this context and these objects for all five exposure trials. Each pig was exposed to only two of three possible objects (metal basket, wooden cutting board, plastic cone). Objects were attached with metal clips to metal eyehooks (0.04cm diameter) which were drilled into the floor in the centre of Quadrants 2 and 3. During trials, each pig received 2min in the holding pen, 10min in one context with objects, 5min in the holding pen, and 10min in same context with same objects in the same positions. Each pig received only one exposure trial (i.e., holding pen, 10min exposure, holding pen, 10min exposure) per day. No data was recorded during the habituation or exposure trials.

Once the exposure trials were completed, pigs were tested for their response to a novel object. Each pig received 2min in the holding pen, 10min in same context and with same objects and positions as in exposure trials, 5min in holding pen, and 10min in same context as first 10min except with one of the previous objects replaced with a novel object. The novel object was one of the three (metal basket, wooden cutting board, plastic cone) to which the pig had not been exposed previously. For novel object test trial, duration spent with the novel object and familiar object was recorded for each pig.

Results

Object Preference

A 2 X 3 X 6 ANOVA (Sex X Trial X Object Type) was carried out to determine if objects used in the episodic-like memory trials (horseshoe, basket, cone, rake, cutting board, coat hanger) were equally preferable to pigs. When the exposure phases of the three episodic-like memory trials (during which each object was presented alongside an object of similar material; i.e., wooden cutting board presented alongside wooden coat hanger) were combined, pigs showed a significant difference between the durations spent with each object [$F(5, 20) = 9.92$, $p < 0.01$; Figure 2.1; Table 1]. Pigs spent significantly more time with the plastic rake when this object was compared to all other objects combined [$t(5) = 3.65$, $p = 0.02$].

A one-way ANOVA was used to examine time spent with each object during the test phases of the three episodic-like memory trials (in which each object was presented alongside an identical object; i.e., wooden cutting board presented alongside an identical wooden cutting board). There were no significant differences in durations spent with each object [F(5, 12) = 2.40, p = 0.10; Figure 2.2; Table 2]. However, further analysis showed that, during the test phases, pigs spent more time with the plastic rake when it was compared against all other objects combined [F(1, 12) = 64.70, p < 0.01].

Time Spent in Object Locations

A 2 X 2 ANOVA (Object Location X Object/Location/Context Familiarity) showed no significant difference in time spent with objects attached to pole in the centre of the context and objects attached to front wall of context [F(1, 5) = 0.29, p = 0.61; Figure 2.3; Table 3]. There was no significant interaction between object location and object/location/context familiarity [F(1, 5) = 1.57, p = 0.27; Table 3]. These results indicate that both locations used to anchor objects were equally preferable to pigs.

Duration with More and Less Familiar Object/Location/Context

A 2 X 3 X 2 ANOVA (Sex X Trial X Object/Location/Context Familiarity) showed no significant differences between time spent by pigs with less familiar object/location/context and more familiar object/location/context during the episodic-like memory tests [F(1, 4) = 0.05, p = 0.84; Figure 2.4; Table 4]. Also, there was no significant difference in time spent with more and less familiar object/location/context over the three episodic-like memory trials [F(2, 8) = 1.61, p = 0.26; Table 4]. However, although not significant, male pigs did show a pattern of spending greater mean time with the less familiar object/location/context during each of the three episodic-like memory trials. Conversely, female pigs spent greater mean time with the more familiar object/location/context during each of the three episodic-like memory trials.

When a 2 X 2 X 2 ANOVA (Sex X Trial X Object/Location/Context Familiarity) was carried out with the (preferred) plastic rake trials removed, no significant differences were found for object/location/context familiarity [F(1, 4) = 3.64, p = 0.13; Table 5], but there was a significant sex by familiarity interaction [F(1, 4) = 7.50, p = 0.05; Table 5]. The same non-significant pattern remained where male pigs spent more time exploring the less familiar object/location/context and female pigs spent more time exploring the more familiar object/location/context.

Response to Novelty

A 2 X 2 ANOVA (Sex X Object/Location/Context Familiarity) showed that both male and female pigs spent significantly more time exploring novel (never-seen) objects as opposed to familiar objects [F(1, 4) = 11.63, p = 0.03; Figure 2.5; Table 6]. There was no significant effect of sex [F(1, 4) = 0.47, p = 0.53; Table 6]. As well, all pigs visited the novel object first, before visiting the familiar object.

Duration in each Quadrant

A 2 X 4 X 15 ANOVA (Sex X Quadrant X Exposure Phase) showed that there was a significant difference in mean time spent by pigs in Quadrants 1, 2, 3 and 4 during the 15 exposure phases carried out before the novel object test [$F(1.08, 4.32) = 9.24$, $p = 0.03$; Figure 2.6; Table 7]. Means indicate that pigs spent more time in Quadrants 1 and 4 than in Quadrants 2 and 3, but there were no significant differences when quadrants were compared. There was no significant interaction between sex and time spent in quadrants [$F(1.08, 4.32) = 0.74$, $p = 0.45$; Table 7]. There was also no significant interaction between time spent in each quadrant and exposure phase [$F(3.81, 15.23) = 1.29$, $p = 0.32$; Table 7].

Movement Between Quadrants

A 2 X 4 X 15 ANOVA (Sex X Quadrant X Exposure Phase) was used to examine pig movement among the four quadrants during the 15 exposure phases carried out before the novel object test. This test showed a significant difference among number of visits to Quadrants 1, 2, 3 and 4 [$F(1.03, 4.11) = 12.42$, $p = 0.02$, Figure 2.7; Table 8]. Post-hoc comparisons demonstrated that pigs visited Quadrant 1 more frequently than Quadrant 2 [$t(89) = 8.64$, $p < 0.001$] and Quadrant 3 [$t(89) = 10.05$, $p < 0.001$), and that they visited Quadrant 4 more frequently than Quadrant 3 [$t(89) = 9.63$, $p < 0.001$]. There was no significant sex difference [$F(1.03, 4.11) = 3.50$, $p = 0.13$; Table 8]. There was also no significant interaction between visits to each quadrant and exposure phase [$F(3.61, 14.44) = 2.81$, $p = 0.07$; Table 8]

Discussion

The results of Experiment 1 provide insight into pigs' response to objects and the testing arena, identify potential differences in the episodic-like memory between sexes, and confirm pigs' tendency to explore novelty preferentially. Interestingly, one particular object, the plastic rake, was preferred over the other objects, leading to a significant difference in durations spent with objects. This preference was likely due to the rake having a greater surface area than the other objects, which meant that pigs could explore it using their whole body (i.e., tossing it into the air and allowing it to fall on their back). Due to this preference, the rake was not used in subsequent experiments. Durations spent with each of the other objects were not statistically different from one another. Therefore, the level of comparability among other objects was high. However, comparability between objects was not of great concern because two copies of the same objects were used in the actual test phases of these experiments.

Pigs tended to spend more time at the front of the test arena (Quadrants 1 and 4), which may be explained by the fact that this was the only wall of the test arena that pigs could see through. The chain-link front wall also contained the door through which pigs entered. Since pigs seemed highly motivated to escape the test arena, attraction to the front wall is not surprising. Therefore, although there was no statistically significant preference for object anchoring locations (pole or front of context), objects used in subsequent experiments were affixed to hooks near the back of the test arena in Quadrants 2 and 3 (i.e., non-preferred quadrants).

The quadrant and object preferences shown by pigs during the episodic-like memory test may have caused the lack of difference in the amount of time pigs spent with the more and less familiar object/location/context configurations. Distraction due to the rake and due to preoccupation with escaping the test arena may have masked any episodic memory effect present. Moreover, the finding that male pigs spent more time with the less familiar object/location/context during each of the three episodic-like memory tests was non-significant because it was offset by the finding that female pigs did the exact opposite (i.e., spent more time with the more familiar object/location/context). When analyzed separately, male and female data still failed to reach statistical significance because of the small sample size (n = 3) for each sex. Therefore, we increased sample size in subsequent experiments.

The significant preference shown by pigs for the novel object as compared to a familiar object, confirms that pigs spent more time with objects that are unfamiliar. This result is consistent with previous findings on pig novelty exploration (Wood-Gush & Vestergaard, 1991; Moustgaard *et al*., 2002). In response to these results and others described above, I developed a more robust pig episodic-like memory test, described in Experiment 2.

CHAPTER 3

Experiment 2: Episodic-Like Memory Tests and Long-term Novel Object Test

Introduction

In this experiment, episodic-like memory in pigs is tested again, incorporating methodological changes suggested by the results of Experiment 1. Specifically, male pigs were tested in Experiment 2, due to the potential sex differences found in the previous experiment. Sample size increased from three males to eight males to increase statistical power. Additionally, extended habituation trials were completed before the start of the Experiment 2 episodic-like memory tests to ensure that pigs were more familiar with the test arena prior to the actual episodic memory tests. Furthermore, since pigs spent significantly more time in Quadrants 1 and 4 during Experiment 1, objects were anchored in Quadrants 2 and 3 during Experiment 2. This eliminated the potential for quadrant preference to confound object/location/context preference results. With these changes, the Eacott and Norman (2004) method was expected to provide a clear measure of object/location/context memory in pigs.

Since human episodic memory occurs over the long term and several definitions of non-human episodic memory require the ability to report on past events over a long term (Schwartz & Evans, 2001), the subject pigs' memory for a familiar object over a long term was also of interest. Since a strong short-term recognition of familiar objects was shown in Experiment 1, repeating the novel object test with a longer interval between object exposure and testing was expected to give greater insight into whether pigs' ability to recognize familiarity persists into the long term. Specifically, our test examined whether pigs were able to recognise an object as familiar one week after last interacting with it, and whether they could differentiate this object from a never-seen object.

Method

Subjects

Four male pigs were born on May 12, 2007 of a Yucatan Miniature sow bred with a Duroc boar. The other four male pigs were born on May 21, 2007 of a Yucatan Miniature sow and Landrace boar. Both breedings were carried out at the Memorial University of Newfoundland Vivarium. Pigs were 36-44 days of age (pre-puberty) at the start of Experiment 2. Pigs were vaccinated, injected with iron and ear-tagged within the first week of life. At this time, pigs also had their needle teeth clipped (to prevent injury to the sow's udder and littermates). Male pigs were not castrated. All procedures and daily husbandry practices were done according to guidelines set out by the Canadian Council of Animal Care.

Housing

Pigs were housed as a group in an indoor pen consisting of a (5.8m X 6.7m) room with a red tile floor and concrete walls. Pigs were fed one meal of Co-op Pig Grower in three black rubber tubs at approximately 8:40am (i.e., before the start of testing) every day. The floor of the pen was washed daily with a hose and pigs were maintained on a 10:14 light/dark schedule, with lights on at 6:00am.

Apparatus

The apparatus used in Experiment 2 was the same as for Experiment 1, except that Quadrants 2 and 3 contained a metal eyehooks (0.04cm diameter) used to anchor test objects. As was done for the Novel Object Test in Experiment 1, eyehooks were drilled into the floor in the back centre part of each quadrant (Figure 1.1). The pole and front wall object anchoring locations were not used in Experiment 2.

Materials

Objects

Due to the preference for the plastic rake identified during Experiment 1, the plastic rake was not used in Experiment 2. However, the other five objects used in Experiment 1 were used again. A metal clip was attached to each object with a cable tie, so that the object could be easily clipped onto the metal eyehooks in the contexts.

Event Recorders

Data recording proceeded in the same manner as in Experiment 1.

Data Analysis

Data was analyzed using repeated-measures Analyses of Variance (ANOVA). In all of these analyses, sex and strain were included as a between-groups variables. In most analyses, sphericity could be assumed, but when it could not be, Greenhouse-Geisser adjusted degrees of freedom were used.

Inter-observer reliability was tested with Pearson's r analysis using data from the Long-term Novel Object Test. It was found to be $r = 1.00$ ($p < 0.01$) and $r = 0.99$ ($p < 0.01$) for duration spent with novel and familiar objects, respectively. For duration spent in each quadrant, it ranged from $r = 0.97$ ($p < 0.01$) to $r = 0.64$ ($p = 0.03$; See Appendix 1).

Design and Procedure

Pig Movement and Scoring

Pig movement and scoring details in Experiment 2 were the same as for Experiment 1.

Cleaning and Odour Control

Cleaning and odour control details in Experiment 2 were the same as for Experiment 1.

Episodic-like Memory Test

Since the pigs in Experiment 1 required multiple habituation trials before they appeared comfortable in the test arena (e.g., reduced escape attempts, etc.), each subject pig in this experiment underwent nine days of habituation before the start of the episodic-like memory testing. Each day of habituation consisted of 2min in the holding pen followed by a 20min habituation trial in a randomly assigned context, then 5min in the holding pen, and finally, another 20min habituation trial in the same context again. Since each pig received two 20min habituation trials per day for nine days, this resulted in 18 habituation trials for each pig. However, quadrant movements were recorded during the first, sixth and eighth days of habituation (i.e., Trials 1, 2, 11, 12, 15, 16) only. Context assignments were counter-balanced over strain and each pig was habituated to both Context 1 and Context 2. No objects were present in either context. Two pigs underwent habituation trials simultaneously in the test room, with one observer at each context. As a potential measure of habituation, the amount of time spent vocalizing was also recorded during days one, six and eight. Vocalization was defined as squealing noises that were louder than the quiet, sustained grunting exhibited by most pigs while they were in the test room.

For the episodic-like memory tests, each pig was tested individually (i.e., only one pig in the test room at a time). The procedure was similar to that of the preliminary episodic-like memory tests in Experiment 1, except that objects were attached to eyehooks in Quadrant 2 or 3 rather than to the pole and front of the context. Each episodic-like memory trial consisted of 2min in the holding pen, 10min in one context containing two objects (Exposure 1), 5min in the holding pen, 10min in other context containing the same to objects in opposite locations (Exposure 2), 5min holding pen, and 10min in one context or the other with two identical objects (Test Phase). For example, for Exposure 1, a pig was placed in Context 1 in which Object A was on the pig's left and Object B was on the pig's right (Location 2; Figure 3.1). The pig's left and right were defined as the pig faced the back wall of the context (i.e., away from the door and observers). The pig was allowed 10min to explore the objects and context and was then removed to the holding pen for 5min. Next, for Exposure 2, the pig was placed in Context 2 with the same objects placed in opposite locations. That is, Object A on the pig's right and Object B on the pig's left (Figure 3.2). The pig was allowed to explore the objects and the context before being removed to the holding pen for another 5min. For the Test Phase, the pig was placed in either Context 1 or Context 2 for 10min and allowed to explore identical objects present on the left and right (e.g., Context 1 with Object A on both left and right; Figure 3.3). By remembering the location and context in which each object occurred during the two exposure phases, it was possible for the pig to allocate its exploration time differentially, based on the familiarity of the object/location/context configurations during the test phase (Figure 3.3).

Test objects were used in different combinations and each pig was tested four times (July 11/12, July 16/17, July 24/25, July 26/27). For the first of these four trials, one metal basket and one plastic cone were present for each exposure phase and two metal baskets or two plastic cones were present for the test phase. The second trial used the same objects (basket and cone), but pigs received the opposite object in the test phase (i.e., if the pig received basket and basket in the first trial test phase, it received cone and cone in the second trial test phase; Figure 3.3, 3.4). For the third trial, three objects were used. Each pig received two of: one wooden coat hanger, one wooden cutting board or one metal horseshoe during each exposure phase, and two identical objects (two hangers, two boards, or two horseshoes) during the test phase. Finally, for the fourth trial, pigs received the same two objects as they were assigned in the third trial and during the test phase the opposite object was used (Figure 3.3, 3.4). For all trials, assigned contexts, objects, test object and context order were counter-balanced according to pig strain (Duroc-Yucatan or Landrace-Yucatan).

Long-term Novel Object Test

The long-term novel object test was done in conjunction with the third and fourth episodic-like memory trials. The rationale behind using three objects in these trials was that each pig was exposed to only two of the three objects (i.e., each pig had a particular object to which it was never exposed). One week after completion of the fourth trial of the episodic-like memory test, each pig was placed in the holding pen for 2min and then placed in a randomly assigned context containing the novel (never-seen) object and a familiar (previously seen in the episodic-like memory test one week ago) object. The pig was allowed to explore both objects for 10min. Duration spent with each object and number of visits to each object were recorded.

Results

Habituation Trials

Duration in each Quadrant

A 2 X 4 X 3 X 2 ANOVA (Strain X Quadrant Duration X Day X Trial) showed a significant difference in durations spent in each quadrant [$F(3, 18) = 23.97$, $p < 0.01$; Figure 3.5; Table 9]. Post-hoc comparisons showed that, similarly to pigs in Experiment 1, Experiment 2 pigs spent significantly more time in Quadrant 1 than in Quadrant 2 [t(47)

$= 5.29$, $p < 0.001$] and Quadrant 3 [$t(47) = 3.25$, $p = 0.02$]. Pigs also spend significantly more time in Quadrant 4 than in Quadrant 2 [$t(47) = 5.95$, $p < 0.001$] and Quadrant 3 [$t(47) = 3.96$, $p < 0.001$]. There was a significant quadrant duration by habituation day interaction [$F(6, 36) = 8.45$, $p < 0.01$; Figure 3.6; Table 9]. Means indicate that pigs spent less time in Quadrants 2 and 3, and more time in Quadrants 1 and 4 as the habituation days progressed. There was also a significant interaction between pig strain and duration spent in each quadrant [$F(3, 18) = 0.489$, $p < 0.01$; Figure 3.7; Table 9].

Movement Between Quadrants

A 2 X 4 X 3 X 2 ANOVA (Strain X Quadrant Visits X Day X Trial) showed that there was a no significant difference in the number of movements between quadrants by pigs over the habituation days [$F(2, 12) = 0.07$, $p = 0.94$; Figure 3.8; Table 10] or over trials within days [$F(1,6) = 1.15$, $p = 0.32$.; Figure 3.9; Table 10]. This finding indicates that pigs continued to move between quadrants at a steady rate (i.e., pigs maintained a steady activity level) as habituation days/trials progressed.

Vocalizations

A 2 X 3 X 2 ANOVA (Strain X Day X Trial) showed no significant difference between duration of loud vocalizations over habituations days [$F(2, 12) = 0.15$, $p = 0.86$; Figure 3.10; Table 11] or over habituation trials within days [$F(1,6) = 2.09$, $p = 0.20$; Figure 3.10; Table 11].

Episodic-like Memory Test

Duration with More and Less Familiar Object/Location/Context

A 2 X 4 X 2 ANOVA (Strain X Trial X Familiarity) showed that pigs spent significantly more time with the less familiar object/location/context [F(1, 6) = 16.74, p = 0.01; Table 12] and that there was a Trial X Familiarity interaction [F(3, 18) = 3.27, p = 0.05; Table 12]. As can be seen in Figure 3.11, the familiarity effect indicated that the pigs showed episodic memory because they spent more time with the less familiar object/location/context as compared to the more familiar object/location/context. However, by the fourth test, this preferred response to the less familiar object/location/context had disappeared (Figure 3.12). There was no significant overall effect of trial [F(3, 18) = 1.00, p = 0.43; Table 12], which indicates that total amount of time spent with objects was constant across trials. Also, there was no significant interaction between familiarity and pig strain [F(1, 6) = 0.80, p = 0.41; Table 12] or between trial and pig strain [F(3, 18) = 0.79, p = 0.52; Table 12].

Data were sorted into 1-minute bins and averaged over the four episodic-like memory trials. A 4 X 2 X 10 ANOVA (Trial X Familiarity X Minute) showed a significant effect of minute [F(9, 63) = 4.93, p < 0.01; Table 13]; there was a linear decrease in the amount of time pigs spent exploring the object/location/contexts as each 10-minute test phase progressed [F(1, 63) = 20.69, p < 0.01]. This pattern appears more defined for the less familiar object/location/context as opposed to the more familiar object/location/context, but the familiarity by minute interaction was not significant [F(9, 63) = 1.75, p = 0.10; Figure 3.13; Table 13].

Number of Visits to Object/Location/Contexts by Pig Strain

A 2 X 4 X 2 ANOVA (Strain X Trial X Familiarity) carried out on the number of visits to object/location/context showed a Trial X Strain interaction [$F(3, 18) = 7.06$, $p < 0.01$; Figure 3.14; Table 14]. There was no significant trend associated with this difference.

Long-term Novel Object Test

As predicted, pigs spent significantly more time with the novel objects than with the familiar objects during the long-term novel object test [$t(7) = 2.35$, $p = 0.05$; Figure 3.15]. Pigs visited the novel objects significantly more often than the familiar objects [$t(7) = 3.36$, $p = 0.01$; Figure 3.16]. As well, 6 out of 8 pigs visited the novel object first, before visiting the familiar object.

Discussion

Episodic-like memory Trials

Pigs spent more time with the less-familiar object/location/context during the test phases of the episodic-like memory trials, indicating that they were able to simultaneously recall memories of what (object), where (location) and which (context). This is so because the separate aspects (object, location, and context) of each object/location/context configuration are equally familiar; it is only the combination of all three aspects that makes one configuration less familiar than another. Therefore, the pigs' significant preference for the *less* familiar configuration cannot be attributed to object preference alone, location preference alone, or context preference alone. Objects in the test phase were identical, pigs had been equally exposed to both locations before the test phase, and pigs had been equally exposed to both contexts before the test phase. Furthermore, the preference for the less familiar object/location/context could not be attributed to object and location alone because objects and locations were counterbalanced for each pig. Thus, each pig received an episodic-like memory trial with two objects "A" in the test phase and a trial with two objects "B" in the test phase. If pigs were ignoring context and making decisions based solely on object and location, half the time pigs would spend more time with the left-hand object and other half of the time pig would spend more time with the right-hand object. This would have resulted in no significant overall preference for either object/location/context. Clearly, the data indicate that this is not the case, leading to the conclusion that pigs formed integrated memories of what/where/which.

The trial by familiarity interaction found for the combined episodic-like memory data reflects the result for the fourth trial, during which pigs did the opposite as they had in the previous three trials. Since the difference between durations spent with more and less familiar object/location/context was significant only for all trials combined and not for any individual trial, the non-significant opposite effect found during the fourth trial is not particularly informative. The change in pattern in the fourth trial does allow speculation that pigs may gradually lose their ability to distinguish less and more familiar object/location/context. Although, on a minute-by-minute basis, pigs significantly decreased their exploration of both objects from the start of a test trial (0min) to the end

(10min), the lack of significant effect of trial suggests that the change in pattern in the fourth trial was not due to a gradual decrease in object exploration over trials. Further experiments are needed to more clearly characterize pigs' episodic-like memory over an increased number of trials.

Long-term Novel Object Test

The long-term novel object test carried out at the end of the episodic-like memory testing indicate that pigs' memories of familiar objects persist for at least one week. Presence of long-term memory for familiar objects suggests that pigs may have the capacity to form episodic-like memories over longer periods than those tested in the current experiment, which indicates that long-term episodic memory testing is warranted.

Habituation Trials

Similarly to Experiment 1, the pigs in Experiment 2 spent significantly more time at the front of the context (Quadrants 1 and 4) during the habituation phase, which suggests that these pigs may have shared the same motivation to escape the context (i.e., through the door at the front of the context). Furthermore, the significant trial by duration interaction found in Experiment 2 indicates that pigs increased time spent in Quadrants 1 and 4 and decreased time in Quadrants 2 and 3 as trials progressed. As well, as days progressed, pigs appeared to show no statistically significant decrease in their level of movement around the context (number of movements between quadrants) or in the frequency of loud vocalizations. Furthermore, no significant difference in movement or vocalizations was found when trials within days were compared. Although these results seem to indicate an absence of habituation, qualitative observations suggest otherwise. Qualitative observations over the habituation trials indicated that Experiment 2 pigs appeared more comfortable in the contexts as days progressed, with some pigs lying down on occasion. Although not formally quantified, pigs also appeared to engage in fewer escape behaviours (jumping at walls, trying to get under walls, etc.) as habituation trials progressed. The discrepancy between quantitative and qualitative results suggests that, contrary to habituation measures for other lab animals, time spent in quadrants, movement around the context and vocalization frequency may not be good measures of pig habituation. It is possible that pigs must be exposed to the test arena for periods longer than 40min at one time in order for decreases in activity and vocalization to be evident. Further study of pig habituation is needed to provide a definitive characterization of pig habituation patterns. For the current study, by the end of habituation trials, qualitative evidence of habituation was robust enough to conclude that the pigs were sufficiently comfortable in the test arenas to allow for the start of episodic-like memory testing.

Effect of Pig Strain

While there appear to be significant differences between Yucatan-Duroc and Yucatan-Landrace pigs in overall time spent with object/location/context, this difference is not particularly informative. The lack of significant trends in this data do not allow for conclusions about the nature of the differences or about what they indicate about pig behaviour. However, the findings do suggest that strain differences warrant consideration in future pig studies and specific experiments are required.

CHAPTER 4

Experiment 3: Female Episodic-Like Memory Tests and Long-term Novel Object Test

Introduction

It is apparent that male-specific and female-specific behaviours exist in most vertebrate species, particularly during reproduction. In contrast, the presence of differences in male and female memory and learning abilities is more controversial (for review, see Sherry, 2006). Sex differences in memory use and/or neural substrates for memory have been demonstrated in some species, such as meadow voles and brood - parasitic cowbirds. Specifically, polygynous male meadow voles, which access females by remembering the spatial locations of female burrows, have a larger hippocampus than female meadow voles (Jacobs *et al.*, 1990). Since the hippocampus is required for spatial memory and a difference in hippocampus size is not found in monogamous prairie voles (Jacobs *et al.*, 1990), it is argued that spatial memory is more developed in male meadow voles (than in female meadow voles) due to males' increased use of, and likely memory for, space. This argument has been extended to other species, since a relatively larger hippocampus is also found in males of several other polygynous mammals (Galea & McEwan, 1999; Jacobs & Spencer, 1994; Sherry *et al.*, 1996). The existence of sex differences in memory ability is further supported by findings that parasitic cowbird females, who lay eggs in nests of other females and must remember the locations of these nests, have a larger hippocampus than do male parasitic cowbirds (Sherry *et al.*, 1993). This disparity in hippocampus size is not found in related, non-parasitic species (Sherry *et al.*, 1993). These findings suggest that there may be sex differences found in other species, with "superior" spatial memory conferred to either males or females, depending on the life history strategies and evolutionary pressures faced by the species.

In pigs, behavioural differences between males and females have also been noted. Bolhuis *et al.* (2006) found that month-old female pigs showed significantly more exploratory behaviour than did month-old male pigs. Lind and Moustgaard (2005) similarly reported that female Gottingen minipigs spent less time exploring novel objects than do male minipigs. As well, in the Yucatan miniature pig, sex differences have been detected in open field behaviours and in pre-training latencies to perform food-rewarded tasks (Walsh, personal communication). In contrast, Weary *et al.* (1999), found no sex differences in the behaviour of month-old pigs in response to separation from their mothers. However, Poletto *et al.* (2006) suggest that age may be a factor in pigs' behavioural response to stress and that sex differences may not be apparent until after sexual maturity. To date, there are no reported studies of sex differences in pig learning and memory.

Due to patterns in female pig behaviour found in Experiment 1 and the preliminary evidence of sex differences emerging in Yucatan miniature pigs, the episodic-memory and long-term novel objects tests described in Experiment 2 were also carried out on four female pigs. These pigs underwent testing simultaneously with the male pigs in Experiment 2. Results were analyzed separately because of the small number of female subjects, which resulted in insufficient statistical power to include sex as a between subjects factor in combined male/female data. It was expected that female data would give further insight into potential sex differences in pig episodic-like memory abilities.

Method

Subjects

Four female pigs were used. Two of these were born on May 12, 2007 of a Yucatan Miniature sow bred with a Duroc boar. The other two were born on May 21, 2007 of a Yucatan Miniature sow and Landrace boar. All other subject details were the same as for Experiment 2.

Housing, Apparatus, Materials, Data Analysis, Design and Procedure

All procedures were the same as Experiment 2.

Results

Habituation Trials

Duration in each Quadrant

A 2 X 4 X 3 X 2 ANOVA (Strain X Quadrant Duration X Day X Trial) showed a significant difference between durations spent in each quadrant [F(3, 6) = 10.09, p = 0.01; Figure 4.1; Table 15]. Post-hoc comparisons showed that, similarly to male pigs, female pigs spent significantly more time in Quadrant 1 than in Quadrant 2 [t(23) = 3.18, p = 0.04] and Quadrant 3 [t(23) = 3.98, p = 0.01]. Pigs also spend significantly more time in Quadrant 4 than in Quadrant 2 [t(23) = 3.48, p = 0.002] and Quadrant 3 [t(23) = 3.74, p = 0.001]. There was a significant Quadrant Duration X Day interaction [F(6, 12) = 3.00, p

= 0.05; Figure 4.2; Table 15]. Means indicate that pigs spent less time in Quadrants 2 and 3, and more time in Quadrants 1 and 4 as habituation days progressed.

Movement Between Quadrants

A 2 X 4 X 3 X 2 ANOVA (Strain X Quadrant Visits X Day X Trial) showed that there was no significant difference in the overall number of visits to quadrants over the nine habituation days [F(2, 4) = 1.32, p = 0.36; Figure 4.3; Table 16] or over habituation trials within days [F(1, 2) = 0.68, p = 0.50; Figure 4.4; Table 16]. This finding indicates that pigs continued to move between quadrants at a steady rate (i.e., pigs maintained a steady activity level) as habituation trials and days progressed. However, there was a significant quadrant visits by day interaction [F(6, 12) = 4.39, p = 0.01; Figure 4.3; Table 16]. Means indicate that pigs made more visits to Quadrants 1 and 4 than to Quadrants 2 and 3 as days progressed.

Vocalizations

A 2 X 3 X 2 ANOVA (Strain X Day X Trial) showed no significant difference between duration of loud vocalizations over nine habituations days [F(2, 4) = 0.12, p = 0.89; Figure 4.5; Table 17] or over habituation trials within days [F(1, 2) = 0.01, p = 0.93; Figure 4.5; Table 17]. There was also a significant trial by day interaction [F (2, 4) = 9.57, p = 0.03]. Means indicate that pigs vocalized more in the first trial on Days 1 and 8 of habituation, and that they vocalized more in the second trial on Day 6 of habituation.

Episodic-like Memory Test

Duration with More and Less Familiar Object/Location/Context

Contrary to findings for male pigs, no effect of familiarity was found when female pig data from all four episodic-like memory trials was combined. A 2 X 4 X 2 ANOVA (Strain X Trial X Familiarity) showed no significant difference in time spent with the less familiar object/location/context versus the more familiar object/location/context [F(1, 2) = 0.03, p = 0.87; Table 18; Figure 4.6]. Means indicate that females spent more time with the less-familiar object/location/context on the first and last trials, and more time with the more-familiar object/location/context on the second and third trials. There was no significant effect of trial [F(3, 6) = 0.41, p = 0.75; Table 18].

Data were sorted into 1-minute bins and averaged over the four episodic-like memory trials. A 4 X 2 X 10 ANOVA (Trial X Familiarity X Minute) showed a significant minute effect [F(9, 27) = 3.08, p = 0.01; Table 19; Figure 4.7]. Specifically, there was a significant linear decrease in the amount of time pigs spent exploring both object/location/contexts as each 10-minute test phase progressed [F(1, 27) = 25.28, p = 0.02; Figure 4.7].

Longterm Novel Object Test

Female pigs showed no significant difference in time spent with the novel object and familiar object [t(3) = 0.88, p = 0.44; Figure 4.8]. There was also no significant difference in the number of visits made by female pigs to the novel object and the familiar object [t(3) = 0.29, p = 0.79]. As well, 3 out of 4 female pigs visited the novel object before visiting the familiar object.

Discussion

Episodic-like Memory Trials and Novel Object Test

Female pigs appeared to behave similarly to male pigs during the habituation phases prior to the episodic-like memory tests. They spent more time in Quadrants 1 and 4 and did not decrease frequency of loud vocalizations as trials progressed. However, during episodic-like memory trials, female pigs did not show any significant difference in time spent with the more familiar and less familiar object/location/context. This finding may be due to the small sample size (n = 4), which lacks statistical power. However, means indicate that female pigs may be legitimately different from male pigs in their episodic-like memory and/or behavioural patterns. Female pigs spent more time with the less familiar object/location/context in the first and last trials and more time with the more familiar object/location/context in the second and third trials. These results are similar to those found for female pigs in the Experiment 1 episodic-like memory trials, suggesting that it is possible that females are responding only to object and location memories (rather than a memory combining object/location/context). Due to counterbalancing of objects and locations and contexts, this strategy would result in no consistent overall effects.

An alternate explanation is that female pigs responded to the novelty aspect of the testing procedure differently than did male pigs, which prevented them from accurately demonstrating memories for what/where/which. This explanation is supported by the results of the long-term novel object test, where female pigs did not show a difference in amount of time spent with the novel and familiar object. Although this lack of difference may be attributed to small sample size, it is consistent with findings on Gottingen minipigs in which female pigs were found to spend significantly less time exploring novel objects than male pigs (Lind and Moustgaard, 2005). In the 2005 study, Lind and Moustgaard argue that duration spent with novel objects is a measure of emotional reactivity, and found a positive correlation between lower emotional reactivity (less behavioural response to novelty) and better performance on a "go/nogo" learning task. Although it is not possible to look for correlations between emotional reactivity and memory in our study (because novelty preference is essential for the completion of the what/where/which memory task), an inherently lower response to novelty reduces the suitability of a novel object recognition test as a measure of female episodic-like memory. It is evident from the linear decrease shown by female pigs in exploration of both object/location/contexts from the first minute of the test phase to the last (10th min) that females were motivated enough to explore both object/location/contexts and that their interest gradually declined. However, female pigs' motivation to explore novelty may have been too low for any preference for the less familiar object/location/context to become apparent. Overall, these results indicate that a replication of this experiment should be carried out with a larger number of females to provide a more reliable description of episodic-like memory in female pigs.

Habituation Trials

During the habituation trials carried out prior to episodic-like memory testing, female pigs showed a similar pattern as the male pigs in Experiment 2 (spending more time in the front of the contexts). Furthermore, the significant quadrant visits by day interaction suggest that pigs were motivated to escape the context (i.e., through the door at the front of the context) and that this motivation increased as days progressed. Durations of loud vocalizations by female pigs did not change significantly over days or trials within days, which is also consistent with the male findings. Also similar to male pigs, was the finding that female pigs moved around the contexts at a steady rate over days and over trials within days, which is reflected by no significant difference in the number of quadrant visits over trials or over days.

The finding that pigs made significantly more visits to Quadrants 1 and 4 over days was unique to female pigs. This finding indicates that female pigs were increasingly more active at the front of the context (i.e., moving between Quadrants 1 and 4), which may reflect the females' increasing motivation to escape the context. Overall, however, female and male pigs showed a similar lack of habituation, as defined by the methods of quantification (durations in each quadrant, vocalizations, and movement around the context). As with males, qualitative observations suggest that female pigs became more comfortable to the contexts as days progressed.

Chapter 5: GENERAL DISCUSSION

General Summary of Results

The results of Experiment 2 contribute to the demonstration of episodic-like memory in pigs by showing that male pigs recognize an object as being more or less familiar due to the location and context in which it occurs. Particularly, since male pigs remembered object/location/context simultaneously, they met the requirements for the what/where/which definition of episodic-like memory outlined by Eacott and Norman (2004). The presence of episodic-like memory in male pigs is further supported by our finding that male pigs' memories for familiar objects persist into the longterm, which allows for speculation that they are also be capable of remembering object/location/context into the longterm. Memories that persist in the longterm are a key component of human episodic memory.

Experiments 1, 2 and 3 provide insight into the nature of episodic-like memory in pigs and identify important considerations for future research involving pigs. Both experiments found that female pigs responded differently than male pigs during episodic-like memory testing, which suggests that female pigs may not be capable of episodic-like memory or that they may respond to testing methods differently than male pigs. Since only a small number of female pigs were tested, further study is required to fully characterize female episodic-like memory. However, findings do suggest that potential sex differences are an important consideration in future episodic-like memory tests. Also, findings of strain differences in Experiment 2 indicate that further consideration of pig strain is also warranted.

Besides characterizing episodic-like memory, Experiments 1, 2 and 3 provide insight into pig behaviour that is useful for design of future studies. Particularly, they identify that pigs are biased to spend more time in the area of a test arena nearest to the door (or potential escape route), which is an important consideration when deciding locations of test objects/apparatus. As well, our findings indicate that, vocalizations and amount of movement around the test pen are not good measures of habituation in pigs. Therefore, other measures, such as reduced escape attempts, may be more suitable for quantifying pig habituation.

Defining Non-human Episodic Memory

The what/where/which episodic memory testing method employed in this study is considered particularly simple and efficient because it requires no pre-training and does not require animals to demonstrate a sense of time (i.e., time is replaced by context). Although context has not been as widely used as time in episodic memory studies, the rat-based studies of Eacott and Norman (2004), and Eacott and Gaffan (2005) provide a convincing case for the equivalence of time and context. Their main argument is that the role of time in human episodic memory to act as an occasion specifier (i.e., to identify an event as being unique), is a role that can also be filled by context. This view is supported by Friedman (1993), Gaffan and Harrison (1989), and Gaffan (1994). Furthermore, there are countless anecdotal examples of humans using memories of context instead of time during episodic recall, such as the fact that I remember that I had cereal for breakfast, at the kitchen table, at my house. I do not remember the exact time that this event happened, but I remember that it occurred at my house rather than when I got to school. The use of context in the definition for episodic-like memory is also found in tests that demonstrate a sense of time by requiring animals to remember the order in which events occurred (e.g., Fortin *et al.,* 2002). Similar to context, remembering sequence does not involve the recall of the exact time that an event occurred. Rather, it separates events by their occurrence in relation to other events, which is arguably a contextual cue. That is, subjects recognise an event by what it is, where it is, and the context (order in relation to others) in which it occurs.

The above argument for the equivalence of object/location/context-based episodic memory studies and sequence-based episodic memory provided a good example of the variation that exists in the definition of non-human episodic memory. Although numerous methods have been developed for demonstrating non-human episodic memory, the lack of an agreed-upon definition for non-human episodic memory makes it difficult to decide if methods truly show what researchers claim they do. The absence of a standard definition and methods has led to a tangled series of demonstrations of non-human episodic memory that may or may not be equivalent to one another. While it is undeniable that the different streams of episodic memory research have provided insight into non-human memory, variability in definitions has caused many non-human episodic memory tests to stray from the requirements for human episodic memory (Suddendorf & Busby, 2003). Although it can be argued that diversions from the human episodic memory definition may reflect fundamental differences between human and non-human episodic memory, the need for more explicit definitions does not change. The need for definitions is most evident in the work of Clayton and Dickinson (1998), who appear to have found a type of memory that extends beyond semantic, but does not fully meet the requirements of human episodic memory. This memory was initially termed "episodic-like" and defined as the integrated recall of what/where/when, but extensive use of the term "episodic-like" in other memory studies has led to confusion over its meaning (Clayton, Bussey & Dickinson, 2003; Suddendorf & Busby, 2003). This problem suggests that the field of "episodic-like memory" research has matured to a point where agreed definitions of non-human episodic memory are necessary to prevent conclusions from being stalled by semantic arguments.

Building a single definition from the wide range of research that claims to explore episodic memory is likely an impossible task. Therefore, it seems most practical to provide three increasingly complex definitions that encompass a spectrum ranging from basic what/where/when memory to human episodic memory. Specifically, these definitions could include "What/Where/When Memory," "Episodic-like Memory," and "Mental Time Travel (MTT) Episodic-like Memory." The first level of non-human episodic memory definition, What/Where/When Memory, requires only the demonstration of simultaneous recall of the what, where and when (or which) circumstances of a past event. This definition serves as a sort of "catch-all" for studies that attempt to explore non-human episodic memory, but do not meet all of the requirements for higher definitions. The second level of definition, Episodic-like Memory, enters into the realm of Clayton and Dickinson's (1998) research. It requires the demonstration of subjects' ability to unexpectedly recall a personally experienced event over a long term. In this definition, recall of an event involves either integrated memory of what/where/when (i.e., Clayton & Dickinson, 1998; Babb & Crystal, 2006) or physical/symbolic communication of the details of a past event (i.e., Menzell 1999; Mercado *et al.*, 1998). This definition also requires, when possible, that evidence of dependence on the hippocampus be demonstrated. The third level, which is most closely related to human episodic memory, is Mental Time Travel (MTT) Episodic-like Memory. This definition requires the same criteria as Episodic-like Memory plus distinct evidence that the subject can travel backward and/or forward in time to mentally re-create the remembered event.

Further Investigation of Episodic Memory in Pigs

The series of definitions outlined above lay the groundwork for further progress in the study of episodic memory in pigs. The current study meets the requirements for What/Where/Which (When) definition. Furthermore, due to the spontaneous recall involved in the novel object recognition test, the current study also meets a main requirement for the Episodic-like Memory definition. However, to qualify for this second level of definition, the methods of the current study must be expanded to demonstrate long term memory and reduced memory performance in hippocampally-impaired pigs. As was done with rats by Eacott and Norman (2004), methods used in the current study should be expanded to include delays of variable length between the exposure and test phases. Having longer and shorter delays will provide information regarding how long what/where/which memories persist over time, and whether they extend into the long term. Testing whether performance on the what/where/which novel object recognition test depends on the integrity of the hippocampus can be achieved similarly to Eacott and Norman (2004). That is, the current experiment could be repeated with hippocampally-damaged pigs, so that their what/where/which memory performance can be compared with healthy pigs.

In addition to meeting the three main terms of the Episodic-like Memory definition, another dimension can be added to the current testing methods by hiding objects during the testing phase. This approach was used successfully with rats by Eacott *et al.*, (2005) in a follow-up to Eacott and Norman (2004). This method would involve exposing pigs to objects, locations and contexts in a similar way as in the current study. The pig would be exposed to objects A and B in one context, then objects A and B in opposite locations in the other context. Unlike the current methods, during the holding pen phase (that occurs directly before the test phase), the pig would be given either object A or object B to explore. In the test phase, the pig would enter a randomly assigned context with object A and object B in the same location as when the pig was last in that context. However, both object A and B will be hidden from view as the pig enters the context. This will force the pig to identify the location of the less familiar object without first having seen it. Familiarity, in this experiment, will depend on the object that was introduced during the last holding pen phase (i.e., the introduced object will be more familiar). If the pig is able to remember, from previous exposure phases, the locations of object A and object B within the given context, it should be able find and prefer the less familiar object. The pig's first choice during the test phase compared to the duration it spends with each object will provide valuable insight into whether familiarity plays a role pig's ability to identify object/location/context.

In addition to laying a foundation for more pointed episodic-like memory testing methods, the current study also identifies a need for further study of female episodic memory. Unlike the findings of the Eacott and Norman (2004) and Eacott *et al.* (2005) studies on rats, the current study revealed potential differences between male and female pigs in their response to more and less familiar object/location/context configurations. In the Experiment 1 episodic memory test, females showed a non-significant tendency to spend more time with *more* familiar object/location/context configurations. Consequently, male pigs were the focus of the main episodic memory test. The few female subjects in the Experiment 3 episodic memory test showed no significant preference for either the familiar or non-familiar object/location/context. This finding may suggest that females may have reduced or altered episodic-like memory, as compared to males. However, the lack of effect may also be lack of statistical power arising from small sample size (n = 4). Furthermore, rather than a difference in memory, the contradictory results for female pigs may reflect that they respond differently to the testing procedure than do male pigs. Repeating the current study with a larger number of females may provide interesting insight into the nature of episodic memory in pigs.

Future Direction: Mental Time Travel in Pigs

The criteria for the MTT Episodic-like Memory definition mentioned earlier are by far the most rigorous and difficult to attain, but this definition represents the future direction of non-human episodic memory research. Although the mechanisms of MTT are not completely clear, it appears to play an important role in the mental recreation of an event (Tulving, 1983). Numerous opinions, replies, and reviews have been written on the topic of whether non-humans can engage in MTT (Roberts, 2002; Clayton, Bussey & Dickinson, 2003; Clayton *et al.*, 2003; Suddendorf & Busby, 2005; Zentall, 2005). Of these, most are skeptical of the presence of MTT in non-humans, but they do offer ways of testing the possibility. Roberts (2002) suggests that mental time travel could be demonstrated by having non-human subjects re-enact or pantomime events that they are recalling. He argues the ability to physically re-create an episode requires a corresponding mental re-creation. Zentall (2005) suggests that this type of re-enactment has already been shown in experiments by Mercado *et al.* (1998) in which dolphins were asked to repeat a behaviour that they had done recently. Roberts (2002) also states that hoarding studies in food caching birds should be further pursued. Specifically, he suggests that demonstrating that scrub jays remember the specific location in time during which they hoarded a particular food would provide evidence of MTT. Alternately, Suddendorf and Busby (2005) cite language training in non-human primates as a promising means of testing for MTT.

The above suggestions illustrate that, due to biological and behavioural differences between species, methods for testing MTT will likely be as variable as methods for testing non-human episodic memory as a whole. However, the suggested methods also prompt the question of whether evidence for episodic-like memory and evidence for MTT must be shown simultaneously, or if independent demonstration of each ability is acceptable. Arguably, MTT ability is either present or absent in a species (Suddendorf and Busby 2005). Therefore, if evidence of MTT is shown in one context, it would follow that it occurs in other contexts as well. Even though MTT and episodic-like memory occur together, it may be difficult to *demonstrate* the presence of both simultaneously due to the number of variables involved. The option of demonstrating MTT independently of episodic-like memory may lead to a convincing demonstration of MTT in one experiment and convincing demonstration of episodic-like memory in another experiment. Taken together, these results would provide a strong case for an advanced form of episodic-like memory (i.e., MTT Episodic-like Memory).

Demonstrating MTT independently is also more likely to lead to a generalized non-verbal testing method for MTT. Specifically, Suddendorf and Busby (2005) put forth the idea that MTT is demonstrated through the anticipation of future need. Specifically, this anticipation must be shown in the absence of trial and error learning opportunities, and must occur for behaviours that are not innate or species-typical. Using these guidelines, Suddendorf and Busby (2005) outline a simple design used for testing MTT in young children that could be configured for studying MTT in pigs and other animals. This test, which is called "The Rooms Task," begins with several pre-training sessions in which a subject is placed in Room A with free access to drinks. The subject is then allowed to choose one object (out of a series that does not include anything related to drinking) to take into Room B. In Room B, the subjects are made thirsty (through consuming a salty food), but no drinks are made available. In the experimental phase, the subject is allowed to choose from a novel set of objects including one that is associated with drinking (e.g., drink container) before going from Room A to Room B. Suddendorf and Busby conclude that choosing the drink object indicates anticipation of future need and, thus, MTT. This simple experiment could be modified for different species by altering the "need" and the choice objects, which could lead to a generally accepted method of demonstrating MTT in non-humans. Subsequently, a better understanding of which non-humans have the ability for MTT would provide insight into the implications of current demonstrations of episodic-like memory. That is, non-humans with demonstrated abilities for both episodic-like memory and MTT are more likely to be mentally re-creating events than those without MTT.

The possibilities for experiments exploring Mental Time Travel in pigs that are identified above, combined with a demonstration of several fundamental aspects of Episodic-like Memory in the current study, provides a promising case for further study of episodic memory in pigs. Results of the current study provide solid evidence that pigs can form and spontaneously recall memories that integrate the what/where/which circumstances of a past event. Furthermore, these results suggest that episodic memory may be different in females as compared to males and that pigs are capable of forming long term (week-long) memories of objects. By building on the findings of the current study, future experiments may lead to demonstration of pigs recalling what/where/which memories over the long term, and to evidence that these memories do not rely on familiarity and are dependent on the integrity of the hippocampus. Additionally, further experiments will provide insight into the nature of episodic-like memory in female pigs. Finally, developing further experiments to attempt to demonstrate MTT in pigs represents an ambitious, yet realistic, goal. Demonstration of MTT in pigs would pull non-human episodic memory significantly closer to human episodic memory and leave little doubt of the presence of an episodic-like memory system in pigs.

Conclusions and Implications

The experiments described here are a first step in the exploration of episodic memory in pigs. Evidence that pigs are able to form integrated memories of the what, where and which details of an event support previous anecdotal and empirical evidence that pigs are capable of higher cognitive functions. This finding suggests that cognitive function may play a role in pig well-being, and subsequently, may be important for welfare and handling guidelines. A second implication of this finding, is that it may provide a useful measure for cognitive changes caused by manipulations made in pig physiology models. Since pigs are considered a good model for the human body, they are often used to study the effects of different drugs and dietary manipulations. Lacking an efficient and accurate measure of cognitive function, these experiments currently measure only the physical effects of these manipulations and not the cognitive effects. The cognitive function inherent in episodic memory, combined with the straightforward nature of the what/where/which method, provide a simple and efficient way of measuring changes in cognitive function. Pigs' ability to form integrated memories of what/where/which memories prior to a manipulation (dietary, drug, etc.) can be compared with this same ability after the manipulation. Although this test obviously does not encompass all aspects of cognition, it may expose substantial or broad-spectrum changes in mental function and improve our understanding of the pig brain.

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TABLES

Table 1: Analysis of Variance for object preference during exposure phases of episodic-like memory trials (males and females)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	256.13	1.00	256.13	2.68	0.18
Trial * Sex	Sphericity Assumed	123.25	1.00	123.25	1.29	0.32
Error(Trial)	Sphericity Assumed	382.90	4.00	95.72		
Object	Sphericity Assumed	72673.82	5.00	14534.76	9.92	0.00
Object * Sex	Sphericity Assumed	3408.52	5.00	681.70	0.47	0.80
Error(Object)	Sphericity Assumed	29292.82	20.00	1464.64		
Trial * Object	Sphericity Assumed	1597.88	5.00	319.58	1.30	0.30
Trial * Object * Sex	Sphericity Assumed	400.02	5.00	80.00	0.33	0.89
Error(Trial*Object)	Sphericity Assumed	4905.69	20	245.28		

Table 2: Analysis of Variance for object preference during test phases (males and females)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Object	15909.75	5.00	3181.95	2.39	0.10
Error	15978.94	12.00	1331.58		
Total	61729.93	18.00			

Table 3: Analysis of Variance for durations spent with object attached to pole and object attached to front of context (males and females)						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
ObjectLocation	Sphericity Assumed	163.28	1.00	163.28	0.29	0.61
Error(ObjectLocation)	Sphericity Assumed	2804.47	5.00	560.89		
Object/Location/ContextFamiliarity	Sphericity Assumed	127.88	1.00	127.88	0.20	0.67
Error(Object/Location/ContextFamiliarity)	Sphericity Assumed	3159.35	5.00	631.87		
ObjectLocation * Object/Location/ContextFamiliarity	Sphericity Assumed	2380.04	1.00	2380.04	1.57	0.27
Error(ObjectLocation*Object/Location/ContextFamiliarity)	Sphericity Assumed	7578.71	5.00	1515.74		

Table 4: Analysis of Variance for duration spent by males and females with more and less familiar object/location/context during episodic-like memory test (data for rake included)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	2557.93	2.00	1278.96	1.61	0.26
Trial * Sex	Sphericity Assumed	3509.80	2.00	1754.90	2.21	0.17
Error(Trial)	Sphericity Assumed	6351.36	8.00	793.92		
Object/Location/ContextFamiliarity	Sphericity Assumed	17.36	1.00	17.36	0.05	0.84
Object/Location/ContextFamiliarity * Sex	Sphericity Assumed	1694.69	1.00	1694.69	4.59	0.10
Error(Object/Location/ContextFamiliarity)	Sphericity Assumed	1477.34	4.00	369.33		
Trial * Object/Location/ContextFamiliarity	Sphericity Assumed	666.48	2.00	333.24	1.13	0.37
Trial * Object/Location/ContextFamiliarity * Sex	Sphericity Assumed	828.40	2.00	414.20	1.41	0.30
Error(Trial*Object/Location/ContextFamiliarity)	Sphericity Assumed	2352.58	8.00	294.07		

Table 5: Analysis of Variance for duration spent by males and females with more and less familiar object/location/context during episodic-like memory test (data for rake NOT included)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	162.24	1.00	162.24	1.04	0.36
Trial * Sex	Sphericity Assumed	7.71	1.00	7.71	0.05	0.83
Error(Trial)	Sphericity Assumed	621.61	4.00	155.40		
Object/Location/ContextFamiliarity	Sphericity Assumed	365.04	1.00	365.04	3.64	0.13
Object/Location/ContextFamiliarity * Sex	Sphericity Assumed	752.64	1.00	752.64	7.50	0.05
Error(Object/Location/ContextFamiliarity)	Sphericity Assumed	401.28	4.00	100.32		
Trial * Object/Location/ContextFamiliarity	Sphericity Assumed	1.31	1.00	1.31	0.01	0.94
Trial * Object/Location/ContextFamiliarity * Sex	Sphericity Assumed	40.56	1.00	40.56	0.21	0.67
Error(Trial*Object/Location/ContextFamiliarity)	Sphericity Assumed	762.77	4.00	190.69		

Table 6: Analysis of Variance for durations spent by male and female pigs with novel and familiar objects during novel object test						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Familiarity	Sphericity Assumed	77537.76	1.00	77537.76	11.63	0.03
Familiarity * Sex	Sphericity Assumed	3129.87	1.00	3129.87	0.47	0.53
Error(Familiarity)	Sphericity Assumed	26679.39	4.00	6669.85		

Table 7: Analysis of Variance for quadrant durations during exposure phases before novel object test (males and females)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
QuadrantDuration	Greenhouse-Geisser	3084149.61	1.08	2852733.28	9.24	0.03
QuadrantDuration * Sex	Greenhouse-Geisser	246640.98	1.08	228134.50	0.74	0.45
Error(Durations)	Greenhouse-Geisser	1335716.89	4.32	308873.15		
Exposure Phase	Sphericity Assumed	0.00	14.00	0.00		
Exposure Phase * Sex	Sphericity Assumed	0.00	14.00	0.00		
Error(ExposurePhase)	Sphericity Assumed	0.00	56.00	0.00		
QuadrantDuration * ExposurePhase	Greenhouse-Geisser	185015.13	3.81	48582.76	1.29	0.32
QuadrantDuration * ExposurePhase * Sex	Greenhouse-Geisser	124385.00	3.81	32662.02	0.87	0.50
Error(QuadrantDuration*ExposurePhase)	Greenhouse-Geisser	573924.87	15.23	37676.45		

Table 8: Analysis of Variance for quadrant counts during exposure phases before novel object test (males and females)						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
QuadrantVisits	Greenhouse-Geisser	1391.90	1.03	1354.51	12.42	0.02
QuadrantVisits * Sex	Greenhouse-Geisser	391.83	1.03	381.31	3.50	0.13
Error(QuadrantVisits)	Greenhouse-Geisser	448.42	4.11	109.09		
Exposure Phase	Greenhouse-Geisser	202.31	2.94	68.83	0.68	0.58
Exposure Phase * Sex	Greenhouse-Geisser	689.87	2.94	234.72	2.33	0.13
Error(ExposurePhase)	Greenhouse-Geisser	1182.09	11.76	100.55		
QuadrantVisits * Exposure Phase	Greenhouse-Geisser	459.56	3.61	127.33	2.81	0.07
QuadrantVisits * Exposure Phase * Sex	Greenhouse-Geisser	241.46	3.61	66.90	1.48	0.26
Error(QuadrantVisits*ExposurePhase)	Greenhouse-Geisser	654.58	14.44	45.34		

Table 9: Analysis of Variance for quadrant durations during habituation trials (males)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
QuadrantDuration	Sphericity Assumed	721878.62	3.00	240626.21	23.97	0.00
QuadrantDuration * Strain	Sphericity Assumed	147185.36	3.00	49061.79	4.89	0.01
Error(QuadrantDuration)	Sphericity Assumed	180725.33	18.00	10040.30		
Day	Sphericity Assumed	0.00	2.00	0.00		
Day * Strain	Sphericity Assumed	0.00	2.00	0.00		
Error(Day)	Sphericity Assumed	0.00	12.00	0.00		
Trial	Sphericity Assumed	0.00	1.00	0.00		
Trial * Strain	Sphericity Assumed	0.00	1.00	0.00		
Error(Trial)	Sphericity Assumed	0.00	6.00	0.00		
QuadrantDuration * Day	Sphericity Assumed	596784.04	6.00	99464.01	8.44	0.00
QuadrantDuration * Day * Strain	Sphericity Assumed	161083.08	6.00	26847.18	2.28	0.06
Error(QuadrantDuration*Day)	Sphericity Assumed	424025.25	36.00	11778.48		
QuadrantDuration * Trial	Greenhouse-Geisser	14191.62	1.21	11716.92	0.65	0.47
QuadrantDuration * Trial * Strain	Sphericity Assumed	28374.36	3.00	9458.12	1.30	0.30
Error(QuadrantDuration*Trial)	Sphericity Assumed	130532.32	18.00	7251.80		
Day * Trial	Sphericity Assumed	0.00	2.00	0.00		
Day * Trial * Strain	Sphericity Assumed	0.00	2.00	0.00		
Error(Day*Trial)	Sphericity Assumed	0.00	12.00	0.00		
QuadrantDuration * Day * Trial	Sphericity Assumed	30339.78	6.00	5056.63	0.67	0.68
QuadrantDuration * Day * Trial * Strain	Sphericity Assumed	57321.33	6.00	9553.55	1.26	0.30
Error(QuadrantDuration*Day*Trial)	Sphericity Assumed	273045.72	36.00	7584.60		

Table 10: Analysis of Variance for quadrant counts during habituation trials (males)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
QuadrantCount	Greenhouse-Geisser	147.04	1.31	112.65	15.05	0.00
QuadrantCount * Strain	Greenhouse-Geisser	45.94	1.31	35.19	4.70	0.06
Error(QuadrantCount)	Greenhouse-Geisser	58.60	7.83	7.48		
Day	Sphericity Assumed	13.20	2.00	6.60	0.07	0.94
Day * Strain	Sphericity Assumed	439.03	2.00	219.52	2.18	0.16
Error(Day)	Sphericity Assumed	1207.69	12.00	100.64		
Trial	Sphericity Assumed	130.02	1.00	130.02	1.15	0.32
Trial * Strain	Sphericity Assumed	21.33	1.00	21.33	0.19	0.68
Error(Trial)	Sphericity Assumed	676.06	6.00	112.68		
QuadrantCount * Day	Greenhouse-Geisser	81.43	1.99	40.89	2.66	0.11
QuadrantCount * Day * Strain	Greenhouse-Geisser	28.59	1.99	14.36	0.94	0.42
Error(QuadrantCount*Day)	Greenhouse-Geisser	183.40	11.95	15.35		
QuadrantCount * Trial	Sphericity Assumed	0.85	3.00	0.28	0.08	0.97
QuadrantCount * Trial * Strain	Sphericity Assumed	10.88	3.00	3.63	1.03	0.40
Error(QuadrantCount*Trial)	Sphericity Assumed	63.52	18.00	3.53		
Day * Trial	Sphericity Assumed	236.01	2.00	118.01	1.96	0.18
Day * Trial * Strain	Sphericity Assumed	203.01	2.00	101.51	1.68	0.23
Error(Day*Trial)	Sphericity Assumed	724.06	12.00	60.34		
QuadrantCount * Day * Trial	Sphericity Assumed	36.11	6.00	6.02	1.83	0.12
QuadrantCount * Day * Trial * Strain	Sphericity Assumed	35.78	6.00	5.96	1.81	0.12
Error(QuadrantCount*Day*Trial)	Sphericity Assumed	118.35	36.00	3.29		

Table 11: Analysis of Variance for duration of loud vocalizations during habituation trials (males)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Day	Sphericity Assumed	5532.35	2.00	2766.17	0.15	0.86
Day * Strain	Sphericity Assumed	47677.62	2.00	23838.81	1.29	0.31
Error(Day)	Sphericity Assumed	221267.63	12.00	18438.97		
Trial	Sphericity Assumed	17941.33	1.00	17941.33	2.09	0.20
Trial * Strain	Sphericity Assumed	22102.08	1.00	22102.08	2.57	0.16
Error(Trial)	Sphericity Assumed	51622.25	6.00	8603.71		
Day * Trial	Sphericity Assumed	29688.05	2.00	14844.03	1.35	0.30
Day * Trial * Strain	Sphericity Assumed	2676.60	2.00	1338.30	0.12	0.89
Error(Day*Trial)	Sphericity Assumed	131796.28	12.00	10983.02		

Table 12: Analysis of Variance for duration spent with more and less familiar object/location/context during episodic-like memory test (males)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	8430.48	3.00	2810.16	0.97	0.43
Trial * Strain	Sphericity Assumed	6868.09	3.00	2289.36	0.79	0.52
Error(Trial)	Sphericity Assumed	52387.68	18.00	2910.43		
Familiarity	Sphericity Assumed	4205.52	1.00	4205.52	16.74	0.01
Familiarity * Strain	Sphericity Assumed	200.22	1.00	200.22	0.80	0.41
Error(Familiarity)	Sphericity Assumed	1507.60	6.00	251.27		
Trial * Familiarity	Sphericity Assumed	9331.05	3.00	3110.35	3.27	0.05
Trial * Familiarity * Strain	Sphericity Assumed	2175.01	3.00	725.00	0.76	0.53
Error(Trial*Familiarity)	Sphericity Assumed	17119.84	18.00	951.10		

Table 13: Analysis of Variance for minute-by-minute duration spent with more and less familiar object/location/context during episodic-like memory test (males)						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	843.05	3.00	281.02	1.00	0.41
Error(Trial)	Sphericity Assumed	5925.58	21.00	282.17		
Familiarity	Sphericity Assumed	420.55	1.00	420.55	17.24	0.00
Error(Familiarity)	Sphericity Assumed	170.78	7.00	24.40		
Minute	Sphericity Assumed	2641.17	9.00	293.46	4.93	0.00
Error(Minute)	Sphericity Assumed	3746.49	63.00	59.47		
Trial * Familiarity	Sphericity Assumed	933.11	3.00	311.04	3.39	0.04
Error(Trial*Familiarity)	Sphericity Assumed	1929.48	21.00	91.88		
Trial * Minute	Sphericity Assumed	1961.76	27.00	72.66	1.21	0.23
Error(Trial*Minute)	Sphericity Assumed	11393.49	189.00	60.28		
Familiarity * Minute	Sphericity Assumed	1990.22	9.00	221.14	1.75	0.10
Error(Familiarity*Minute)	Sphericity Assumed	7955.79	63.00	126.28		
Trial * Familiarity * Minute	Sphericity Assumed	2861.40	27.00	105.98	1.33	0.14
Error(Trial*Familiarity*Minute)	Sphericity Assumed	15093.38	189.00	79.86		

Table 14: Analysis of Variance for number of visits to more and less familiar object/location/context during episodic-like memory test (males)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	7.30	3.00	2.43	0.42	0.74
Trial * Strain	Sphericity Assumed	122.17	3.00	40.72	7.05	0.00
Error(Trial)	Sphericity Assumed	103.91	18.00	5.77		
Familiarity	Sphericity Assumed	3.52	1.00	3.52	1.05	0.35
Familiarity * Strain	Sphericity Assumed	4.52	1.00	4.52	1.35	0.29
Error(Familiarity)	Sphericity Assumed	20.09	6.00	3.35		
Trial * Familiarity	Sphericity Assumed	13.30	3.00	4.43	1.64	0.21
Trial * Familiarity * Strain	Sphericity Assumed	14.55	3.00	4.85	1.80	0.18
Error(Trial*Familiarity)	Sphericity Assumed	48.53	18.00	2.70		

Table 15: Analysis of Variance for quadrant durations during habituation trials (females)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
QuadrantDuration	Sphericity Assumed	384606.17	3.00	128202.06	10.09	0.01
QuadrantDuration * Strain	Sphericity Assumed	13085.95	3.00	4361.98	0.34	0.80
Error(QuadrantDuration)	Sphericity Assumed	76217.65	6.00	12702.94		
Day	Sphericity Assumed	0.00	2.00	0.00		
Day * Strain	Sphericity Assumed	0.00	2.00	0.00		
Error(Day)	Sphericity Assumed	0.00	4.00	0.00		
Trial	Sphericity Assumed	0.00	1.00	0.00		
Trial * Strain	Sphericity Assumed	0.00	1.00	0.00		
Error(Trial)	Sphericity Assumed	0.00	2.00	0.00		
QuadrantDuration * Day	Sphericity Assumed	225740.37	6.00	37623.40	3.00	0.05
QuadrantDuration * Day * Strain	Sphericity Assumed	138192.33	6.00	23032.06	1.84	0.17
Error(QuadrantDuration*Day)	Sphericity Assumed	150556.45	12.00	12546.37		
QuadrantDuration * Trial	Sphericity Assumed	9957.30	3.00	3319.10	0.57	0.65
QuadrantDuration * Trial * Strain	Sphericity Assumed	50404.61	3.00	16801.54	2.90	0.12
Error(QuadrantDuration*Trial)	Sphericity Assumed	34777.57	6.00	5796.26		
Day * Trial	Sphericity Assumed	0.00	2.00	0.00		
Day * Trial * Strain	Sphericity Assumed	0.00	2.00	0.00		
Error(Day*Trial)	Sphericity Assumed	0.00	4.00	0.00		
QuadrantDuration * Day * Trial	Sphericity Assumed	59049.56	6.00	9841.59	1.23	0.36
QuadrantDuration * Day * Trial * Strain	Sphericity Assumed	56011.71	6.00	9335.29	1.16	0.39
Error(QuadrantDuration*Day*Trial)	Sphericity Assumed	96264.41	12.00	8022.03		

Table 16: Analysis of Variance for quadrant counts during habituation trials (females)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
QuadrantCount	Sphericity Assumed	72.21	3.00	24.07	12.65	0.01
QuadrantCount * Strain	Sphericity Assumed	1.21	3.00	0.40	0.21	0.88
Error(QuadrantCount)	Sphericity Assumed	11.42	6.00	1.90		
Day	Sphericity Assumed	155.77	2.00	77.89	1.32	0.36
Day * Strain	Sphericity Assumed	21.06	2.00	10.53	0.18	0.84
Error(Day)	Sphericity Assumed	235.25	4.00	58.81		
Trial	Sphericity Assumed	8.17	1.00	8.17	0.68	0.50
Trial * Strain	Sphericity Assumed	8.17	1.00	8.17	0.68	0.50
Error(Trial)	Sphericity Assumed	24.17	2.00	12.08		
QuadrantCount * Day	Sphericity Assumed	34.23	6.00	5.70	4.39	0.01
QuadrantCount * Day * Strain	Sphericity Assumed	3.10	6.00	0.52	0.40	0.87
Error(QuadrantCount*Day)	Sphericity Assumed	15.58	12.00	1.30		
QuadrantCount * Trial	Sphericity Assumed	2.25	3.00	0.75	0.56	0.66
QuadrantCount * Trial * Strain	Sphericity Assumed	17.58	3.00	5.86	4.40	0.06
Error(QuadrantCount*Trial)	Sphericity Assumed	8.00	6.00	1.33		
Day * Trial	Sphericity Assumed	320.40	2.00	160.20	3.08	0.16
Day * Trial * Strain	Sphericity Assumed	39.52	2.00	19.76	0.38	0.71
Error(Day*Trial)	Sphericity Assumed	208.33	4.00	52.08		
QuadrantCount * Day * Trial	Sphericity Assumed	21.94	6.00	3.66	1.95	0.15
QuadrantCount * Day * Trial * Strain	Sphericity Assumed	6.98	6.00	1.16	0.62	0.71
Error(QuadrantCount*Day*Trial)	Sphericity Assumed	22.50	12.00	1.88		

Table 17: Analysis of Variance for duration of loud vocalizations during habituation trials (females)						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Day	Sphericity Assumed	1544.81	2.00	772.41	0.12	0.89
Day * Strain	Sphericity Assumed	6377.16	2.00	3188.58	0.51	0.63
Error(Day)	Sphericity Assumed	24942.99	4.00	6235.75		
Trial	Sphericity Assumed	48.73	1.00	48.73	0.01	0.93
Trial * Strain	Sphericity Assumed	823.68	1.00	823.68	0.15	0.73
Error(Trial)	Sphericity Assumed	10815.10	2.00	5407.55		
Day * Trial	Sphericity Assumed	7144.32	2.00	3572.16	9.57	0.03
Day * Trial * Strain	Sphericity Assumed	2066.61	2.00	1033.31	2.77	0.18
Error(Day*Trial)	Sphericity Assumed	1493.57	4.00	373.39		

Table 18: Analysis of Variance for duration spent with more and less familiar object/location/context during episodic-like memory test (females)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Trial	Sphericity Assumed	7361.41	3.00	2453.80	0.41	0.75
Trial * Strain	Sphericity Assumed	9976.11	3.00	3325.37	0.55	0.67
Error(Trial)	Sphericity Assumed	36207.50	6.00	6034.58		
Familiarity	Sphericity Assumed	112.50	1.00	112.50	0.03	0.87
Familiarity * Strain	Sphericity Assumed	4753.13	1.00	4753.13	1.46	0.35
Error(Familiarity)	Sphericity Assumed	6503.91	2.00	3251.95		
Trial * Familiarity	Sphericity Assumed	4857.03	3.00	1619.01	0.59	0.64
Trial * Familiarity * Strain	Sphericity Assumed	6675.59	3.00	2225.20	0.82	0.53
Error(Trial*Familiarity)	Sphericity Assumed	16332.02	6.00	2722.00		

Table 19: Analysis of Variance for minute-by-minute duration spent with more and less familiar object/location/context during episodic-like memory test (females)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Familiarity	Sphericity Assumed	11.25	1.00	11.25	0.03	0.87
Error(Familiarity)	Sphericity Assumed	1125.70	3.00	375.23		
Trial	Sphericity Assumed	736.14	3.00	245.38	0.48	0.71
Error(Trial)	Sphericity Assumed	4618.36	9.00	513.15		
Minute	Sphericity Assumed	1419.76	9.00	157.75	3.08	0.01
Error(Minute)	Sphericity Assumed	1384.01	27.00	51.26		
Familiarity * Trial	Sphericity Assumed	485.70	3.00	161.90	0.63	0.61
Error(Familiarity*Trial)	Sphericity Assumed	2300.76	9.00	255.64		
Familiarity * Minute	Sphericity Assumed	978.65	9.00	108.74	1.29	0.29
Error(Familiarity*Minute)	Sphericity Assumed	2270.35	27.00	84.09		
Trial * Minute	Sphericity Assumed	1643.96	27.00	60.89	0.74	0.81
Error(Trial*Minute)	Sphericity Assumed	6676.00	81.00	82.42		
Familiarity * Trial * Minute	Sphericity Assumed	3720.97	27.00	137.81	1.12	0.34
Error(Familiarity*Trial*Minute)	Sphericity Assumed	9938.64	81.00	122.70		

FIGURES

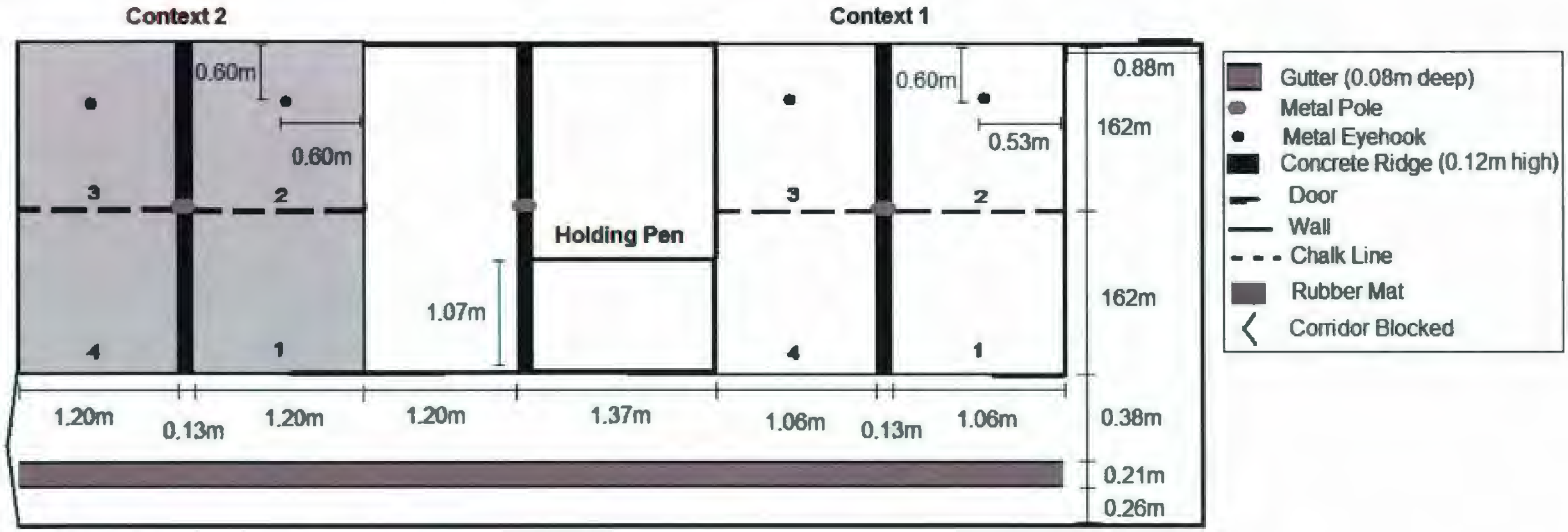

Figure 1.1: Diagram of test room

Figure 1.2: Photos of test objects

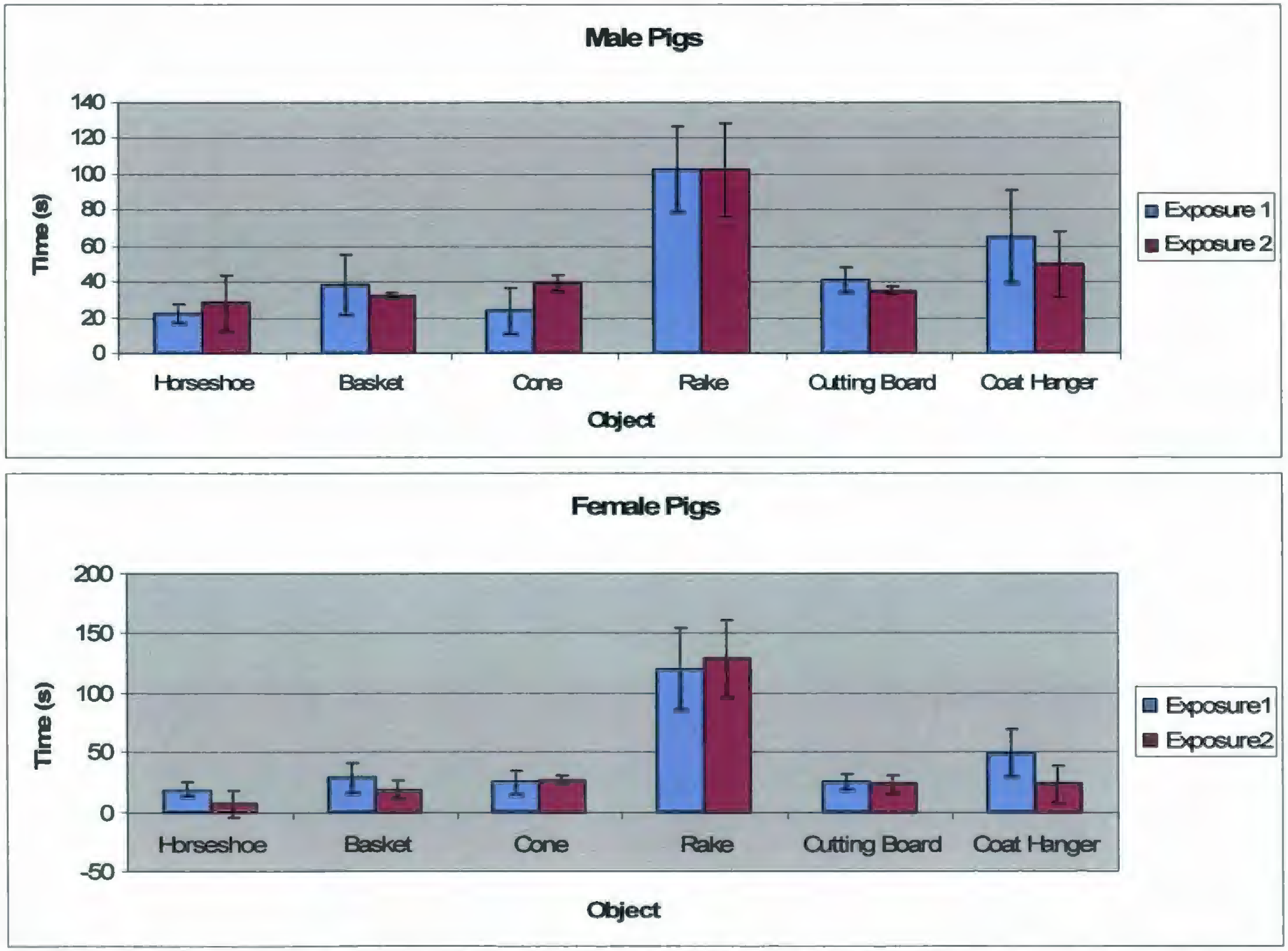

Figure 2.1: Mean time spent by male and female pigs with each object during exposure phases of episodic-like memory trials in Experiment 1.

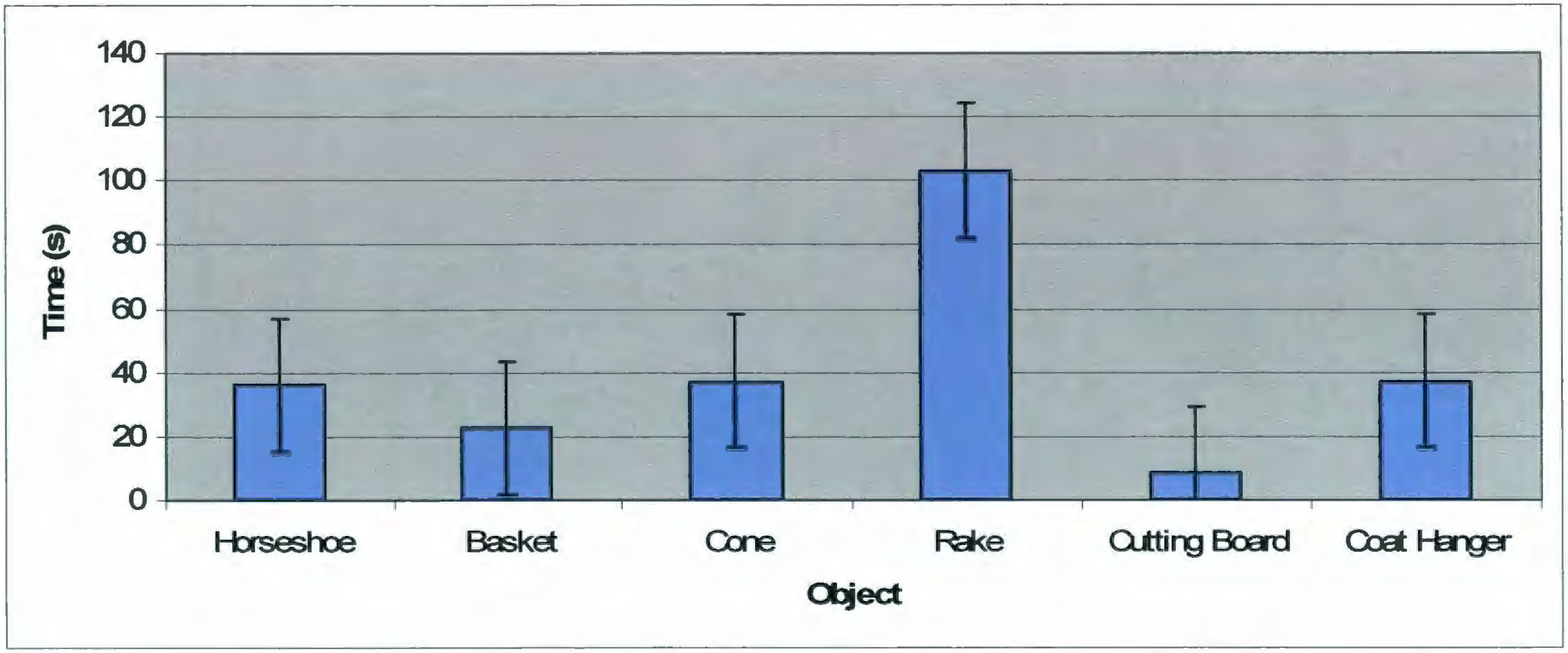

Figure 2.2: Mean time spent by male and female pigs with each object during test phase of episodic-like memory trials in Experiment 1.

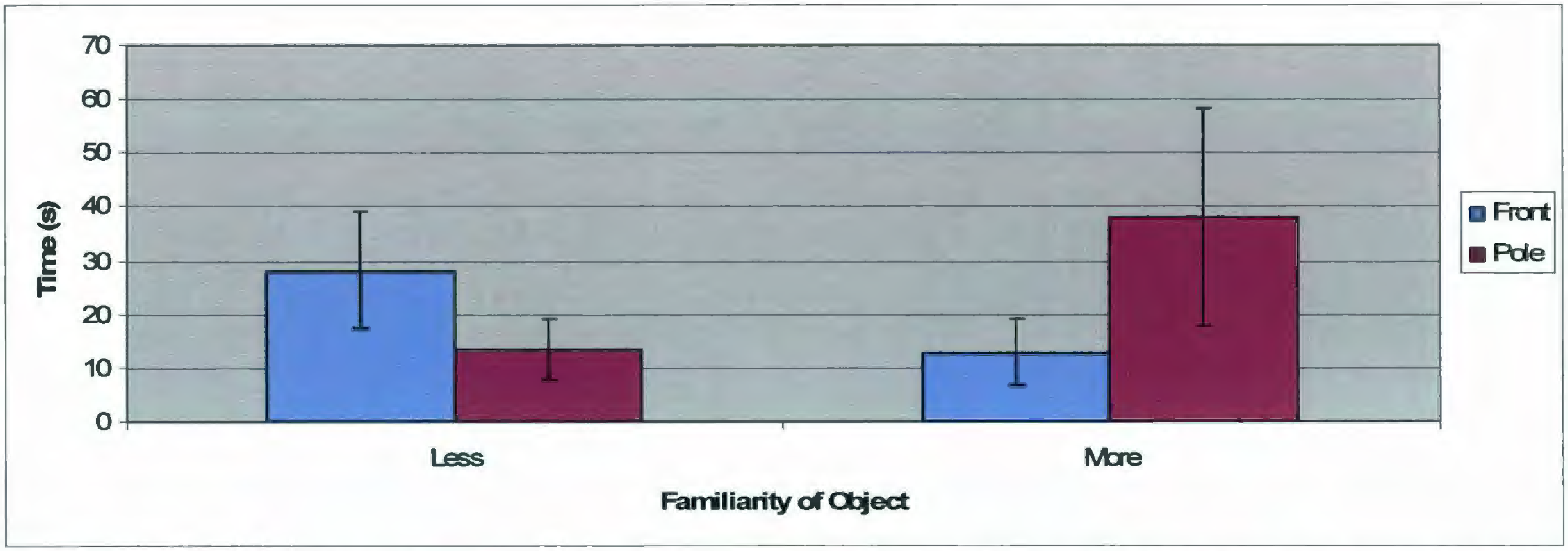

Figure 2.3: Mean time spent by male and female pigs with more and less familiar objects located at the front of the context and on the pole at the centre of the context in Experiment 1.

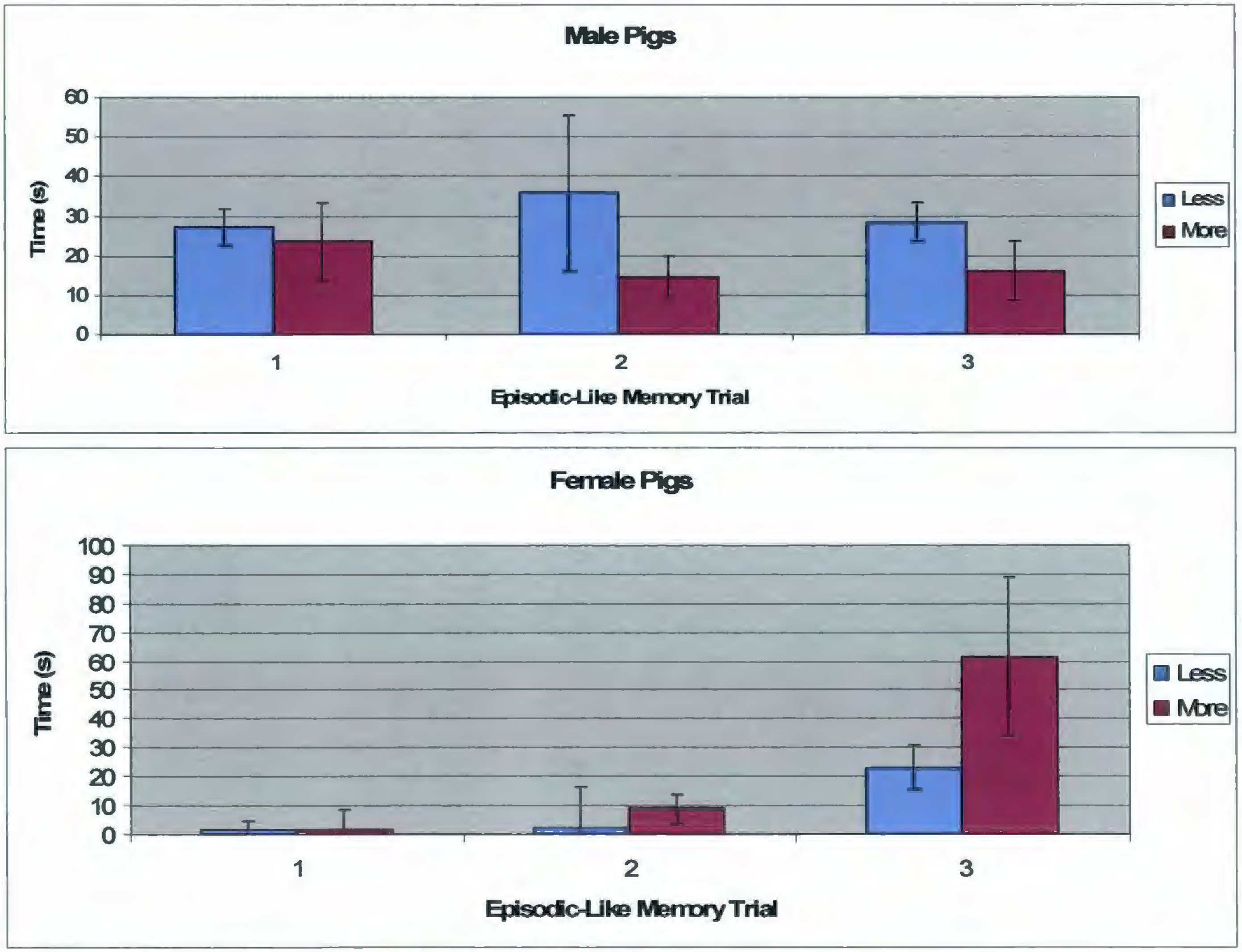

Figure 2.4: Mean time spent by male and female pigs with more and less familiar object/location/context during each episodic-like memory trial in Experiment 1.

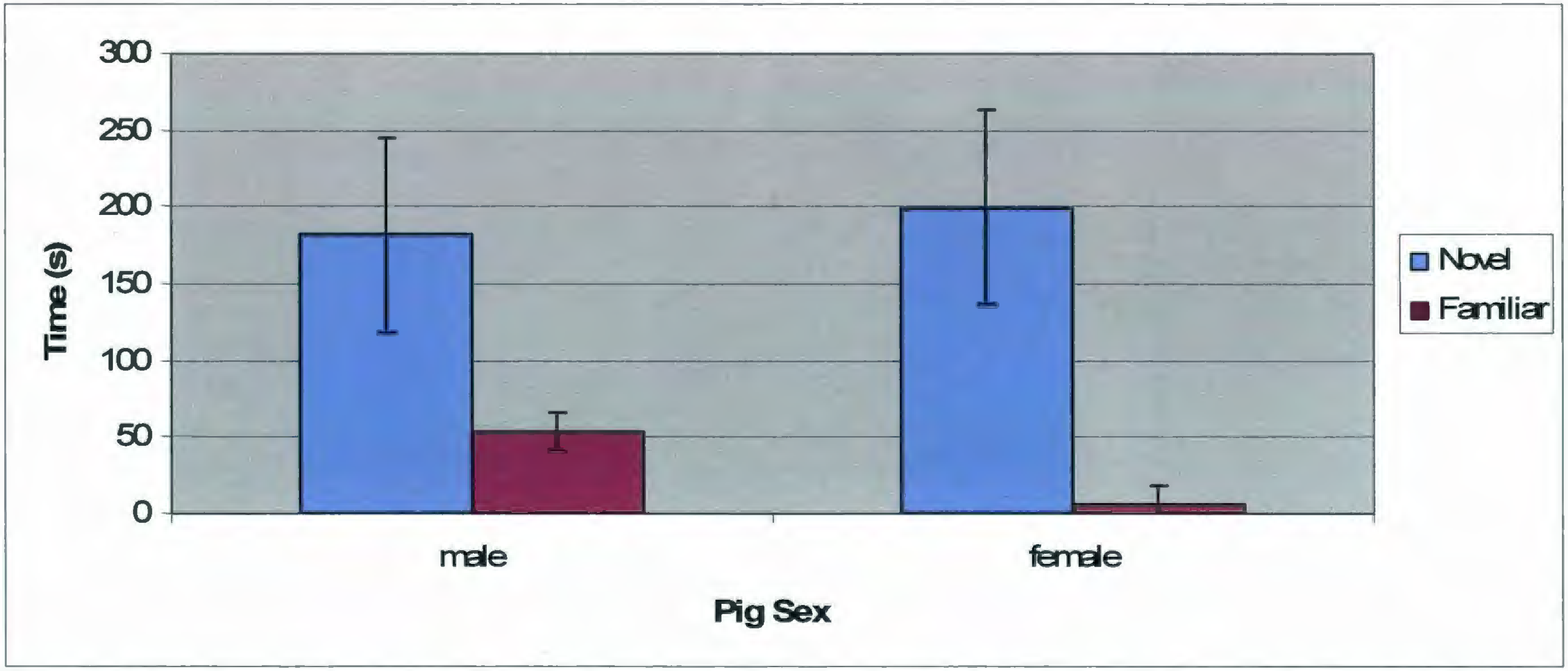

Figure 2.5: Mean time spent by male and female pigs with novel and familiar objects during novel object test in Experiment 1.

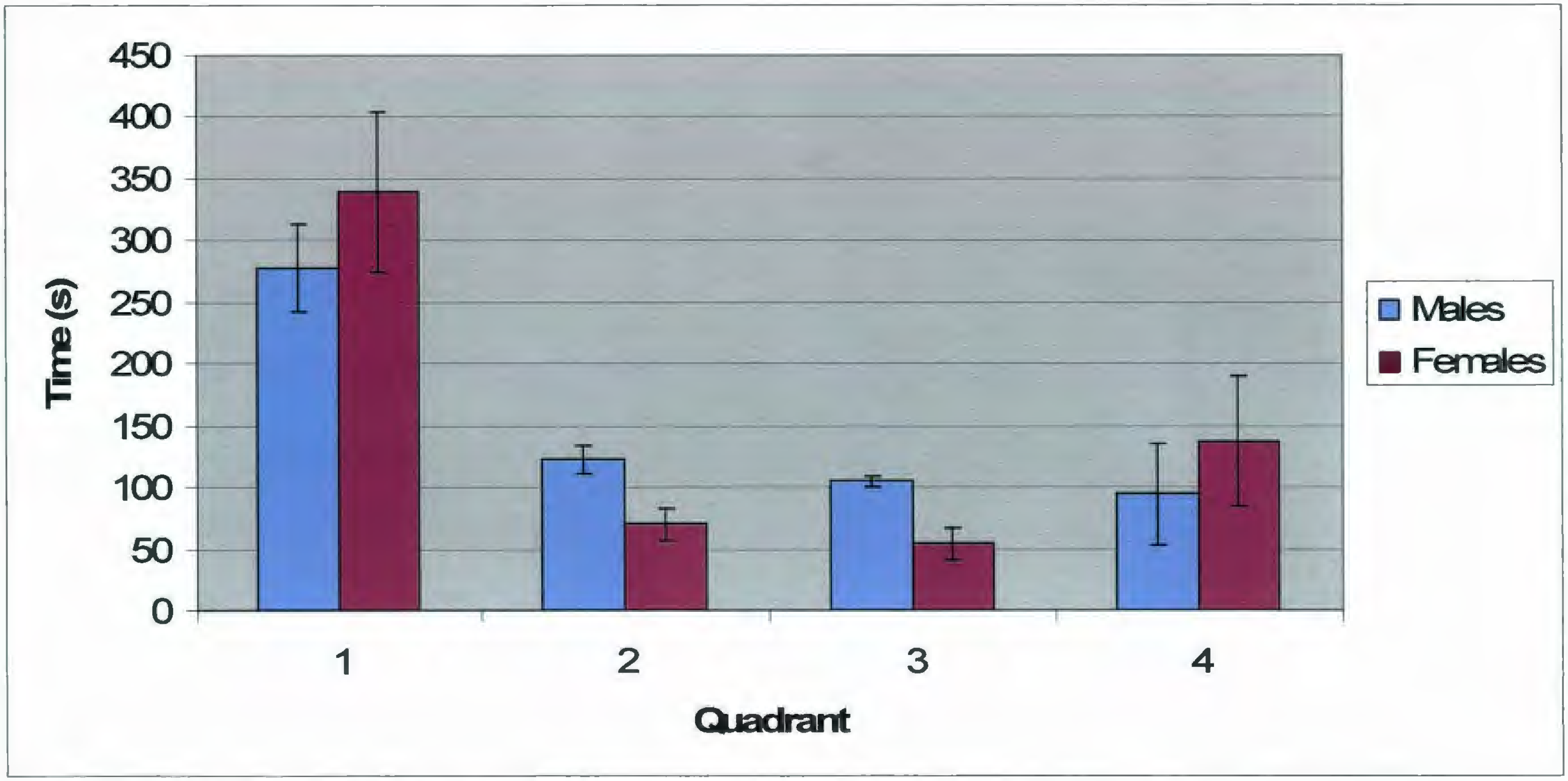

Figure 2.6: Mean time spent by male and female pigs in each quadrant during episodic-like memory trials in Experiment 1.

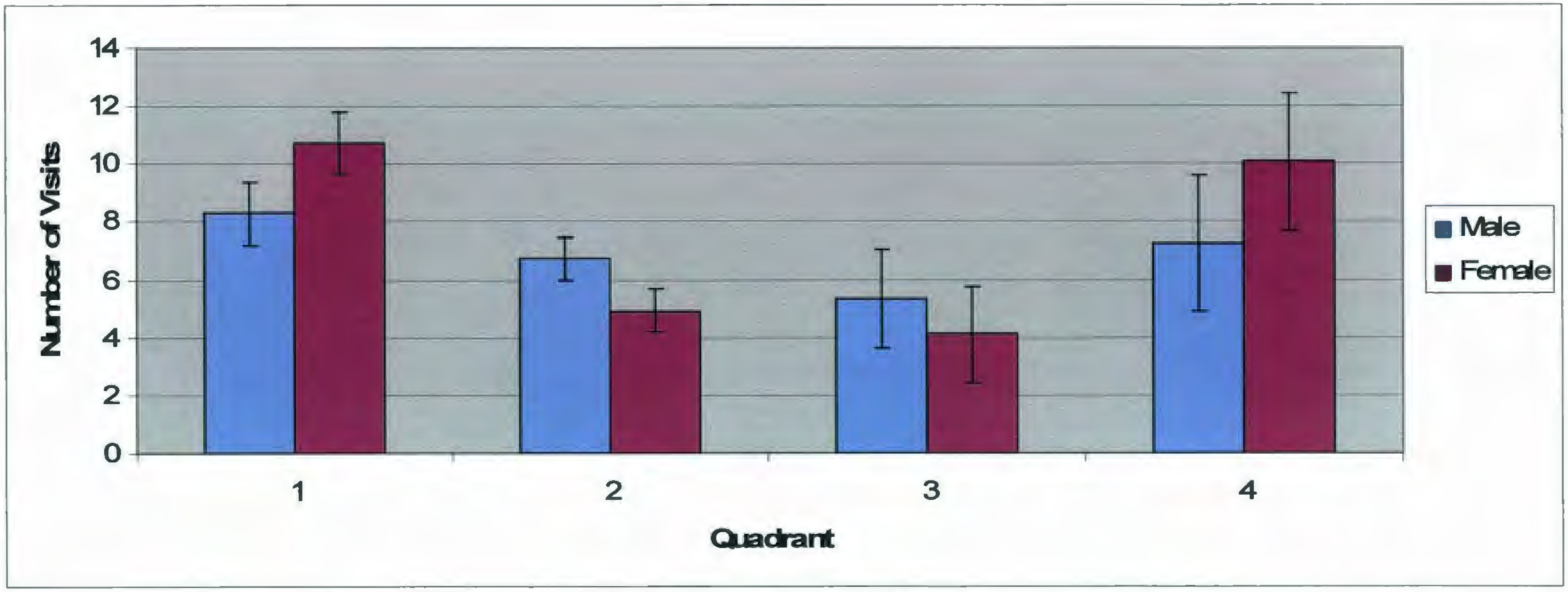

Figure 2.7: Mean number of visits by male and female pigs to each quadrant during exposure phases of episodic-like memory trials in Experiment 1.

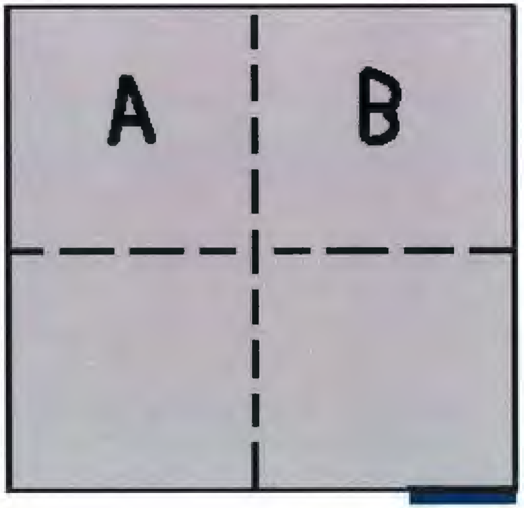

Figure 3.1: Example of first exposure phase during episodic-like memory test (Pig randomly assigned Context 2 with Object A in Location 1 and Object B in Location 2).

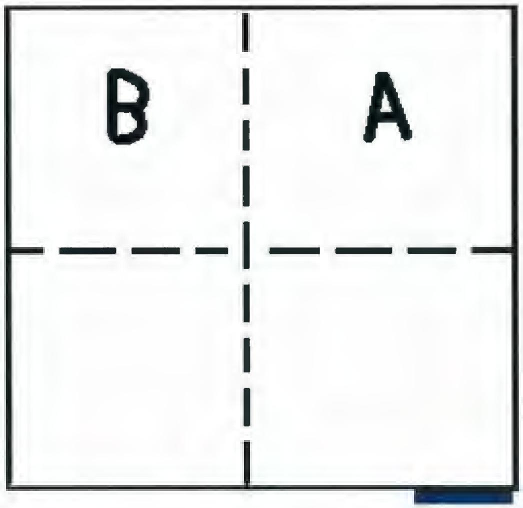

Figure 3.2: Example of second exposure phase during episodic-like memory test (Pig assigned opposite Context (1) with Object B in Location 1 and Object A in Location 2)

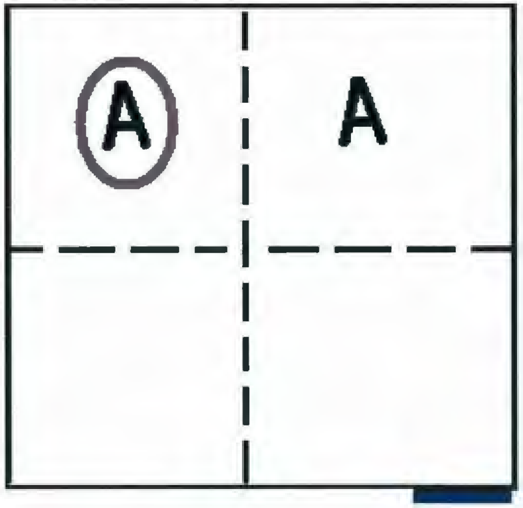

Figure 3.3: Examples of possible test phase configuration during episodic-like memory test (Pig randomly assigned Context 1 with Object A in Location 1 and in Location 2). Less familiar object/location/context marked with circle.

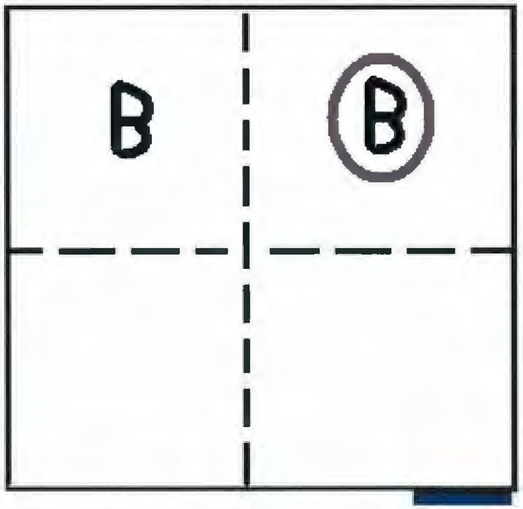

Figure 3.4: Example of test phase configuration during episodic-like memory test that counterbalances test phase configuration depicted in Fig. 3.3. (Pig randomly assigned Context 1 with Object B in Location 1 and in Location 2). Less familiar object/location/context marked with circle.

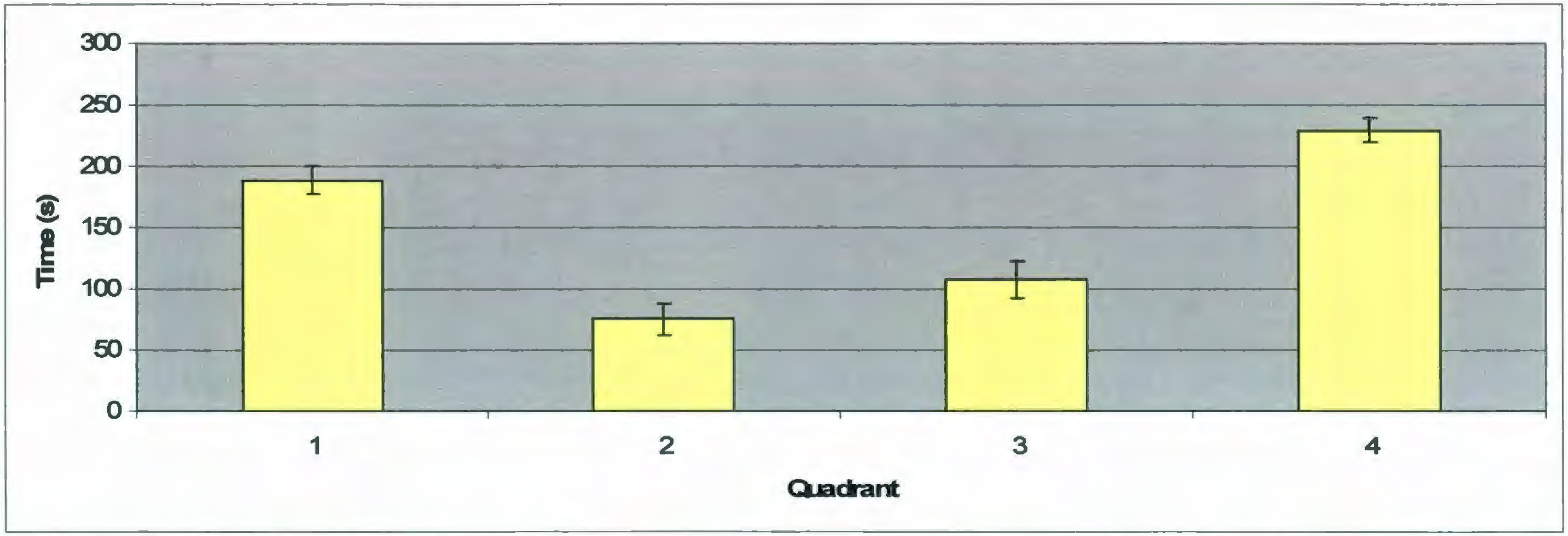

Figure 3.5: Mean time spent in each quadrant by male pigs over eighteen 20min habituation trials (with no objects present) in Experiment 2.

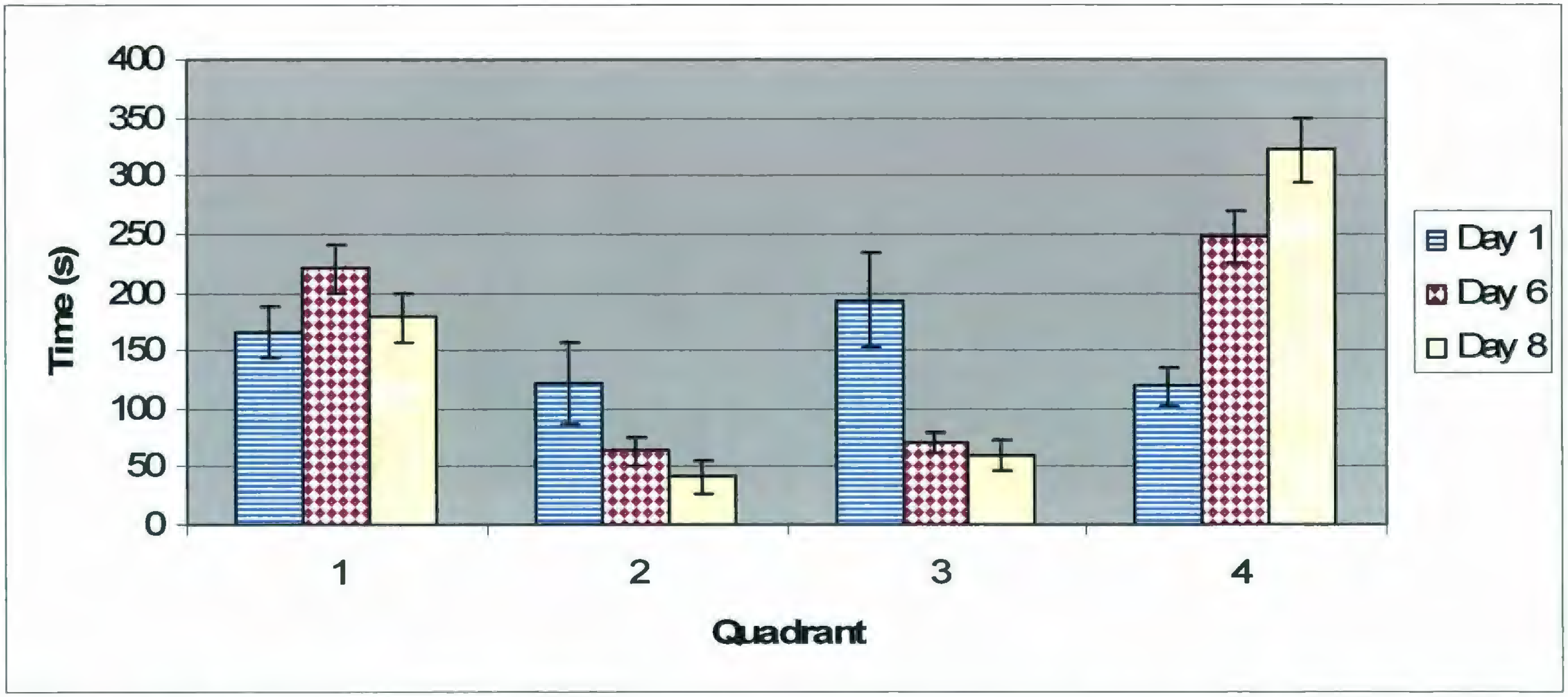

Figure 3.6: Mean duration spent by male pigs in each quadrant over 9 habituation days (two 20min trials/day; data from Days 1, 6, and 8) in Experiment 2.

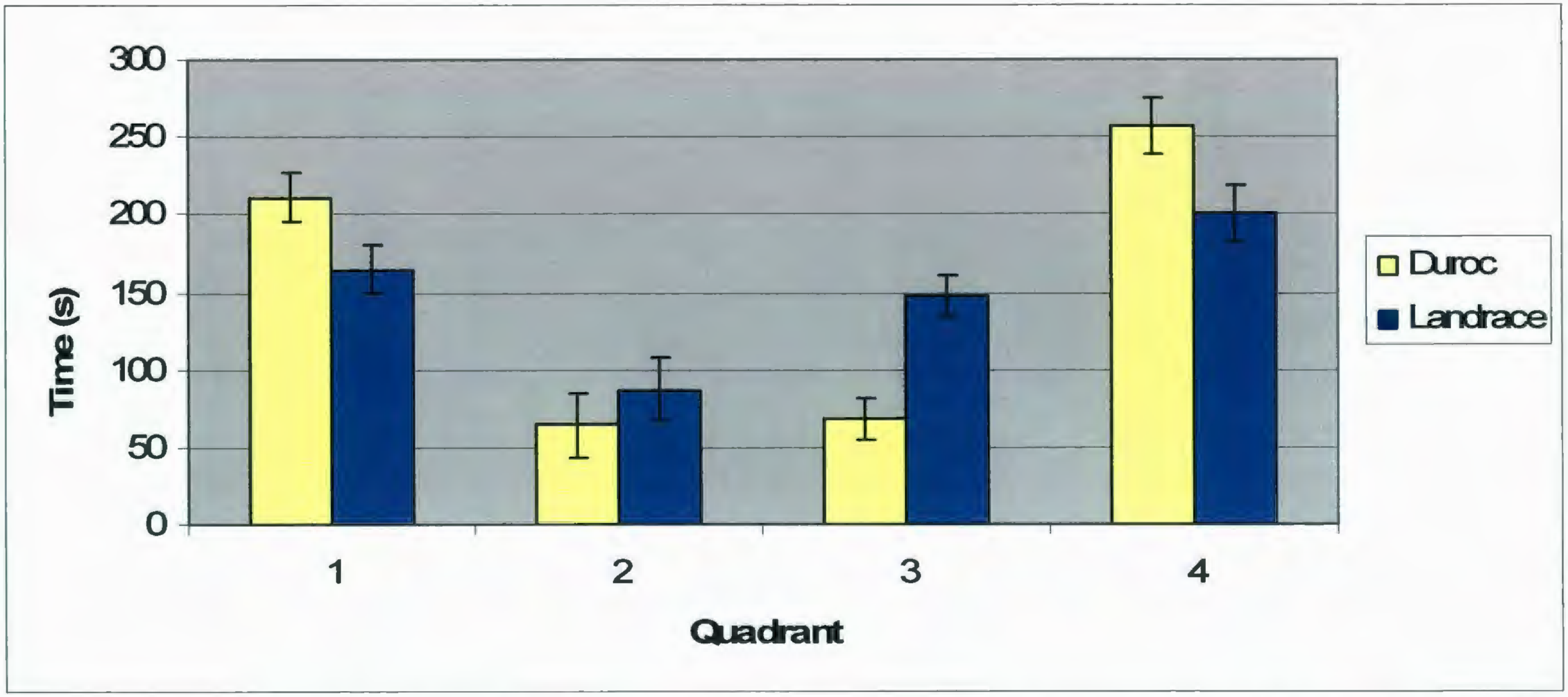

Figure 3.7: Mean time spent by male pigs in each context quadrant over eighteen 20min habituation trials (data from Trials 1, 2, 11, 12, 15, 16) in Experiment 2.

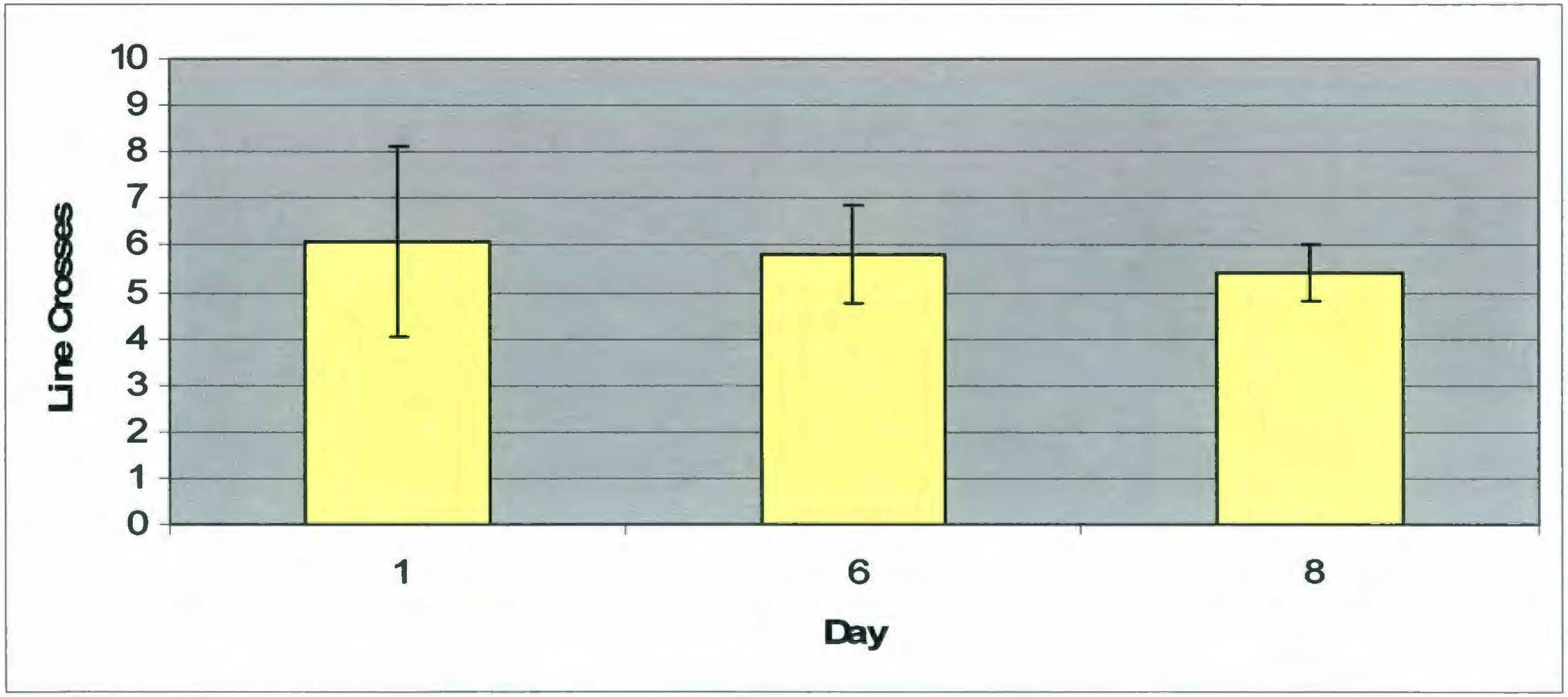

Figure 3.8: Number of movements between quadrants by male pigs over nine days of habituation (data from Days 1, 6, and 8) in Experiment 2.

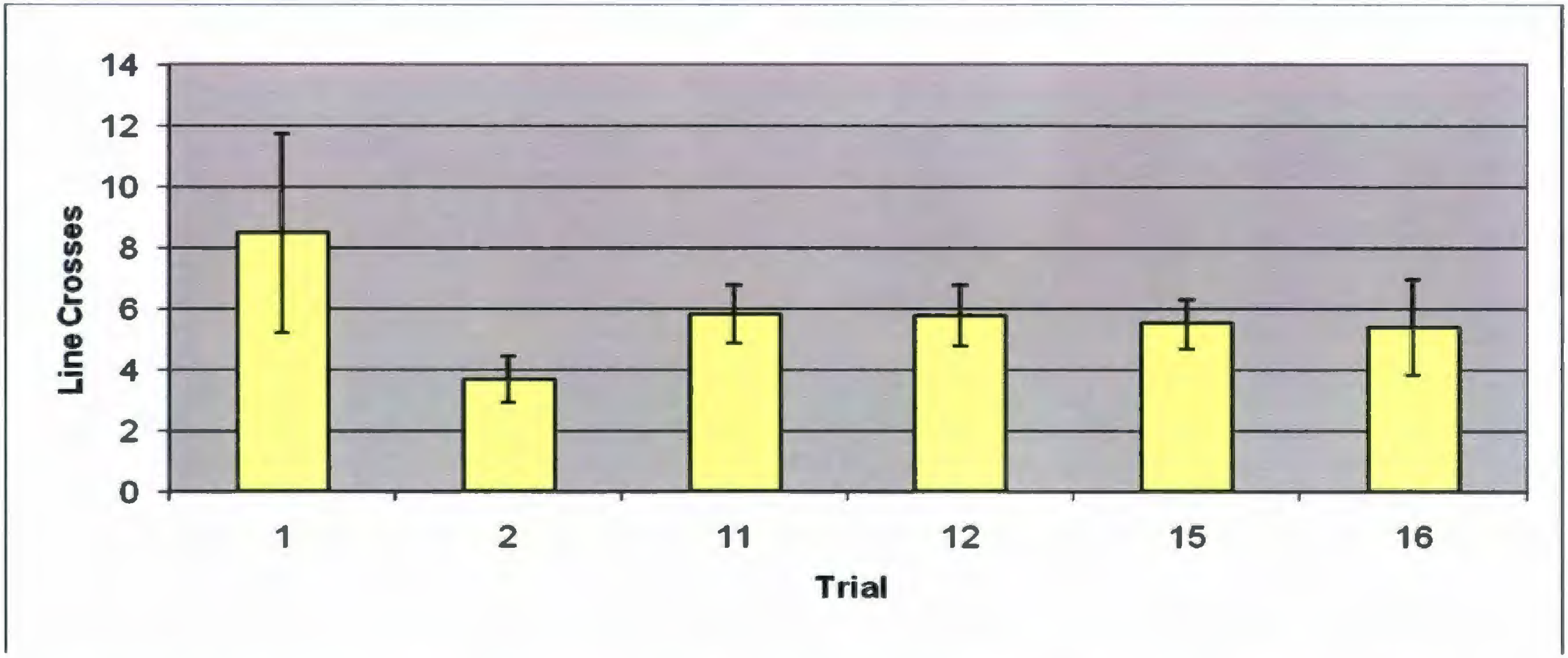

Figure 3.9: Number of movements between quadrants by male pigs over eighteen 20min habituation trials (data from Trials 1, 2, 11, 12, 15, 16) in Experiment 2.

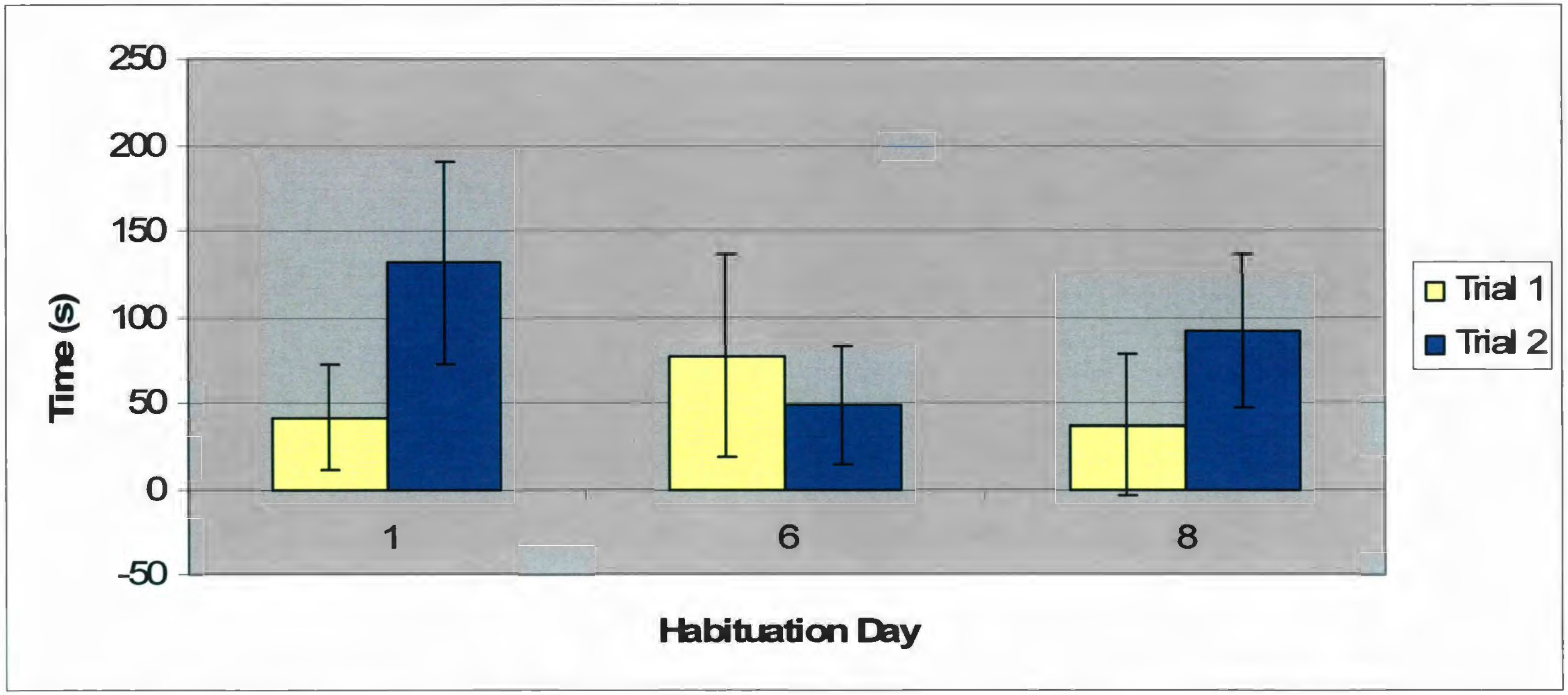

Figure 3.10: Mean duration of loud vocalizations by male pigs over 9 habituation days (data from Days 1, 6, and 8) in Experiment 2.

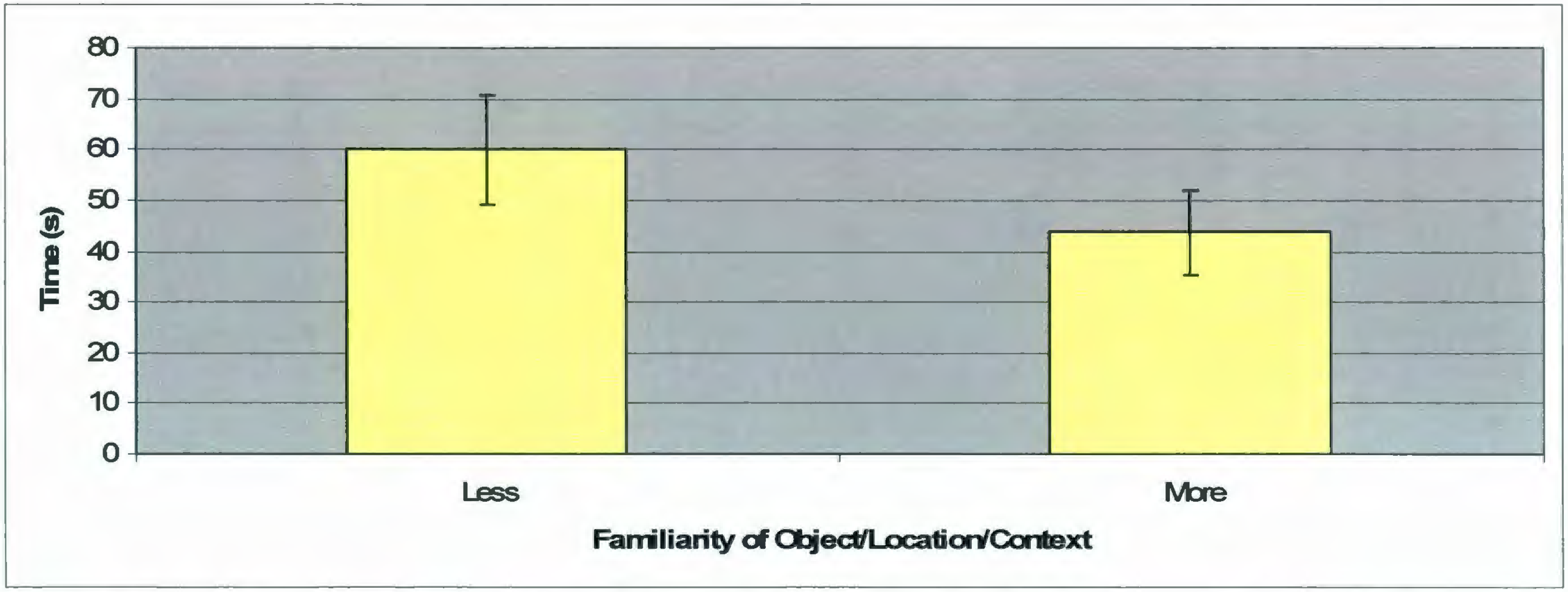

Figure 3.11: Mean time spent by male pigs with less familiar and more familiar object/location/context for all episodic-like memory trials in Experiment 2 combined.

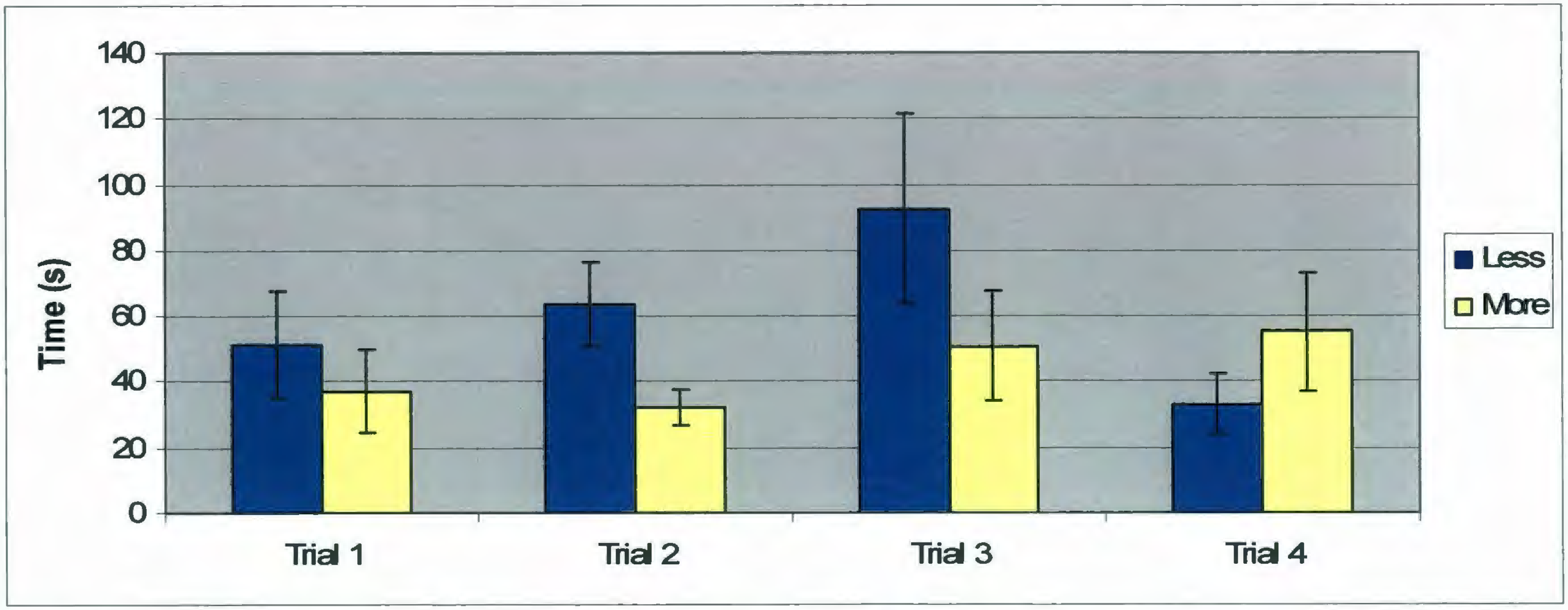

Figure 3.12: Mean duration spent by male pigs with less familiar and more familiar object/location/context over four episodic-like memory trials in Experiment 2.

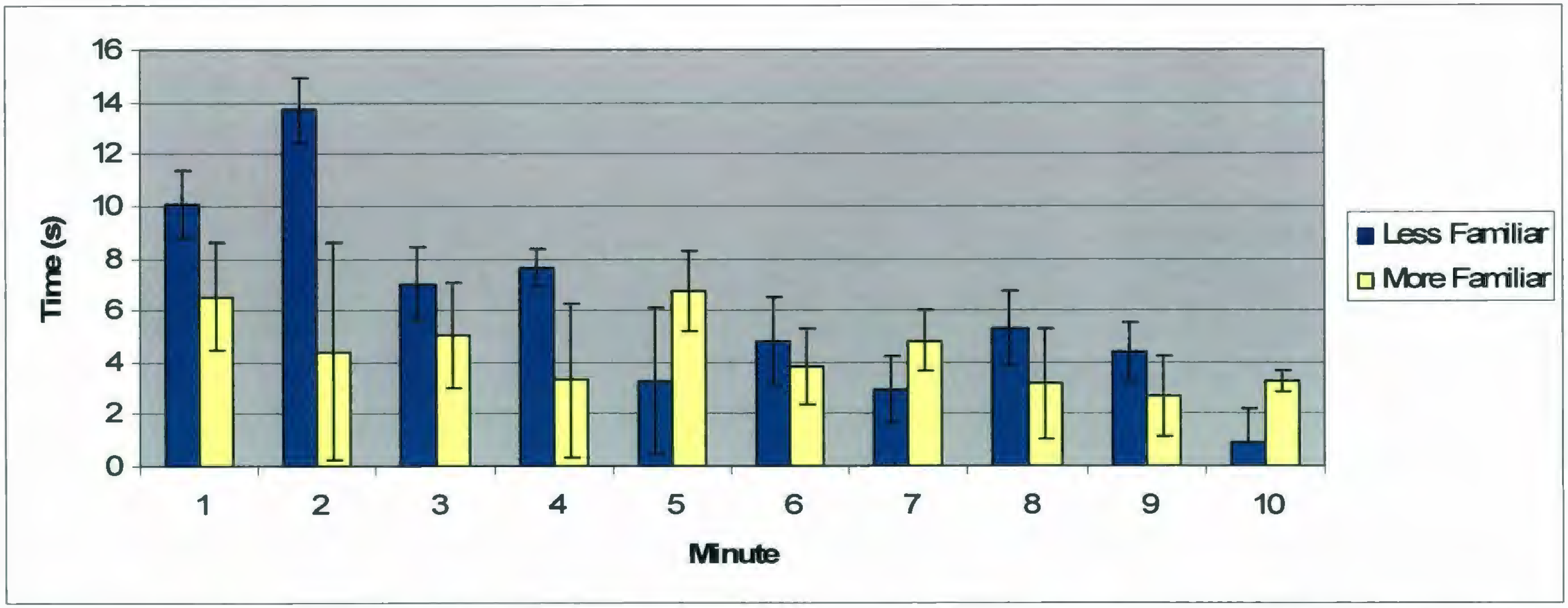

Figure 3.13: Mean duration spent by male pigs with more familiar and less familiar object/location/context during each minute of 10-minute episodic-like memory test phases in Experiment 2.

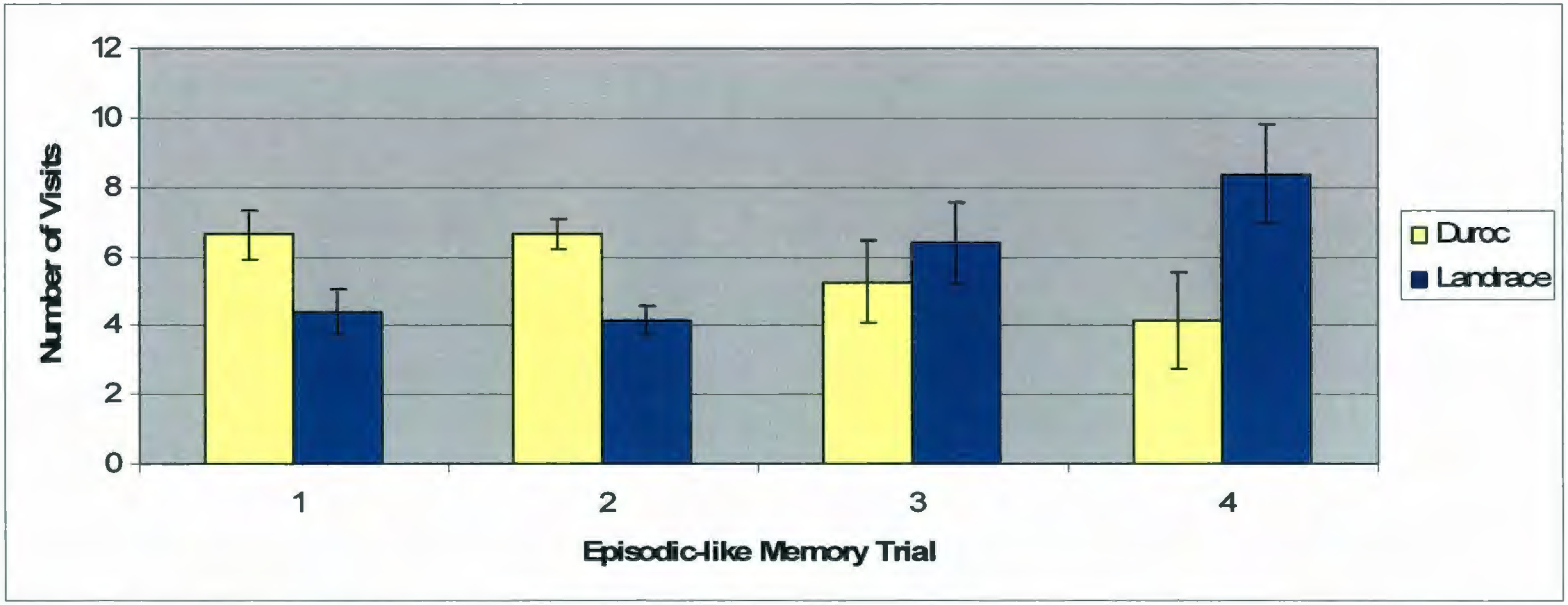

Figure 3.14: Mean number of visits by Duroc-Yucatan and Landrace-Yucatan male pigs to more familiar and less familiar object/location/context during each episodic-like memory trial in Experiment 2.

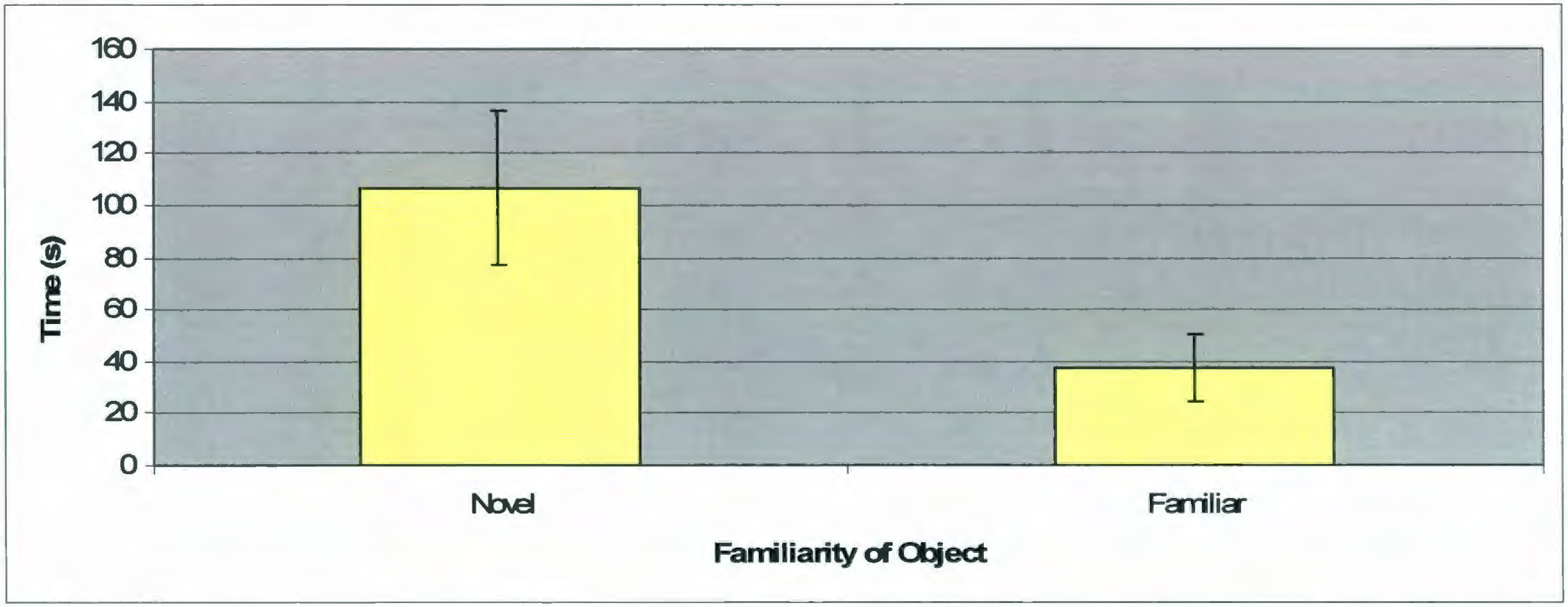

Figure 3.15: Mean time spent by male pigs exploring novel and week-old familiar objects during long-term novel object test in Experiment 2.

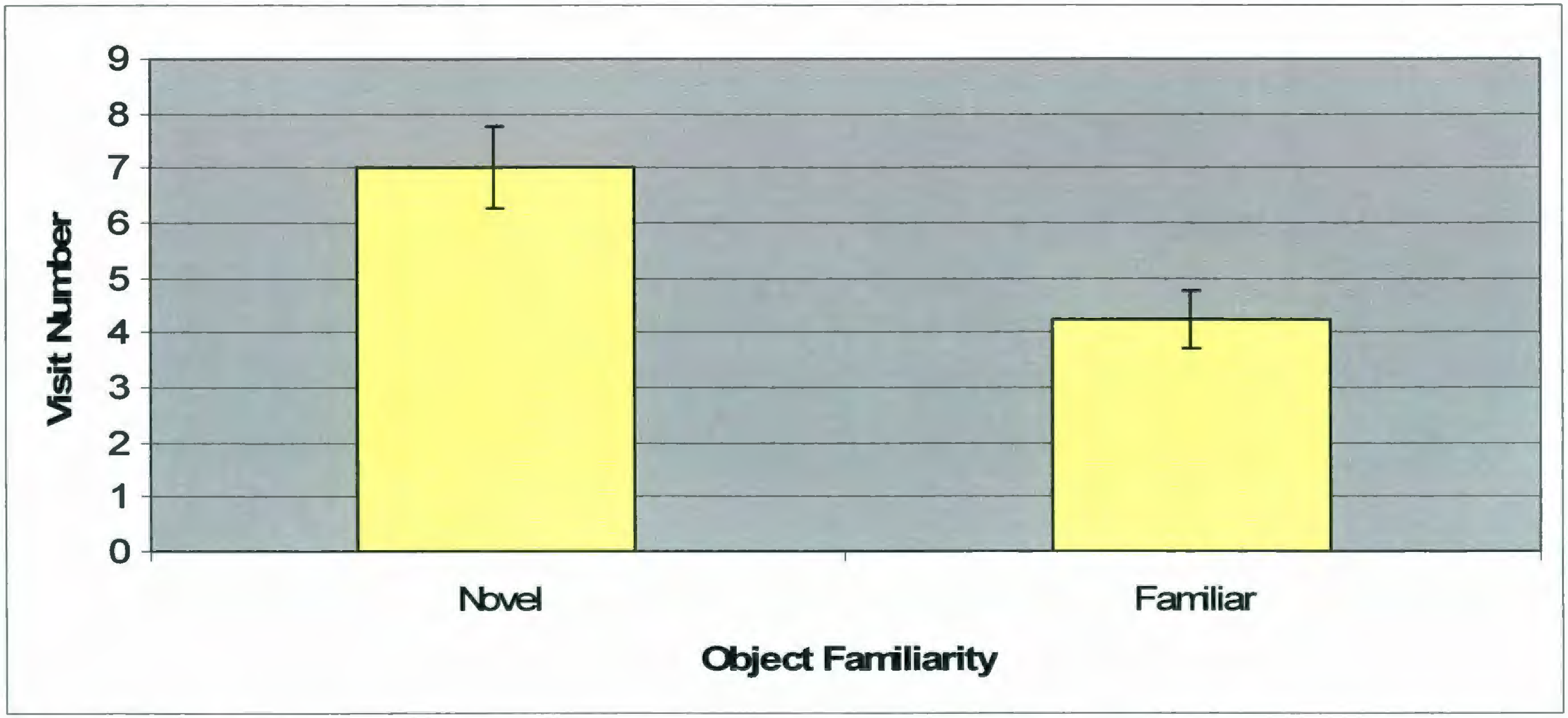

Figure 3.16: Number of visits by male pigs to novel and week-old familiar objects during long-term novel object test in Experiment 2.

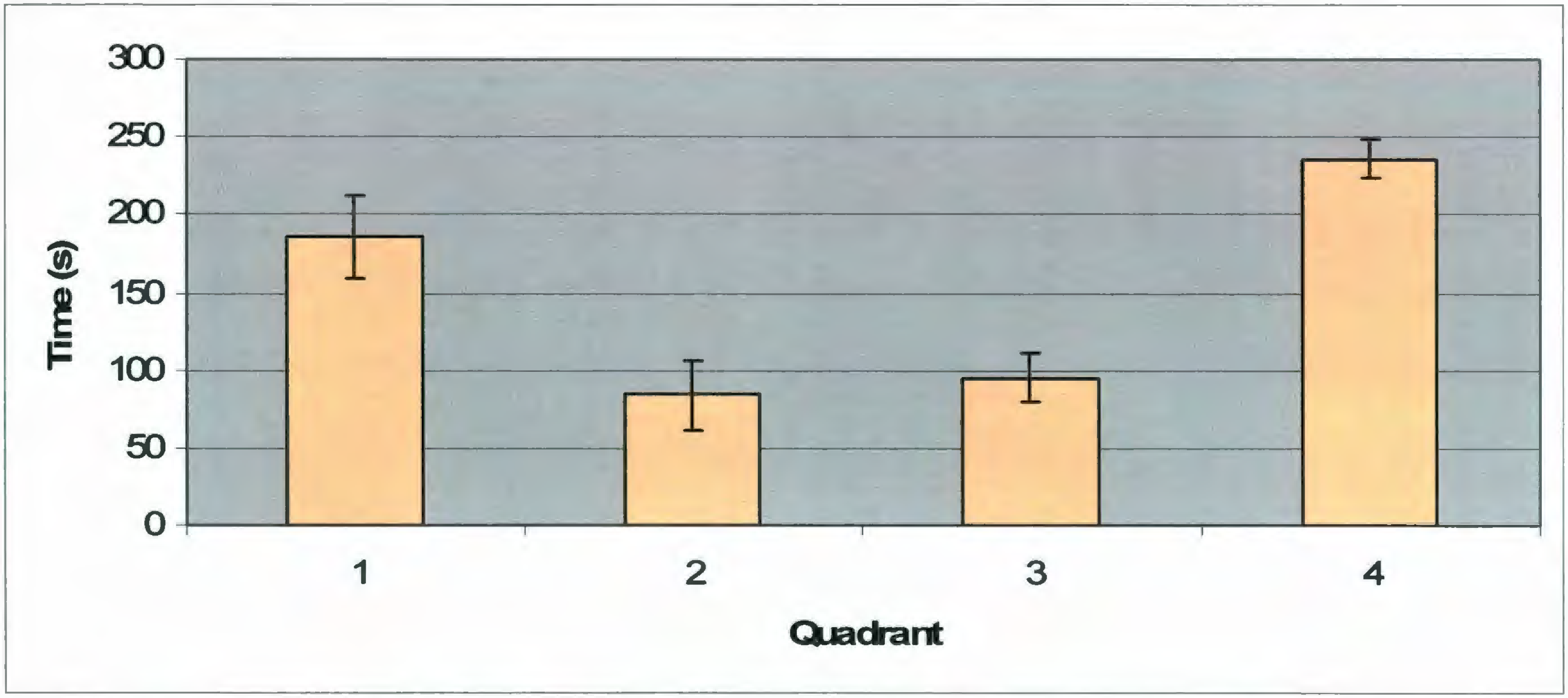

Figure 4.1: Mean time spent in each quadrant by female pigs over eighteen 20min habituation trials (with no objects present) in Experiment 3.

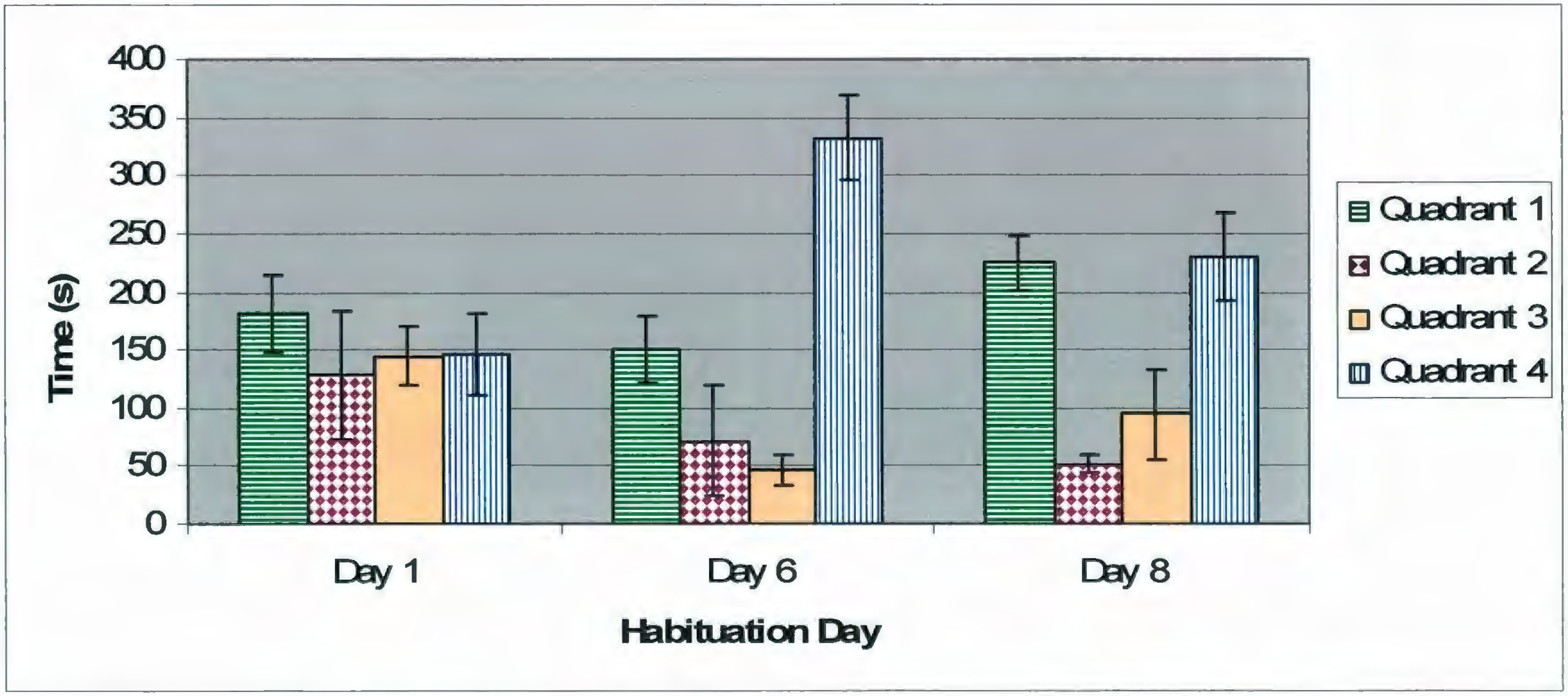

Figure 4.2: Mean duration spent by female pigs in each quadrant over 9 habituation days (two 20min trials/day; data from Days 1, 6, and 8) in Experiment 3.

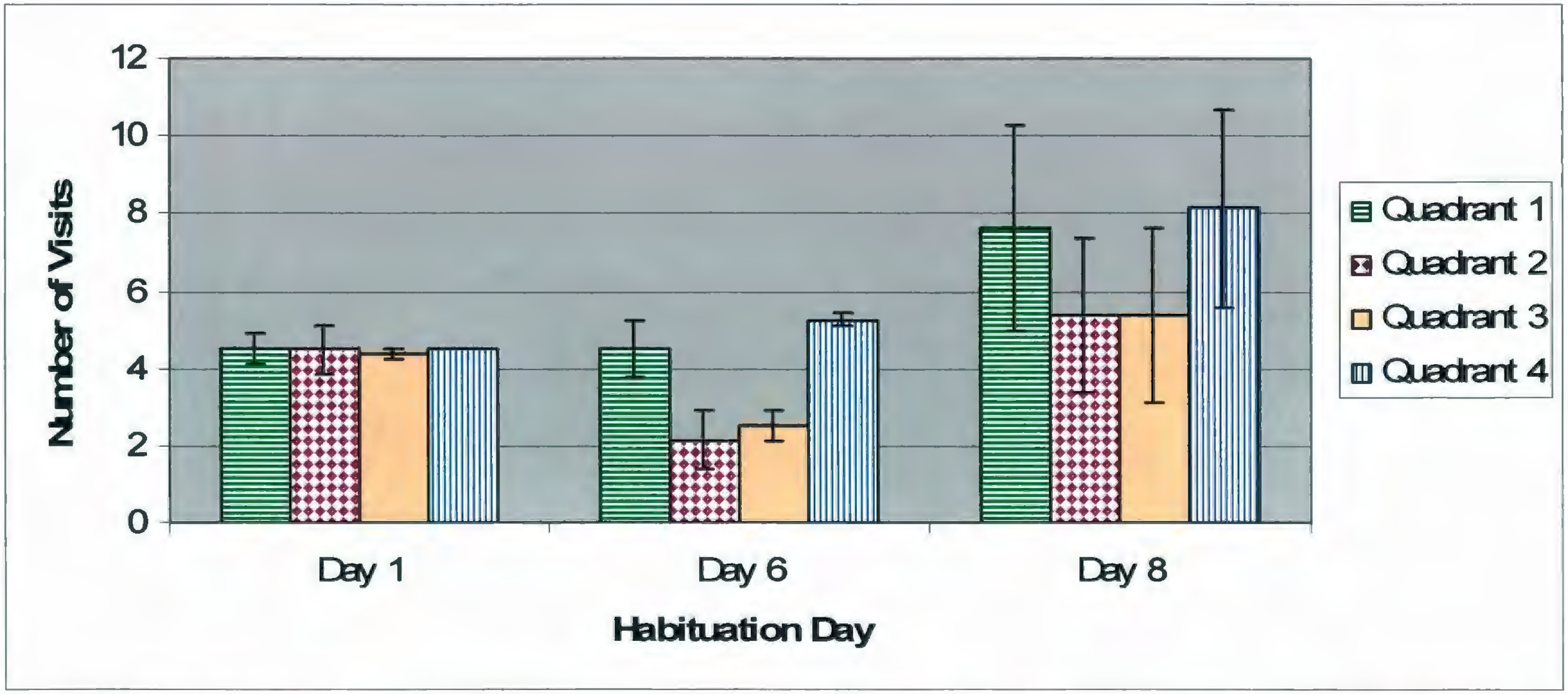

Figure 4.3: Mean number of visits by female pigs to each quadrant over nine days (two 20min habituation trials/day; data from Days 1, 6, and 8) in Experiment 3.

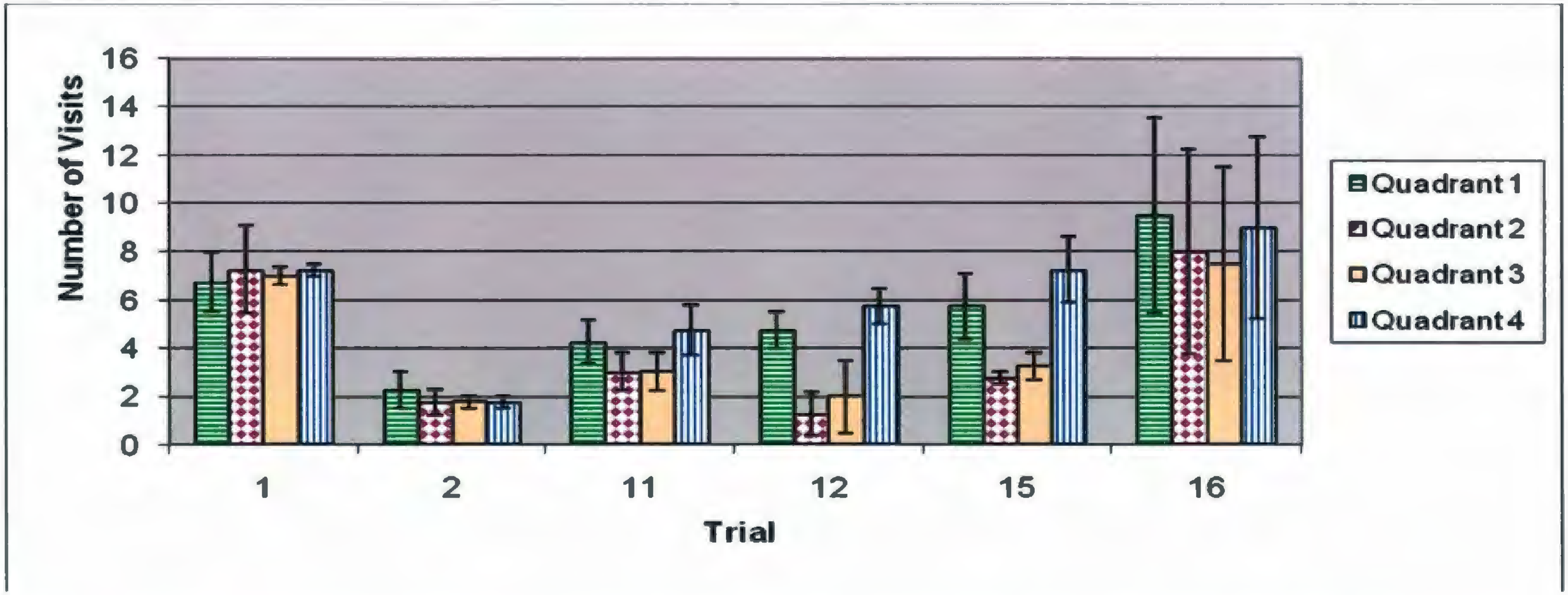

Figure 4.4: Number of movements between quadrants by female pigs over eighteen 20min habituation trials (data from Trials 1, 2, 11, 12, 15, 16) in Experiment 3.

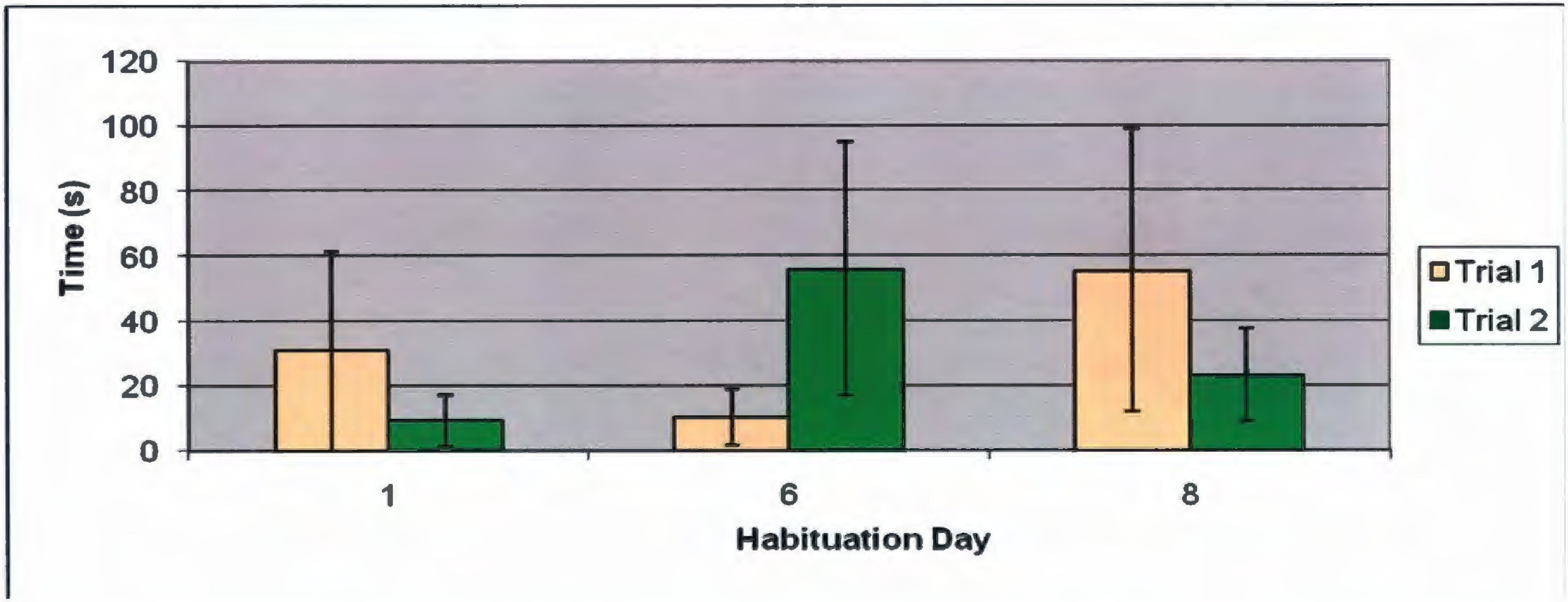

Figure 4.5: Mean duration of loud vocalizations by female pigs over 9 habituation days (data from Days 1, 6, and 8) in Experiment 3.

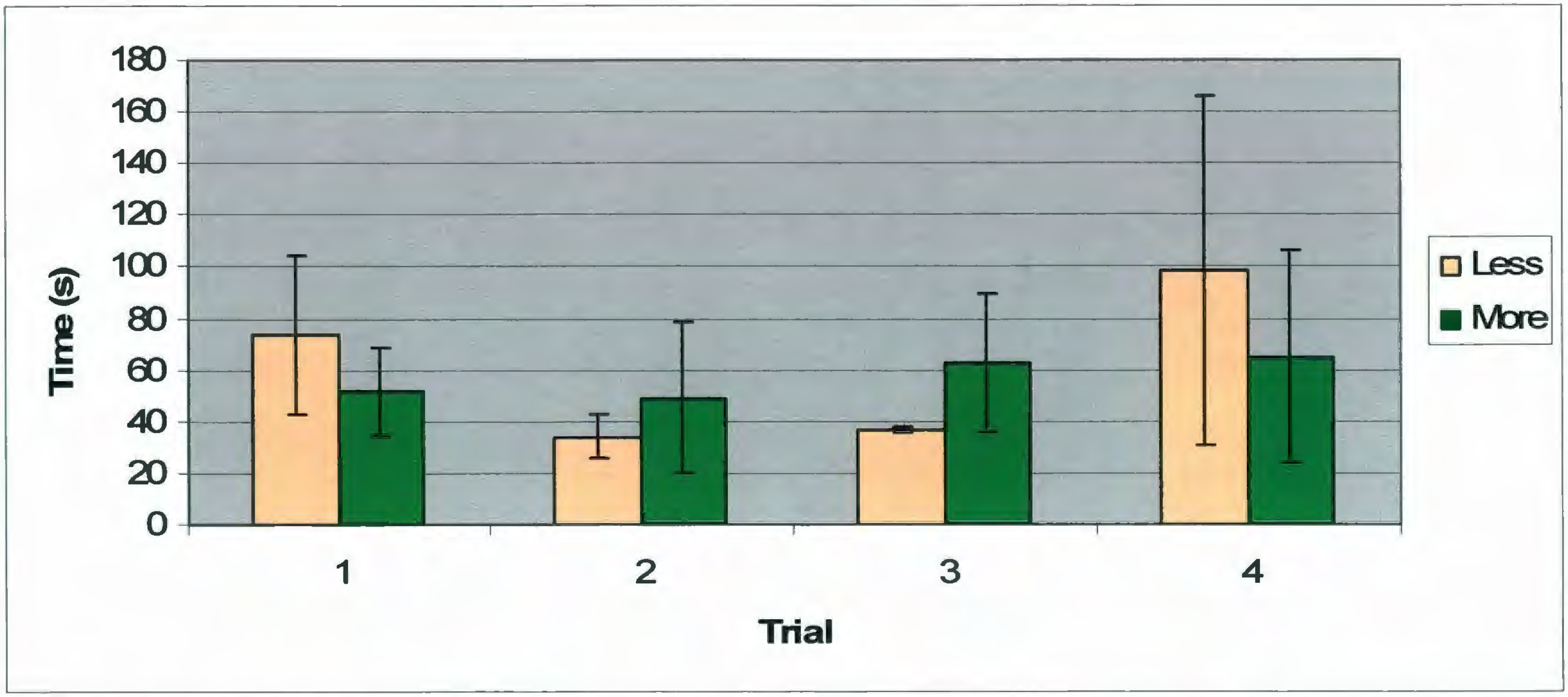

Figure 4.6: Mean time spent by female pigs exploring more and less familiar object/location/context over four episodic-like memory trials in Experiment 3.

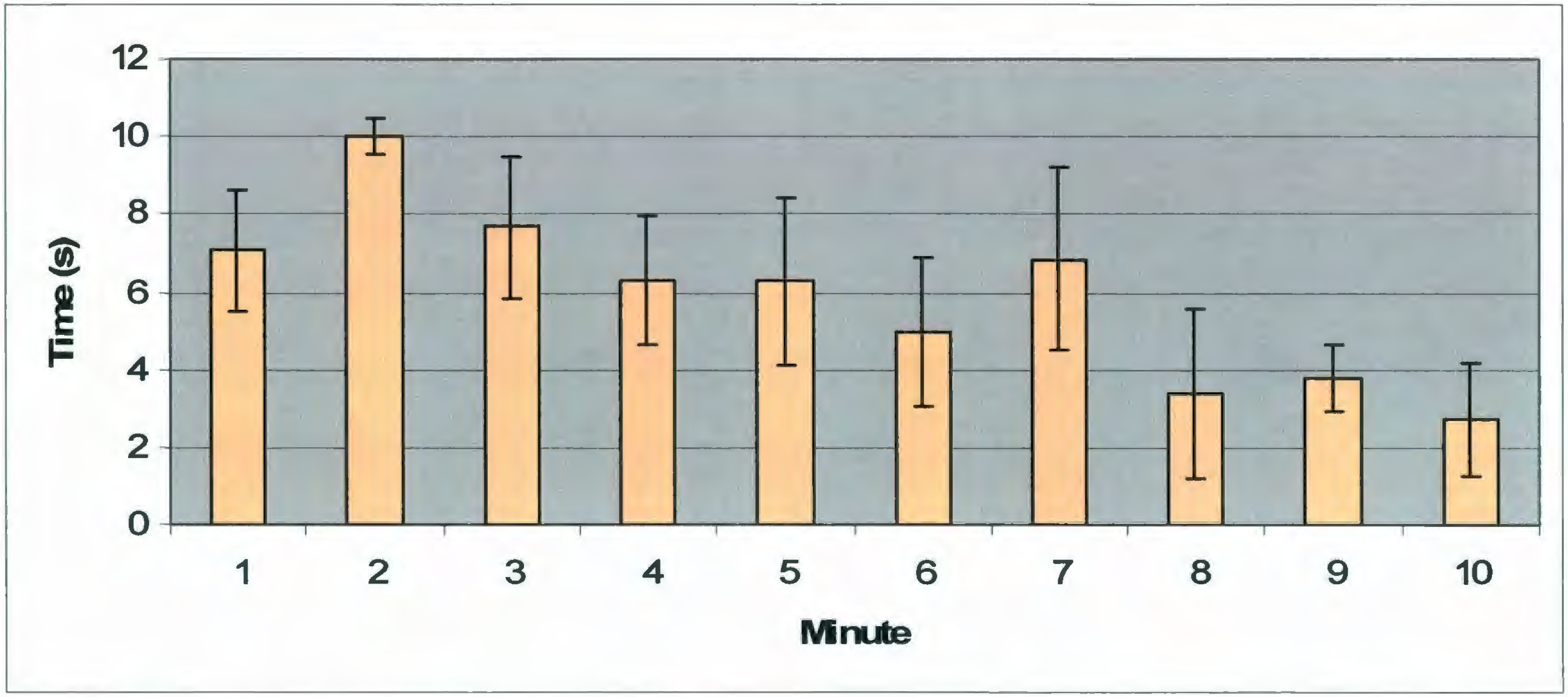

Figure 4.7: Mean time spent by female pigs exploring both object/location/context during each minute of 10-minute episodic-like memory test phases in Experiment 3.

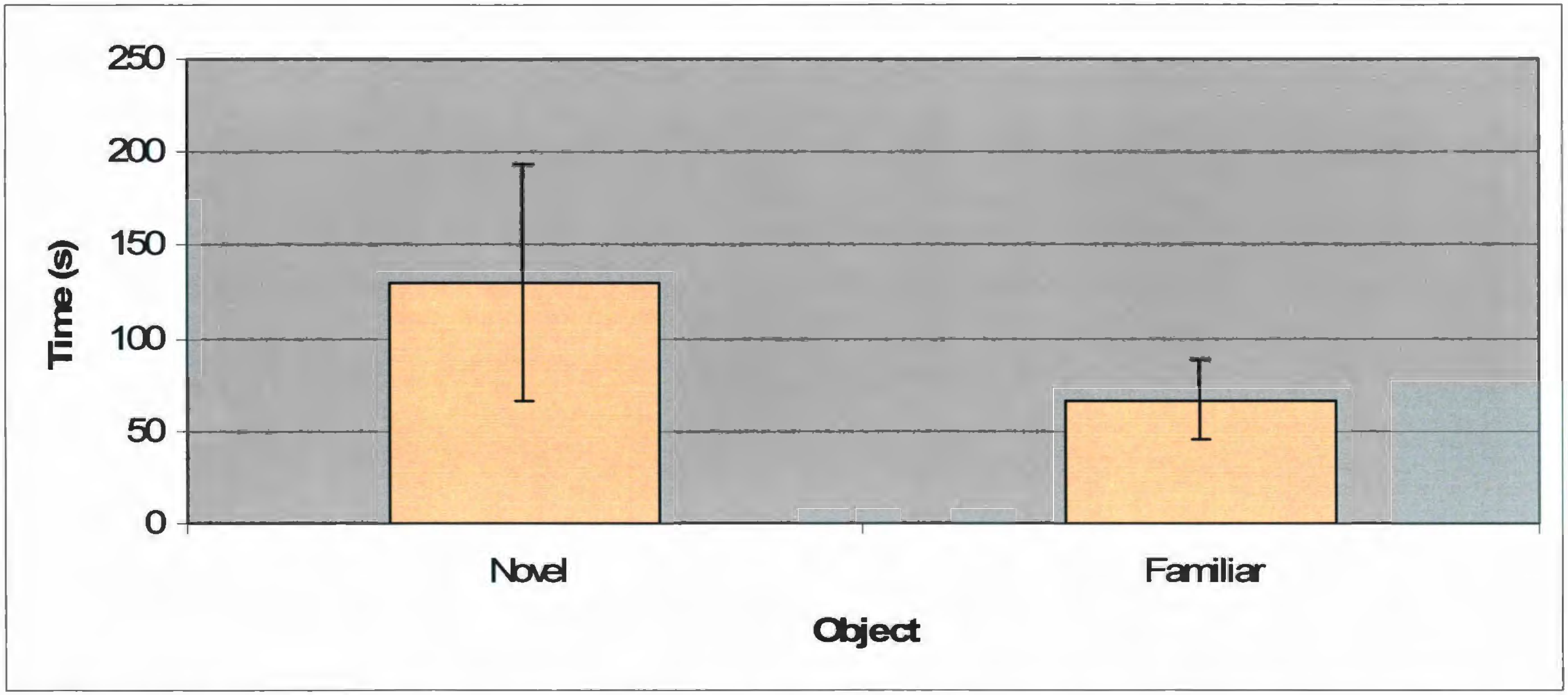

Figure 4.8: Mean time spent by female pigs exploring novel and week-old familiar objects during longterm novel object test in Experiment 3.

APPENDIX 1: Inter-observer Reliability Values

Table 1.1: Pearson r values obtained during inter-observer reliability analysis of data collected during Episodic-like Memory Test (Experiment 1).

Observation Type	Pearson's r value	p-value
Object Exploration Duration	0.96	<0.01
Quadrant Exploration Duration	0.94	<0.01
Visits to Objects	0.95	<0.01
Visits to Quadrants	0.92	<0.01

Table 1.2: Pearson r values obtained during inter-observer reliability analysis of data collected during Long-Term Novel Object Test (Experiment 2).

Observation Type	Pearson's r value	p-value
Duration with Novel Object	1.00	<0.01
Duration with Familiar Object	0.99	<0.01
Visits to Novel Object	0.97	<0.01
Visits to Familiar Object	0.88	<0.01
Duration in Quadrant 1	0.68	0.02
Duration in Quadrant 2	0.97	<0.01
Duration in Quadrant 3	0.96	<0.01
Duration in Quadrant 4	0.64	0.03
Visits to Quadrant 1	0.98	<0.01
Visits to Quadrant 2	0.93	<0.01
Visits to Quadrant 3	0.83	0.00
Visits to Quadrant 4	0.90	<0.01