ASSESSING OBJECT RECOGNITION MEMORY IN THE DOMESTIC PIG

By

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A thesis submitted in the partial fulfillment of the requirements for the degree of

Master of Science in Animal Sciences

Washington State University
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May 2005

To the faculty of Washington State University:

The members of the committee appointed to examine the thesis of Amanda Kristyne Gifford find it satisfactory and recommend that it be accepted.

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ACKNOWLEDGEMENT

There are many people I want to acknowledge who have helped me finally reach this day. First, I would like to thank my husband Robert for encouraging me to begin in the first place, and putting up with all the late nights and the occasional pig odors. To Ruth Newberry, the best advisor and mentor a student could ask for, thank you for encouraging and challenging me to be a better student, and for the countless hours of time you have so generously given along the way. I want to thank Sylvie Cloutier for her willing and cheerful helpfulness, Rich Alldredge for statistical advice, and Ragen Trudelle-Schwartz McGowan for sharing in the joys and pains of graduate school with me. I thank my committee members Kris Johnson and Samantha Swindell for their helpful insight and encouragement. To Theresa Tassell and all the staff at the Washington State University Swine Center, I am grateful for your expertise. I also want to recognize the Department of Animal Sciences for providing me with a teaching assistantship.

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Abstract

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May 2005

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This thesis was undertaken to examine the performance of pigs in a spontaneous object recognition test, whereby preference for a novel stimulus instead of a previously experienced stimulus is a measure of recognition of the familiar stimulus. Knowledge of the robustness of a pig's memory for objects and the factors affecting it could be useful in a variety of contexts. I first review what is known about the cognitive abilities of pigs and why this might be important from an animal welfare as well as an animal production standpoint. I then focus on recognition memory, including how it is tested in various species and what is known of the neural mechanisms mediating recognition memory. Next I discuss novelty preference as a specific way to assess recognition, and describe two experiments using the spontaneous object recognition test to investigate object recognition memory in pigs. In the first experiment, I hypothesized that object recognition memory in the pig would be mediated by length of initial exposure to the object, and the delay between initial exposure and re-exposure. I predicted that increasing the exposure time would result in novelty preference at longer

delays, and that novelty preference would decrease as delay increased. Pigs were exposed to two sample objects for 10 minutes and 2 days, respectively, and tested at delays of 1 hour, 3 hours, 5 days, and 6 days. Pigs did not demonstrate recognition of the 10-minute sample object at any delay, as indicated by a lack of preference for the novel object. Pigs demonstrated no recognition of the 2-day sample object at the 1-hour delay, but did at the 3-hour and 5-day delay, and novelty preference increased from the 1-hour delay to the 5-day delay for the 2-day sample object. In the second experiment, I refined the test methodology and further tested the prediction of decreased novelty preference over increasing delays using a single short (5-minute) exposure time and separate groups for time delays of 1 hour, 24 hours, and 5 days. Pigs did not demonstrate a novelty preference at any delay. Overall, the results indicate a need to reduce within treatment variability in responding and to establish a stable novelty preference before testing. They also cast doubt on the interpretation that a lack of novelty preference indicates failure to recognize the sample object.

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INTRODUCTION

Recent research in the field of farm animal welfare has focused on the role of animals' cognitive abilities in their perception of the environment and interaction with the environment (Spinka et al., 1998; Kendrick et al., 2001; Croney et al., 2003). "Cognitive abilities" are usually loosely defined in the welfare literature, and can range from conscious awareness of self and others (Kendrick, 1998), to how well an animal can form associations and how long they are remembered (Held et al., 2002). It is thought that only by understanding what an animal is or is not capable of mentally can we begin to make any valid assessments of an animal's potential to "suffer" (Nicol, 1996).

Scientists have recently shown a renewed interest in pigs as subjects for studies of cognitive abilities in farm animals (Laughlin et al., 1999; Kristensen et al., 2001; Croney et al., 2003). This is primarily because of the belief that the pig is fairly intelligent, but also due to concern over the implications of housing these animals in intensive, relatively barren environments if pigs do have high levels of cognitive ability (Croney, 1999). Spatial memory has been one cognitive aspect measured. Laughlin et al. (1999) explored the ability of adult pigs to remember where food was baited in an eight-arm radial maze. The authors found that imposing common husbandry practices during the delay interval, such as isolating the animal or exposing it to a novel pig, impaired performance in the task. The

authors concluded that even mild husbandry practices can act as stressors, which interfere with a pig's spatial memory. They extend their results to speculate that the same stressors may have similar disrupting effects on social memory, as evidenced by the observation of increases in aggression between familiar pigs after short absences due to husbandry practices.

However, research in the field of rodent memory indicates that spatial memory is moderated by a separate brain area than memory for discrete stimuli (Steckler et al., 1998b; Wan et al., 1999; Bussey et al., 2000), of which social memory would be a part. Damage or impairment of one memory type is independent of the other type. If this dichotomy is exclusive (i.e. there is no overlap of functions between brain regions), studies of non-spatial recognition memory should be undertaken in pigs, as they may shed light on those memory processes not governed by the spatial memory-influencing brain regions.

For example, understanding a pig's capabilities regarding recognition of individuals, including potential disrupters of social memory, could help producers when moving and reintroducing animals. Likewise, knowledge of the robustness of object recognition memory in the pig, including when and how recognition fades, would be important for producers using objects as part of an enrichment program. Looking at aspects of animal cognition such as object recognition in pigs may bring us one step closer to elucidating their mental capabilities and limitations, and enable us to utilize this information for the benefit of the animals and producers alike.

LITERATURE REVIEW

Cognitive Studies in Pigs

Why Pigs?

Proponents of animal welfare consider more than just the physical health and reproductive functioning of an animal when determining "welfare". Also of interest is the psychological well-being of an animal (Dawkins, 1990). To assess whether animals can "suffer," knowledge of their cognitive, or mental, capabilities is needed (Nicol, 1996). By understanding what an animal can or cannot perceive, how they learn and what they remember, and if they are capable of "higher" cognitive functions, scientists and producers are better able to either refute or validate claims of animal suffering.

Another reason for measuring cognitive abilities in farm animals in particular would be to improve production of animals, or make the management process more pleasant for both the animals and their handlers. Temple Grandin's insights on facility design and handling procedures based on knowledge of livestock perception are an excellent example of this principle (Grandin, 1989a; Grandin, 1994). The design of facilities and handling procedures should be based not only on ease for the producer, but also on knowledge of how they may affect perception, learning, or memory in the animal, as this can alter productivity.

Scientists have recently shown a renewed interest in pigs as subjects for studies of cognitive abilities in farm animals (Laughlin et al., 1999; Kristensen et al., 2001; Croney et al., 2003). This is because the pig is fairly intelligent, and concern about the implications of housing these animals in intensive, relatively barren environments if they do have high levels of cognitive ability (Croney, 1999). Several behavioral problems, such as tail biting and stereotypies, are thought to be associated with the relatively barren environment in which intensively managed pigs are raised (Broom and Johnson, 1993; Wemelsfelder, 1993). More information about the mental abilities of pigs is needed to address the animal welfare concerns.

Pigs may also have their cognitive abilities taxed in unexpected ways during routine management. Some pigs are expected to operate mechanical feeders at weaning, which requires operant learning (Held et al., 2002). Maneuvering around pens housing large groups of pigs requires spatial memory for the location of resources (Mendl et al., 1997). Stable dominance hierarchies lead to low levels of aggression, and may necessitate the use of recognition (Ewbank et al., 1974; Gheusi et al., 1997; Held et al., 2002). Studies of cognitive abilities in pigs may help to facilitate learning in these cases, or discover how susceptible they are to disruption from common husbandry practices (Mendl et al., 1997).

The following sections explore what is already known about pig cognitive abilities, and encompass the areas of sensory perception, learning, and memory abilities.

Sensory Capabilities

Sensory perception and discrimination entails what an animal's sense organs are capable of detecting, as well as how the animal perceives these signals and chooses to act. Tests of perception are usually operant conditioning tasks, where animals are trained to respond to one stimulus for a food reward, then tested with stimuli that vary slightly from the original stimulus on some dimension. If an animal perceives a difference between the varied stimuli and the original stimulus, it will behave in a different manner than if it perceives the stimuli to be the same (Domjan, 2003, p. 221).

Pigs can perceive wavelengths of light between 420 and 760 um, and are thought to have color vision similar to humans (Klopfer, 1966). Pigs can also make visual discriminations using color to find food under buckets in a foraging task (Croney et al., 2003). Little information exists on the range of auditory sensitivity in the pig, even though pigs have a rather large vocabulary of vocalization they rely on for communication (Houpt, 1998). Piglets may be able to recognize their mother from other sows by variations in vocalization patterns (Blackshaw et al., 1996). As for taste discrimination, Kennedy and Baldwin (1972) found that pigs could perceive the difference between concentrations of

sucrose solutions, and preferred sucrose, glucose and saccharin solutions over water.

Pigs have very developed olfactory sensory abilities. Piglets can discriminate between their mother's and another sow's fecal odors, as shown by a preference for the mother's fecal odor (Morrow-Tesch and McGlone, 1990). This ability develops between birth and 12 hours after birth. Olfactory discrimination also seems to be important for sows to detect strange piglets. Sows that had their olfactory bulb removed at a young age did not react aggressively to strange piglets when introduced after parturition, while non-bulbectomized sows did react aggressively to strange piglets (Meese and Baldwin, 1975). Females can identify intact male boars by using chemical signals in the preputial secretions or saliva (Houpt, 1998). Pigs also discriminate between the urine of a stressed and a non-stressed animal, and can use this information to avoid the location where the stressor occurred (Vieuille and Signoret, 1992). In a foraging task, pigs were able to discriminate between odors to find a food reward (Croney et al., 2003).

Recognition of conspecifics may involve many sensory modalities. Pigs are able to discriminate between socially unfamiliar pigs and familiar pigs, normally will investigate an unfamiliar pig for longer than a familiar pig, and will react more aggressively to strange pigs than towards familiar pen-mates (McGlone, 1986, 1990; Zayan, 1990). It is believed that this recognition is mediated by a combination of olfactory and visual stimuli provided by the other pig. Pigs can discriminate between familiar and unfamiliar pigs, a familiarity

discrimination, or between two familiar or two unfamiliar pigs, an individual discrimination, using only olfactory cues (Meese et al., 1975; Kristensen et al., 2001; Mendl et al., 2002), but recognition is impaired when visual cues are blocked by hoods or opaque contact lenses (Ewbank et al., 1974; Friend et al., 1983). Tanida and Nagano (1998) found that miniature pigs used all three sensory cues (visual, auditory, and olfactory) to discriminate between a human handler distributing rewards and a stranger, although olfactory cues contributed the least. These findings suggest that a combination of sensory cues is used to obtain information about both pig and human identity.

Learning Abilities

Early studies of learning ability in pigs demonstrated that pigs could learn both classically-conditioned and operantly-conditioned associations (Yerkes and Coburn, 1915; Myers, 1916; Marcuse and Moore, 1946; Karas et al., 1962). Classical conditioning involves learning an association between an unconditioned stimulus and a neutral stimulus paired with the unconditioned stimulus. Presentation of an auditory cue was paired with shock, and eventually came to evoke a shock-avoidance response in pigs, an example of classical conditioning (Karas et al., 1962; Hammell et al., 1975). Pigs can also learn to associate both negative (hitting or shouting) and positive (gentle stroking, food reward) handling with the presence of human beings (Hemsworth et al., 1996a; Hemsworth et al., 1996b; Tanida et al., 1995), although there is contrary evidence on whether pigs

generalize to all humans or discriminate between their particular handler and other humans (Rushen et al., 1999).

Operant, or instrumental, conditioning involves learning an association between a response and a consequence. Pigs can learn to press a panel with their snout for various reinforcements (heat-Baldwin and Ingram, 1967; food-Baldwin and Stephens, 1973; light-Baldwin and Meese, 1977). The ability of pigs to use electronic feeding stations and feeders with hinged covers are examples of operant learning in the farm setting (Houpt, 1998). The type of sensory reinforcement is important when using pigs in operant conditioning paradigms. Changes in illumination and stimulation of the brain were reinforcements for which the pigs would perform an operant response, but not pig noises (Houpt, 1998).

Pigs are able to learn left-right (spatial) discriminations and black-white (visual) discriminations to obtain a food reward (Klopfer, 1966; Lien and Klopfer, 1978; Sobotka and Brown, 1986; Moustgaard et al., 2004), and learn to reverse their response to the stimulus more quickly for spatial than visual discriminations (Moustgaard et al., 2004). Interestingly, Klopfer (1966) found that pigs fed in the same place since weaning lose the ability to associate visual stimuli with food reward.

In an early study of operant conditioning requiring pigs to use different rules for choosing which door to enter, Yerkes and Coburn (1915) found that pigs learned to choose the correct door to receive a food reward when the door was always on the right or the left, the second door from the left, or alternated from

right to left or left to right, which indicates the ability to learn simple rules. However, pigs did not reach the learning criterion if the door was located in the middle of more than three doors, which might indicate an inability to learn concepts such as "middle".

Social learning involves the ability to use information obtained by observing group members. Pigs show some evidence of social cognitive abilities in their foraging behavior. When paired with a pig with knowledge of the location of a food resource, an untrained pig gave up independently searching and began following the trained pig (Held et al., 2000). Although, in that study there was no control pairing two uninformed foragers together to tease out social cognition from simple social facilitation. Pigs also learn about novel foods more quickly after watching a social companion eating the novel food (Nicol and Pope, 1994). However, pigs do not seem to have the ability to infer what another animal can or cannot see or use that information to find a food resource (Held et al., 2001).

Memory Abilities

Memory involves the ability to "respond to or recount information that was experienced earlier" (Domjan, 2003, p. 318). Studies of memory differ from learning studies in that they are more focused on the effects of the delay interval on the retrieval of the stored information, not the initial acquisition of the information (Domjan, 2003).

Spatial memory ability of pigs has been the most commonly studied. Pigs could remember the location of a food reward from among 10 possible locations after delays of 10 minutes and 2 hours, with relatively fewer errors than expected for an animal searching by chance (Mendl et al., 1997). In a more complex task using an eight-arm radial maze, pigs were able to remember where food was located in 4 out of 8 possible locations after a 10-minute delay (Laughlin et al., 1999). Memory for this task was disturbed by a task-related stimulus (confinement in the center compartment of the maze) imposed in the delay interval.

I shall now turn to a more general review of the topics surrounding recognition memory, as this is the type of memory focused on in the two experiments discussed in this thesis.

Recognition Memory

Defining Recognition Memory

Recognition is defined as 'the act of recognizing,' and to recognize means to 'know or identify from past experience or knowledge' (American Heritage Dictionary, 1983). Therefore, recognition memory refers to the ability to judge whether one has encountered something (object, location, idea, person, situation) in the past based on stored memories (Murray, 2000). In more scientific terms, Steckler et al. (1998a) define recognition memory as "neural process(es) by which a subject is aware that a stimulus has been previously experienced" and define

recognition as "the behavioural outcome of these processes." To recognize a stimulus, it is necessary to 1) perceive and identify the unique sensory cues about the stimulus and 2) judge whether it has been experienced before or not (Mandler, 1980).

Recognition memory is different from recall of information about stimuli or events. Recall can be defined as "generating information about a past event in the absence of the original event" (Ruggiero and Flagg, 1976, p. 1), whereas recognition memory occurs in the presence of the original stimulus. Because of our reliance on verbal or written responses to show recall, it is very hard to demonstrate that an animal possesses recall memory.

There are several different types of recognition memory. Since recognition involves the judgment of prior occurrence (Mandler, 1980), it is possible to recognize the recency of occurrence, whether exposure has occurred previously or not (familiarity), and the context (where and when) associated with the prior occurrence (Brown and Xiang, 1998). Separate and distinct populations of neurons are activated when humans and animals view and recognize objects (Wan et al., 1999; Kreiman et al., 2000), faces (Bruce et al., 1981; Kendrick and Baldwin, 1987), spatial arrangements of objects (Zhu et al., 1995; Wan et al., 1999), and changes to these stimuli from past exposures (Brown and Xiang, 1998).

Individual recognition is by definition the ability to discriminate between equally familiar or equally unfamiliar individuals. The ability to recognize a familiar conspecific from an unfamiliar conspecific has been termed social

recognition, and is a familiarity discrimination, which can be explained by a simpler cognitive process than individual recognition (Zayan, 1987, p. 321).

Types of Recognition Memory Tests

There is a wide array of possible recognition memory tests, and each differs in methodology and the type of recognition memory tested. Some tests may not be measuring recognition memory at all, but recall memory instead. Despite the differences, however, there are similar properties shared among all the tests. Most tests of recognition involve three trials including (i) a sample phase, where the subject is presented with the stimulus and required to store the information about it; (ii) a delay phase, where mnemonic load can be manipulated; and (iii) a choice phase, where the subject compares the choice stimuli against the stimulus already stored in memory, and acts on this comparison (Steckler et al., 1998a). In most cases the subject must use one of two rules for governing their responses: matching- or non-matching-to-sample. However, this is not true for spontaneous preference tasks, where subjects are allowed to respond naturally to a choice between two stimuli varying in familiarity (Hughes, 1997).

It is beyond the scope of this review to recount all the types of tests and variations used to measure recognition memory. An excellent review of the variety of tests used in the rodent literature is provided by Steckler et al. (1998a). Here I shall focus on the most common tests used in the species primarily tested

for recognition memory, namely humans (adults and infants), non-human primates, and rodents.

Recognition memory in adult humans usually involves exposure to lists of words, photographs, or visual patterns that must be committed to memory. In later tests the subjects are given new lists of stimuli, containing both old and new stimuli, and asked to indicate if a particular stimulus has been seen before or not (Mandler, 1980).

Since human infants are unable to express verbally whether they recognize something as familiar or not, non-verbal methods have been devised. The paired visual comparison (PVC) test is used to test infant visual recognition memory (Fagan, 1992). Duration of spontaneous looking behavior is the response measured. Infants are familiarized with a pair of identical visual stimuli in the first phase, and looking behavior is recorded. After a delay period, infants are then presented with the previously experienced stimulus again, this time paired with a novel visual stimulus. Preferential looking at the novel stimulus indicates recognition of the familiar stimulus. Adults have also been tested with this procedure, and have been found to respond in a manner similar to infants (Richmond et al., 2004).

A similar task is the habituation-dishabituation procedure, which measures habituation of the looking response to the sample stimulus between one presentation and the next (Courage and Howe, 1998). If no decrease in looking time is seen after proper familiarization, then it is assumed that the infant does not

recognize the stimulus. The PVC test and the habituation-dishabituation procedure have also been used with non-human primates and rodents (Arletti et al., 1997; Murray, 2000).

Object recognition memory in primates is most widely tested with a trial-unique delayed non-matching-to-sample test (DNMS) (Murray, 2000). During the training phase, subjects are presented with a three-holed apparatus, with a sample object covering the middle hole which contains a food reward. The subjects must displace the sample object to receive the food reward beneath. Delays ranging from seconds to minutes to days can be imposed, before the subject is retested with a copy of the sample object and a novel stimulus covering the outer holes of the apparatus. In the DNMS task, subjects must displace the object they have not encountered before to receive a food reward. A variation of this task, the delayed matching-to-sample (DMS) test, requires the subject to choose the familiar sample object (Murray, 2000). Accurate performance requires a subject to recognize which sample it has seen before, as well as learn and apply the "choose same" or "choose different" rule.

Rodent studies of recognition memory have used DNMS and DMS tests (Steckler et al., 1998a), habituation-dishabituation (Arletti et al., 1997), as well as spontaneous object recognition, which is similar to the PVC task except that it measures exploration of 3-D objects (Ennaceur and Delacour, 1988).

Neuronal Mechanisms

Several different techniques can be used to study the role of brain areas in recognition memory. These include: lesion/ablation studies, where the brain area of interest is damaged (e.g. Bussey et al., 1999; Bussey et al., 2000); single-cell recording (e.g. Young et al., 1997); functional imaging techniques (e.g. Kreiman et al., 2000); measurement of protein products of immediate early genes like c-*fos* (e.g. Zhu et al., 1995; Zhu et al., 1996); or administration of agonistic or antagonistic drugs (e.g. Sargolini et al., 2003).

Contrary to a previously accepted theory that the hippocampus was involved in all forms of recognition and working memory (Olton et al, 1979), researchers using varied procedures have found a dichotomy in the brain areas needed for spatial and non-spatial recognition tasks (Aggleton et al., 1986; Ennaceur and Aggleton, 1997; Ennaceur et al., 1997; Galani et al., 1998; Easton et al., 2001; Mumby et al., 2002). Based on these studies, two distinct neural networks have been proposed to account for spatial and non-spatial recognition memory. The first one, which mediates spatial memory, includes the hippocampus, mammillary bodies, anterior thalamic nuclei, and prelimbic frontal areas. The second, mediating memory for objects, includes the temporal cortical association areas, rhinal cortex, and mediodorsal thalamic nuclei (Steckler et al., 1998b). The nucleus accumbens, which receives input from both the hippocampal and rhinal cortex regions, has been implicated in both memory tasks in mice (Sargolini et al., 2003). Performance on one type of task is independent of

performance on the other, and it is possible to produce deficits in one type of memory and not the other. For example, damage to the hippocampus in rats produced deficits in a spatial alternation task but not a non-spatial DNMS task (Aggleton et al., 1986). On the other hand, rhinal cortex lesions impaired object recognition in rats but left performance in a Morris water maze, a measure of spatial memory, intact (Bussey et al., 1999; Ennaceur and Aggleton, 1997).

Beyond the fact that different brain regions regulate spatial versus non-spatial recognition memory, within non-spatial recognition certain brain areas are specifically responsible for the detection of novelty, or familiarity recognition. According to Berlyne (1960), "novel stimuli cannot be distinguished by physiochemical properties," meaning that novelty is not an inherent property of a stimulus. Rather, novelty is a transient feature that seems to disappear with prolonged or repeated exposure to the stimulus, and is based on previous experience. So how then does the brain recognize if a stimulus is novel or not, and which brain areas are responsible for this process? Cognitive theories and mechanisms for the recognition of familiarity will be presented in the next section. Here I shall focus on the brain areas that are implicated in the process of familiarity recognition.

The novelty (or familiarity) of individual objects is detected by the perirhinal cortex and cortical areas (TE1, TE2, TE3) of the temporal lobe. Novel arrangements of familiar objects activate the postrhinal cortex and hippocampus (Wan et al., 1999). This is to be expected, since the latter requires spatial memory,

which uses a different brain region than non-spatial memory. Rats shown a novel environment had higher activation of their hippocampal neurons, while those shown a novel object in a familiar environment had higher activation of perirhinal neurons (Zhu et al., 1997). Similar findings have been reported for monkeys (Parker et al., 1998). The detection of novelty or familiarity might be modulated by the differential responding of neurons to the second presentation of a stimulus (Brown and Xiang, 1998).

Many of the above-mentioned studies were testing for visual recognition memory. However, similar results with regard to the dichotomy of neural substrates have been found using an odor-recognition DNMS paradigm (Young et al., 1997).

Novelty Preference

The Theory of Novelty Preference

As was mentioned above, recognition can be interpreted from the spontaneous differential responding of animals towards stimuli they either have or have not been exposed to previously. This involves the phenomenon of novelty preference, which seems to be a widespread behavioral pattern of organisms to preferentially explore aspects of their environment that are novel or unfamiliar for longer than those they consider familiar. Since different responses to stimuli require the ability to tell the two stimuli apart based on some variable, increased

responding towards novelty indicates an ability to discriminate between stimuli based on the variable "familiarity."

The phenomenon of novelty preference is usually seen during the course of exploratory behavior. Interest in the motivation of exploratory behavior in animals was prominent in the early 1950s through the 1960s. Of special interest was exploratory behavior that seemed to have no immediate functional relevance, such as to find food or water. This type of exploratory behavior was studied by many researchers of the time, but perhaps the most influential researcher was D.E. Berlyne. Berlyne (1960) defined this type of exploratory behavior as "intrinsic exploration", and described several factors that he believed influenced its expression, of which novelty of the stimulus was highly important (Berlyne, 1960, p. 18). Fowler (1965) also detailed factors affecting intrinsic exploratory behavior, and these two books launched a flurry of new studies into the motivation behind exploration of novelty.

The earliest theories to explain the exploration of novel stimuli tried to link the behavior to the search for primary reinforcement (e.g. food or water), with the novel stimuli serving as secondary reinforcement directly linked to a primary reinforcer (Inglis, 1983, p. 72). However, studies of novelty exploration in monkeys by Harlow and his associates showed that the monkeys would explore novel puzzle boxes for long periods of time although their primary needs were met and the puzzles were never associated with any primary reinforcers (Harlow, 1950).

Later theories, still following drive-reduction models, proposed that exploration was motivated by its own drive: either a "curiosity" or a "boredom" drive. The curiosity drive (Berlyne, 1950; Harlow, 1950) was activated by a novel stimulus, and exploration diminished the drive. However, this did not explain how animals could learn to respond for novelty, so a "boredom" drive was formulated, whereby repeated exposure to familiar stimuli increased the motivation to experience environmental change and access to that change decreased the "boredom" (Fowler, 1965). Combinations of these theories tried to account for problems with each theory used independently, but they still failed to explain all the results adequately (Russell, 1983). Optimal arousal theories, which assume animals will seek to maintain an optimal level of arousal (in this case environmental stimulation), have been difficult to interpret, as "arousal" level is hard to measure (Russell, 1983).

Several cognitive comparator models have also been proposed to explain responses to novelty. Many of these theories use the idea of internal comparator models to filter external information and determine behavioral responses. Sokolov (1960) described how the orienting response to stimuli is regulated by physiological changes, which are evoked by the reception of unexpected sensory input. The greater the discrepancy between incoming stimuli and the internal comparator, the longer the orienting response lasts and the longer it takes before the subject ignores the stimulus. Salzen (1970) also proposed internal

representations as comparators, where discrepancy activates behavioral systems with the goal of eliminating the discrepancy.

Similar explanations for novelty preference are found in the cognitive models of O'Keefe and Nadel (1978) and Inglis (1983). In Inglis' model, every animal has an inherent need to gather information, in order to form a representation of and expectancies about the world. Attention is preferentially biased towards gathering information from stimuli furthest from the expected output of the comparator mechanism (Inglis, 1983, p. 80). O'Keefe and Nadel's model (1978) is specifically concerned with the formation of cognitive maps, but is similar with regard to comparison of external stimuli to internal representations.

Exploration of novel stimuli may be adaptive in many different ways. The "maintenance of familiarity" of the home range, or an animal's normal environmental location, would be necessary to use the resources in that environment effectively and to avoid dangerous situations (Russell, 1983, p. 23). Investigation of novel stimuli, then, may serve to reduce unfamiliarity in an animal's environment. Along with reducing unfamiliarity, preferential exploration of novel stimuli might be adaptive through the possible encountering of biologically relevant stimuli. Chamove (1983) states that contact with novel stimuli may increase preferential access to potentially desirable resources, even though risk may be higher.

Whatever the motivation behind this behavior, the fact remains that it is observed in a wide range of species, and under controlled conditions is quite

robust. In the next section I will discuss how this behavioral propensity has been used to develop a specific test of object recognition memory, and what factors affect performance on this task.

The Spontaneous Object Recognition Test

In 1988, A. Ennaceur and J. Delacour developed what they referred to as "a new one-trial test for neurobiological studies of memory in rats." This test was developed in part to provide a true 'trial-unique' memory test that relied on spontaneous novelty preference. Previous experimenters before this time trying to establish a 'trial-unique' memory test relied on delayed-matching or non-matching to sample tests (Mumby, 2001) which provided a unique stimulus for each trial. However, as Ennaceur and Delacour (1988) point out, performance deficits may be hard to interpret, because subjects have to learn a common rule (choose same or choose different) during a pre-training stage, through repeated stimulus-response-reward associations. Therefore, any deficits in performance could be due to a failure in memory, or a failure to adequately learn the rule. Also, in the matching or non-matching tests, it is necessary to use positive or negative reinforcers to produce behavior, which may alter motivational state.

Because of the limitations of the delayed-matching- and non-matching-to-sample tests to provide a test of memory for unique events, the spontaneous object recognition test (SORT) was developed for rats. It is based on the rats' spontaneous preference to explore novel stimuli over previously experienced ones,

as seen in earlier studies (Berlyne, 1950). Since this test relies on a naturally occurring behavior that rats are motivated to perform without reinforcers, outside of introducing the objects, it requires no training phase or rule-learning by the rats. The test consists of the same three phases as most of the other recognition tests: the sample phase, the delay phase, and the choice phase. In the sample phase, subjects are exposed to two identical copies of an unfamiliar sample object in a familiar test arena. They are allowed to explore these objects for a specified time period, and are then removed back to their home pen. Their exploration time for each object is recorded. After the delay phase, subjects are re-exposed to the test arena in the choice phase, which now contains a copy of the sample object from the sample phase, along with a completely novel object unfamiliar to the subject. Again, the duration of exploration of each object is measured. By comparing the difference between exploration of the sample object and the novel object in the test phase, it is possible to determine if the subjects exhibited a preference for one object over the other. It is assumed that a preference for the novel object demonstrates recognition of the sample object, since rats will spend less time exploring objects with which they are familiar. By manipulating the amount of time between exposures to the objects, it is possible to test for memory of the sample objects at different delay intervals.

This type of test is not only used in the rodent world, but also finds correlates in non-human primate and human research, particularly infant research. The PVC task used with humans and non-human primates uses the same principle

of spontaneous novelty preference, but for looking times for visual stimuli, instead of manipulation of 3-D objects. In non-human primate research, spontaneous preference for novel objects has been measured with a complex grid of sample objects, to which novel objects can be added, or sample objects moved into novel positions (Platt and Novak, 1999).

Factors Affecting Object Recognition and Novelty Preference

There are several factors affecting memory for the objects in the spontaneous object recognition test. The first factor is the delay interval, which is the amount of time between the first exposure to the sample objects and the second exposure in the test phase. Ennaceur and Delacour (1988) found that, as the delay interval increased, rats began to show no discrimination between the novel and the sample object. For example, rats given three minutes to explore the sample objects in the first phase showed discrimination between the objects when re-tested one minute and one hour later, but not 24 hours later.

The second factor affecting discrimination between novel and sample objects is exposure time to the sample objects. The longer a subject is exposed to the sample objects in the first phase, the longer they can be remembered. Ennaceur and Delacour (1988) showed this in their study by manipulating the amount of time rats were given to explore the sample objects in the first phase. When the rats were given only 20 seconds to explore, they showed a novelty preference when tested after a delay of one minute, but not after delays of one

hour, four hours, or 24 hours. On the other hand, when rats were given three minutes to explore sample objects, they showed a novelty preference at delays of one minute and one hour, but not 24 hours. Another related aspect of exposure time is the extent of habituation to the stimulus. If a subject is not allowed enough time to familiarize and fully habituate to the sample stimulus, no novelty preference and even preference for the sample object has been seen on subsequent tests of recognition (Hunter et al., 1982; Hunter et al., 1983).

The effects for both of these factors, delay interval and exposure time, correspond to the 'trace decay hypothesis' (Roberts and Grant, 1976). This hypothesis states that the presentation of a stimulus causes changes in the nervous system, which gradually decay after the removal of the stimulus. So over time, the memory trace fades, and is no longer able to be recalled. Also, the longer a stimulus is presented for in the first exposure, the stronger the initial memory trace, and the longer that trace is able to be remembered. The memory trace also seems to be made stronger by repeated presentations of the sample stimulus on discrete occasions. Mumby et al. (2000) found that this pattern of exposure to the sample object increased rats' abilities to discriminate between novel and sample objects from a few hours to several weeks.

Besides qualities such as color, texture, shape, odor, and taste, the spatial location of an object in an environment is also an important feature used by animals to identify the object. When a sample object is moved between trials to a new location in the same environment, the object seems to regain some novelty.

In one experiment, when two objects from a set of five sample objects were moved to a new location in the test arena, rats spent more time exploring these objects than the unmoved ones (Galani et al., 1998). Other experiments with rats, as well as monkeys, also show preferential exploration towards objects moved in space (Platt and Novak, 1999).

The fact that objects can regain some novelty by changing location in space is thought to play a role in the lack of familiarity discrimination when a sample object is initially presented in one environment, and then presented in a novel environment during testing. Human subjects showed impaired discrimination between novel and sample visual stimuli when they were tested in a different room than where initial exposure to the sample stimulus took place (Richmond et al., 2004). On the other hand, rats did not show impairment in discrimination between samples and novel stimuli when they were exposed to the sample objects in two separate environments, and then tested in a third environment (Besheer and Bevins, 2000). The same results were obtained regardless of how familiar the third environment was to the rats. The authors interpreted these results to indicate that exposing subjects to the sample object in more than one location reduces the effect of differences in exposure and testing environments on memory for familiar objects.

There has been some controversy over the interpretation that failure to show a novelty preference in the test situation demonstrates a failure to recognize the sample object (Sophian, 1980). While this is the explanation put forth in many

studies using spontaneous novelty preference to test object recognition, there are possible alternative explanations. For one, the treatment imposed could have altered the natural preference and biased it towards the sample object. This result was found in rats receiving perirhinal cortex lesions, where they showed a preference for the sample object after a 15-minute delay, while control rats exhibited the usual novelty preference (Mumby et al., 2002). In another case, prolonged exposure to sensory deprivation (such as a barren environment) led to a reduction in preference for novelty or changes in stimulation over time (Inglis, 1975a, 1975b; Inglis and Freeman, 1976), which may alter performance on the spontaneous recognition task.

The familiarity of the testing environment may also alter novelty preference independently of recognition. Sheldon (1969) found that the familiarity of the testing environment impacted the preference of rats for novel or familiar stimuli. In an unfamiliar environment, rats showed a preference to explore previously encountered objects. When the environment was familiar or it became more familiar, the rats chose to explore the unfamiliar, or novel, objects.

Studies in infants have led to the conclusion that failure to show novelty preference after a delay may not be due to lack of recognition, but to the state of the memory trace for the sample object (Bahrick and Pickens, 1995; Courage and Howe, 1998). The four-phase theory proposed by Bahrick and Pickens (1995) states that over time, the memory trace for an object fades, and the subject tries to reinstate it by increasing attention to the sample stimulus. Tests at early delays

should find novelty preference, since the memory trace for the sample object will be strong. As the delay interval increases, the memory trace begins to fade to an intermediate level, and tests during this time should reveal no preference for either the novel or sample stimulus as interest in the sample stimulus is beginning to increase. During the third phase the subject tries to reinstate the failing memory trace, so exhibits familiarity preference. Finally, in the last phase at the longest delays, the memory trace for the sample object is inaccessible, so no preference is seen.

The four-phase theory has been supported by several studies. In 3-6-month old infants, experimenters observed a return to familiarity preference after long delays, with novelty preference at the shortest delays and no preference shown at intermediate delays (Courage and Howe, 1998; Rose et al., 2004). Since familiarity preference would indicate discrimination between the two stimuli presented and recognition of the sample stimulus, the result of no preference at earlier delays could not have indicated memory loss for the sample stimulus.

These results seem to suggest the fallacy of using spontaneous novelty preference to test recognition memory. However, the authors conclude that this test may still be useful if it is known in what phase(s) results are generated (Bahrick and Pickens, 1995; Courage and Howe, 1998). Another consideration is that these results have not been demonstrated in any non-human animal studies to my knowledge. This may be due to the lack of use of long-term delays, or because this theory does not explain novelty preference in animal models. Whichever the

case, clear discrimination between the stimuli, shown by novelty or familiarity preference, indicates recognition memory, while the interpretation of null results needs to be made carefully.

Spontaneous Object Recognition in Pigs

There are a few different reasons why scientists and producers might be interested in object recognition memory in the domestic pig. Knowledge of a pig's recognition abilities, including how long they can remember an object presented to them and when that object becomes novel again, as well as what factors affect this ability, would be useful for producers using a rotational environmental enrichment program with objects. Another use may be to understand how this form of memory is susceptible to disruption. Since the recognition of objects is mediated by brain regions also responsible for the recognition of the familiarity of conspecifics, disruptions in one memory type may be affected by the same factors that disrupt the other type of memory. Finally, this test may be used as one of a compliment of tests to determine overall cognitive ability in the pig, either to compare with other species or to determine the effects of treatments on learning and memory ability in general. The pig seems especially suited for studies using this test, since pigs display a preference for novelty and will explore novel objects for a longer time than highly familiar objects (Wood-Gush and Vestergaard, 1991). As my experiments were mainly concerned with

the use of the test for designing environmental enrichment programs, this topic will be described in more detail.

Application to Environmental Enrichment Programs

Much of the concern over the welfare of pigs used for commercial meat production stems from the practice of housing pigs in relatively barren indoor environments, with little ability to express behavior shown by pigs in the wild such as rooting and exploratory behavior (Wood-Gush, 1983, p. 201). It is believed that pigs are highly motivated to express these behaviors in a variable and complex environment (Mench, 1998), and limiting their ability to do so could result in negative behavioral consequences. Evidence of this has been seen in studies measuring behavior in groups of pigs housed in "enriched" versus "impoverished" environments, whereby the enriched environment could include the provision of toys (Schaefer et al., 1990), straw or peat (Beattie et al., 1996; de Jong et al., 1998; Beattie et al., 2000), or exposure to an outdoor pen (Grandin, 1989b), and the impoverished condition was usually the standard pen. In most of these studies, enriched animals showed a reduction in negative behaviors like aggression compared with the impoverished animals, although the type of enrichment stimuli used tempered the effect.

A moderate amount of the research on the effects of environmental enrichment in animals, including pigs, has focused on the introduction of novel objects or toys into the home environment. Although there has been criticism of

the use of such arbitrary objects without regard to biological relevance (Newberry, 1995), and some studies failed to find significant benefits from their use such as decreases in stereotypy or aggression or increases in species-typical behavior patterns (Pearce et al., 1989; Hill et al., 1998), the use of these items is still popular in many enrichment programs. This is most likely because solid, indestructible objects can be inexpensive to purchase and maintain, there are fewer hygiene concerns than with provision of rooting substrates such as straw and soil, and they are compatible with liquid waste management systems (Mench, 1998; Van de Weerd et al., 2003).

One large problem with using objects as environmental enrichment is how quickly animals habituate to them (Wemelsfelder and Birke, 1997). The decrease in exploration after extended exposure would limit the object's usefulness as an enrichment item if high levels of exploratory behavior were a desired outcome of the enrichment paradigm (Van de Weerd et al., 2003). Ways need to be found to reduce this effect. The 'information primacy' theory (Inglis, 1983) postulates that animals adapted to living in stochastic environments have an inherent need to gather information about changes in their environment. If this theory is correct and motivates exploratory behavior in animals, then environmental variability and continuing novelty are important aspects to consider when designing environmental enrichment (Mench, 1998). Indeed, Van de Weerd and colleagues (2003) found that novelty of objects was an important variable for initiating and maintaining exploration in pigs. So one way to increase the effectiveness of

objects as enrichment items might be to provide objects for the animals to explore and replace them frequently so that novelty is retained.

However, this strategy may require the repeated use of objects over time, unless producers were able to accumulate an endless supply of new objects to introduce. If objects are repeatedly presented, and pigs recognize them, they may not explore them to the same extent that they would completely unfamiliar objects. How often then should objects be rotated, and when can an object already seen by the pigs be placed back in the pen? Unfortunately, there is very little empirical evidence of the memory ability of pigs that might allow us to answer the question of how long a pig can remember an object, and which factors affect this memory ability.

Objectives

In collaboration with Dr. R.C. Newberry and Dr. S. Cloutier, I conducted two experiments, presented below as two manuscripts formatted for submission to Applied Animal Behaviour Science. It was the purpose of our first experiment to discover how the effects of initial exposure time and delay interval affect object recognition memory in the pig using a modified spontaneous object recognition test. We hypothesized that pig object recognition memory would be affected by exposure time and delay interval, with the specific predictions that longer exposure times would lead to recognition, as shown by novelty preference, at longer delays and that novelty preference would decrease as delay interval

increased. In our second experiment, we sought to investigate the performance of pigs in a more standardized test of spontaneous object recognition by using only one exposure time and independent groups to investigate effects of delay interval. This was done to help explain some of the confusing results from the first experiment, as well as to determine if the behavior of pigs in this task was similar to the behavior observed in rodent studies, and if this test could be used as part of an overall battery of cognition tests in the pig. We hypothesized that pigs would respond similarly to rats in this study and show a gradual decrease in novelty preference as the delay interval increased.

EXPERIMENT ONE

Effects of Object Exposure Time and Delay Interval on Object Recognition Memory of the Domestic Pig

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Abstract

A modified spontaneous object recognition test was used to examine object recognition memory in the domestic pig. This test uses preference for a novel object over a previously encountered sample object as indicating recognition of the sample object, and no preference as indicating no recognition. Two factors hypothesized to affect object recognition are exposure time to the sample stimulus and the delay interval before re-exposure. Increased exposure time should allow for recognition at longer delays, and increasing the delay interval should cause a decrease in recognition. We exposed pigs to different sample objects in their home pens for 10 minutes and 2 days, respectively. Then we tested pigs at four delay intervals after initial exposure: 1 hour, 3 hours, 5 days, and 6 days. Littermate pairs were placed in a test pen, and snout contact with a sample object and a completely novel object was recorded for 10 minutes. Half the pairs were tested with the 2-day sample at the 1-hour delay; the other half received the 10-minute sample object. For the 3-hour delay, pairs were tested with the opposite sample object. Pairs were also tested with the 2-day sample at the 5-day delay and the 10-minute sample at the 6-day delay. We predicted that pigs would show a preference for the novel versus the 2-day sample object at all three delays, but would only prefer the novel object over the 10-minute sample object at the 1- and 3-hour delays. Data were analysed using a mixed model repeated measures ANOVA, planned comparisons, and Wilcoxon signed-ranks tests to determine

preference at each delay and exposure time. Pigs did not show a novelty preference in the presence of the 10-minute sample object at any delay. A novelty preference in the presence of the 2-day sample object occurred at the 3-hour ($P < 0.05$) and 5-day delays ($P < 0.001$), but not the 1-hour delay. These results indicate that longer exposure times do enable pigs to recognize objects for longer delays, but shed doubt on the interpretation that a failure to show novelty preference is an indication of a recognition failure.

Keywords: Pig; Memory; Object Recognition; Novelty; Enrichment

1. Introduction

An important non-spatial memory ability not well described in pigs is memory for objects. Knowledge of the robustness of object recognition memory in the pig, including when and how recognition fades, would be important for producers using objects as part of an enrichment program. While the use of objects has produced inconsistent effects in the enrichment literature, and habituation to "toy" objects occurs rapidly in pigs, reducing the object's usefulness in initiating exploration (Wemelsfelder and Birke, 1997), many producers find the use of such objects necessitated by economics, hygiene, or other concerns (Mench, 1998).

Van de Weerd and colleagues (2003) showed that object novelty is an important characteristic for initiating and maintaining exploration in pigs. Novelty can either be incorporated into the design of the object (Van de Weerd et al., 2003), or can be achieved by changing objects over time, such as in a rotational system. Realistically, pig producers would not have an endless supply of new objects to use, and objects would most likely be used more than once. If pigs recognized the repeated object as familiar, they might not interact with it as they would a novel object, or they may habituate to the object much more quickly. Little is known, however, about how long a pig remembers objects it has seen, or when they become novel again. Therefore, it is the focus of this paper to address object recognition abilities in the pig.

A specific test of object recognition memory exists in the rodent, non-human primate, and human literature. This test is based on a spontaneous behaviour, whereby the subject will focus on or explore a novel object for a longer duration than a familiar object (Ennaceur and Delacour, 1988; Fagan, 1992). Since the behaviour is spontaneous, it is not necessary to train the animals to learn a rule (i.e. choose "same" or choose "not-same") before testing. This reduces the amount of time needed to obtain results.

While several studies have looked at the exploratory behaviour of pigs towards novel objects presented alone (e.g. Wood-Gush et al., 1990; Hemsworth et al., 1996), almost no studies have looked at the exploratory behaviour of pigs when both a novel and a previously seen sample object are presented together. In

a rare example of the second type of study, Wood-Gush and Vestergaard (1991) trained 5-week-old pigs to explore side pens attached to the home pen in order to find and interact with objects placed there. One side contained a highly familiar object, while the other side pen contained a novel object. The authors found that pigs showed a preference for choosing the side pen containing the novel object first, and explored the novel objects for a longer period of time than a highly familiar object. From this study it seems that pigs can exhibit a spontaneous preference to explore novel objects over more familiar objects. Therefore, it should be possible to modify the object recognition test developed for rodents to test pig object recognition memory.

In the rodent studies, two main factors affect recognition of a previously encountered sample object in a memory test. The first is the initial amount of time a subject is exposed to the object. The second is the delay between the first exposure and the second exposure. As initial exposure time increases, a subject is able to remember an object for longer delays. Also, as the delay interval increases, subjects show decreased recognition of the sample object, regardless of exposure time. Both of these factors could be manipulated in an enrichment program for swine facilities. Shorter exposure times and longer delays between reintroduction of the same objects should increase an object's novelty at reintroduction, and therefore an object's effectiveness at stimulating exploration.

To better understand the effect of exposure time and delay intervals on pig object recognition memory, we exposed pigs to sample objects for a short

exposure time (10 min) and a long exposure time (2 d), and then tested them at different delay intervals (1 h, 3 h, 5 d, 6 d). Their response to a novel object and a copy of a sample object was recorded in the test phase. We predicted that pigs would show a preference for the novel object over the sample object (indicating recognition of the sample object) at short delays (1 h and 3 h) for both exposure times, but only for the long-exposure time (2 d) object at longer delays (5 d and 6 d).

2. Methods

2.1 Subjects, Housing and Management

Subjects were 36 crossbred domestic piglets (sows of Large White x Yorkshire x Landrace lineage, boars of Duroc x Hampshire lineage) from 9 different litters housed at the Washington State University Swine Center. These litters were recruited from three different farrowing windows over the course of one year, forming three batches of test litters. After farrowing, sows and litters were kept in fully slatted farrowing crates (2.1 m long x 1.8 m wide). According to standard practices in the United States, piglets were weighed at birth, and had their teeth clipped, tails docked, and ears notched on day 1. Males were castrated at 7 days of age. Litters were weighed and weaned at approximately 21 days of age, and four randomly selected piglets per litter, two males and two females, were

moved to fully slatted nursery pens (1.5 x 2.4 m), where they were housed as a litter group. Standard starter food (19.4% crude protein, 1.42% lysine, 3405 kcal DE/kg) and water were available *ad libitum*. The temperature in the nursery room averaged 26 ± 5 ºC. The average light intensity from the fluorescent bulbs was 416 lux and the photoperiod was 8L:16D, with lights on at 6 am. At the end of the experiment, all pigs were weighed (approx. 42 days of age), and remained in the herd until they reached market weight.

2.2 Test Pens

Initial presentation of the sample objects occurred in the home pen of the pigs. Tests of recognition were conducted in one of two test pens (1.5 x 1.7m) made of plywood, painted black, with metal joiners in the corners. Test pens were located in empty pens in the same room as the nursery pens. Two black rubber mats formed the floor of the test pen, and the mats were covered with a layer of fresh straw. One wall of each test pen was slanted outwards to allow for a clear view of the entire pen by a video camera mounted to a tripod and suspended from the ceiling directly above the slanted wall. The camera was connected to a video-recorder and monitor housed in a moveable wooden cart, which was placed to the side of the test pen. The pigs were extensively habituated to the test pens prior to testing.

2.3 Object Recognition Memory Tests

The experiment consisted of two phases, an initial presentation phase, where pigs were exposed to sample objects for the first time, and a recognition test phase, where pigs were re-exposed to the sample objects along with a completely unfamiliar object. The initial presentation phase began when pigs were approximately 35 days old. Two identical sample objects were secured to the walls of the nursery home pen of each litter with plastic electrician's ties. Within each batch of litters, each litter received a different type of object. The objects were left in the home pen for approximately 45 hours (2 days), and became the long exposure time sample objects, designated LE. Two additional identical sample objects, of a different type than the first two objects, were secured in the home pens for the last ten minutes of the 45-hour period. These were labeled the short exposure time sample objects (SE). At the end of the 45-hour period, all four objects were removed. Objects used during initial presentation and testing are listed in Table 1.1.

Long Exposure Samples (LE)	Short Exposure Samples (SE)	Novel
Garden hose, Cotton rope, Red juice bottle, Orange juice bottle, Strip of tire inner tube	Pink plastic utensils, Wooden spoons with colored tops, Tiki Punch soda can, Plastic grocery bag	Newspaper roll, Cardboard paper towel roll, Garden hose, Cotton rope, Plastic grocery bag, White dishcloth, Pink plastic utensils, Squirt soda box, Safeway Apple Cider packets cardboard box, Squirt soda can, Hefty EZ foil pie tin

Table 1.1: Objects used as long exposure (placed in pen for 2 days), short exposure (placed in pen for 10 minutes), and novel objects in Experiment 1.

The first recognition test (Delay 1) began one hour after the removal of all four sample objects from the home pen. A duplicate of one of the sample objects, either LE or SE, was attached securely to one side of the test pen with an electrician's tie looped through two holes in the pen's side and around the object. A completely novel object was attached similarly to the opposite side of the pen. All four pigs from a litter were tested simultaneously in one of the two test pens in the following way: one male and one female from the litter were marked with wax stock markers with their respective identification numbers, and placed simultaneously in a test pen, opposite each other, with their heads pointed toward the middle of the sides of the pen without objects. One test pen was set up with LE, while the other was set up with SE. Which side the novel and sample objects were on, and which test pen received LE versus SE in Delay 1, was counterbalanced across test pens and litters. Once pigs were placed into the pens,

video recording was started, and experimenters left the room. Test sessions ran for 10 minutes. At the conclusion of the test session, the video-recorders were stopped, pigs were placed back in their home pens, and new sample and novel objects were secured in place of the ones just used. Then a new litter was tested in an identical fashion to the first litter in a predetermined random order, until all litters within the batch had been recorded. The timing of the tests was staggered for the different litters within a batch to maintain a 1-hour delay for all pairs.

Delay 3 tests were carried out after a delay of two hours from the time the first test ended (i.e. 3 hours after last seeing the sample objects in their home pen). The same pairs of pigs were tested in the same sequential order, following the same procedure as described above. At this delay, pairs of pigs that had received LE in the first delay received a duplicate of SE, and vice versa.

Delay 5 tests were conducted five days after the Delay 1 and 3 tests. The procedure on this day was essentially the same except only LE and a novel object were used for all pairs.

A subset of pigs (n=16) was also tested on the sixth day (Delay 6) after initial exposure to the sample objects. During this test, all pigs received a copy of the SE and a novel object in the test pen, and were tested according to the procedure described above.

During all tests, clean gloves were used to handle objects when placing them in the test pens to avoid contamination with pig and human odours. Duplicates of the sample objects were used for each litter at each delay interval

rather than the original objects so that relative attraction to the sample and novel objects was not confounded by pig odours deposited on the sample objects in the home pen or during tests at earlier delays. A range of different object types were used as the LE, SE and novel objects so that the reaction to novelty could be tested rather than the reaction to a specific object type, since some object types might naturally be preferred over other object types.

2.4 Video Analysis

Videotapes of each pig-pair test were analysed to determine the duration of exploration of the sample and novel objects. The ten minutes of each video session were analysed in real time using a hand-held Psion (Psion PLC Inc., UK) data logger. The behaviour of each pig in a pair was analysed separately, and the amount of contact with each object recorded to the nearest tenth of a second. Contact (i.e. object exploration) was defined as touching the object with any part of the snout in front of the eyes, and contact was ended when the pig turned away and took two steps, or 5 seconds had passed since the last contact, as the bout criterion interval was calculated using a log survivorship curve to be 5 seconds. All behaviour data presented as results were collected by the same observer (AKG). Intra-observer reliability was calculated to be 95.1%.

2.5 Statistical Analysis

Data were compiled for all pigs concerning birth weight, weaning weight, and growth rate from weaning to the end of the experiment. Behaviour towards the objects in the tests was averaged for pairs of pigs. Several variables were calculated from measures of duration of contact with the novel and sample object in the test pen as described in Table 1.2.

Variable	Description
D1	(Duration of contact with novel object) – (Duration of contact with sample object in test phase)
S2	(Duration of contact with novel object) + (Duration of contact with sample object in test phase)
D2	D1/S2, to control for differences in total exploratory activity
A1	(Duration of contact with object on left side of pen) – (Duration of contact with object on right side of pen)
B1	(Duration of contact with object nearest entry door into room) – (Duration of contact with object nearest back of room)

Table 1.2: Variables calculated from contact with the novel and sample objects in the test phase.

The variables D1 and D2 are measures of discrimination between the two objects, as commonly reported in the rodent literature. S2 is the total amount of exploration of the two objects in the test phase. Since the two test pens were on opposite sides of the nursery room, two separate measures of location preference were calculated. Side preference within the pen was described by A1, and room orientation bias by B1.

For each exposure time, preference for either the novel or sample object at each delay was tested using Wilcoxon signed-ranks tests for the variable D1. Preference for one side of the pen or one side of the room over the other was also tested using this method for the variables A1 and B1.

The effects of delay interval and order of testing with the long and short-exposure time sample objects were investigated with a mixed model repeated measures analysis of variance (SAS Institute, 1996). Order 1 pairs received the short exposure time sample object in Delay 1 and the long exposure time sample object in Delay 3, and vice versa for Order 2 pairs. Order was the main factor in the model, delay was the repeated measure, litter was a random effect and the subject effect was pair within litter. Because the residuals for the variable D1 were not normally distributed, we applied the mixed linear model to log 10 transformed data for this variable although graphs and tables depict untransformed data. Means comparisons between delays and orders were made based on differences in least-squares means, with p-values adjusted for multiple comparisons using the Tukey option in Proc Mixed.

A priori planned contrasts were conducted to examine differences between the groups receiving the long- or short-exposure time sample objects at the 1-hour and 3-hour delays, as well as changes over delays for each sample object exposure time.

3. Results

Piglets had an average birth weight of 1.84 ± 0.06 kg, an average weaning weight of 6.88 ± 0.17 kg and a growth rate from weaning to the end of the experiment (approximately 42 days of age) of 0.28 ± 0.01 kg/d.

Wilcoxon signed-ranks tests revealed no preference for the novel or sample object at the 1-hour delay in pairs tested with either the long exposure time (2 days) or the short exposure time (10 minutes) sample object. At the 3-hour delay, only pairs tested with the long exposure time sample object showed a significant preference for the novel object (S=18.5, N=9, P=0.03). Pairs tested at the 5-day delay showed a preference for the novel object over the long exposure time sample object (S=60, N=16, P=0.0008), while pairs displayed no preference for either the novel or the short exposure time sample object at the 6-day delay (Figure 1.1). No side bias or room orientation bias was found for any delay or exposure time.

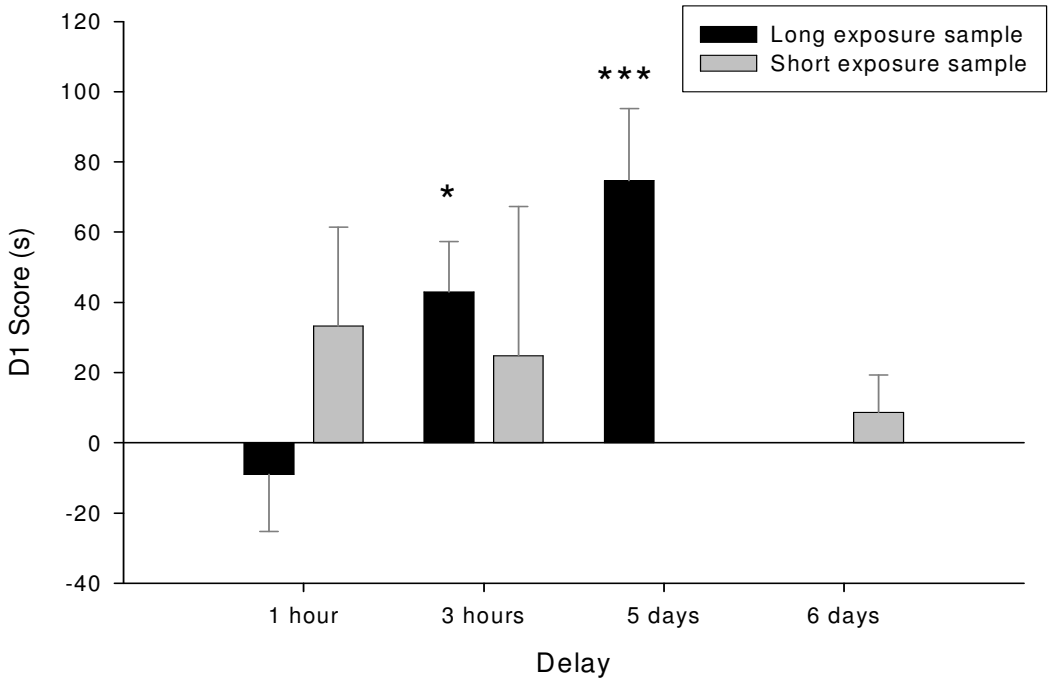

Figure 1.1: Wilcoxon signed-ranks test results for the discrimination score, D1 (mean ± S.E.), at each delay for each sample object exposure time. Values above zero indicate greater time spent with the novel object; below zero, more time spent with the sample object. Asterisks indicate the difference in exploration of the novel and sample objects is significantly greater than zero. (* $P<0.05$, *** $P<0.001$)

No significant main effects of Order or Delay were found for the discrimination variable D1 using the mixed model analysis of variance, although Delay was almost significant ($F_{3,44} = 2.65$; $P = 0.06$). Delay was significant for the second discrimination variable, D2 ($F_{3,44} = 2.98$; $P = 0.04$). D2 scores in Delay 1, regardless of Order (or sample object exposure time) were lower than D2 scores in Delay 5. More specifically, planned comparisons revealed that D1 scores for pairs tested with the long exposure sample object in Delay 1 were significantly

less than the same pairs' D1 scores with the same sample object in Delay 5 (t_{44} = -3.12; P = 0.002). This same response was seen when comparing D2 scores from pairs tested with the long exposure sample object at Delay 1 and Delay 5 (t_{44} = -3.53; P = 0.001). No significant results were found for the variables A1 or B1. The means and standard errors for all the variables calculated are in Table 1.3, separated by exposure time and delay.

	Long exposure sample object			Short exposure sample object		
	Delay			Delay		
Variable	1 hour	3 hours	5 days	1 hour	3 hours	6 days
D1	-9.0 ± 16.21	43.0 ± 14.33	74.7 ± 20.53	33.3 ± 28.15	24.8 ± 42.55	8.7 ± 10.57
D2	-0.2 ± 0.20	0.4 ± 0.10	0.5 ± 0.09	0.2 ± 0.20	0.1 ± 0.20	0.1 ± 0.14
S2	70.2 ± 15.57	120.3 ± 12.10	139.2 ± 18.38	154.5 ± 26.98	165.7 ± 36.16	115.8 ± 24.50
A1	-3.8 ± 16.47	-1.8 ± 20.88	-3.7 ± 28.15	-44.2 ± 26.20	52.6 ± 39.26	-9.0 ± 10.54
B1	11.8 ± 15.99	21.2 ± 19.49	36.8 ± 26.51	-17.2 ± 29.90	10.0 ± 43.30	-11.6 ± 10.17

Table 1.3: Effects of delay and sample object exposure time on measures of interaction with the novel and sample objects in the test phase (mean ± S.E.).

No main effects were found for the total exploration time in the test phase (S2), but there was a significant Order by Delay interaction for total exploration time in the test phase ($F_{3,44}$ = 3.26; P = 0.03). Pairs of pigs tested with the long exposure sample object in Delay 1 (Order 2) explored the novel and sample objects for less time than pairs tested with the short exposure object in Delay 1 (Order 1; t_{44} = -2.49; P = 0.02), and for less time than when tested with the short

exposure object in Delay 3 (t_{44} = -2.82; P = 0.01) and the long exposure object in Delay 5 (t_{44} = -2.48; P = 0.02; Figure 1.2).

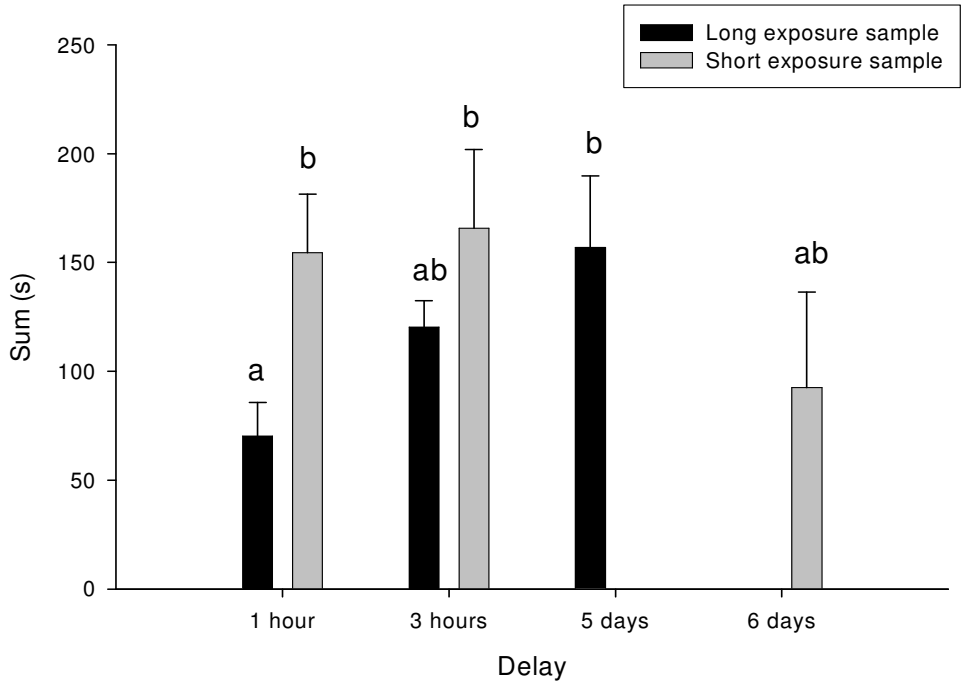

Figure 1.2: Effects of delay and sample object exposure time on mean ± S.E. total time spent exploring the novel and sample objects in the test phase. Differences in least squares means between exposure times and across delays are denoted by different letters (P<0.05).

To visualize the behaviour of the pairs towards the objects in the test phase, duration of contact with the novel and sample objects in each minute of the 10-minute test session was plotted for each delay and sample object exposure time (Figure 1.3).

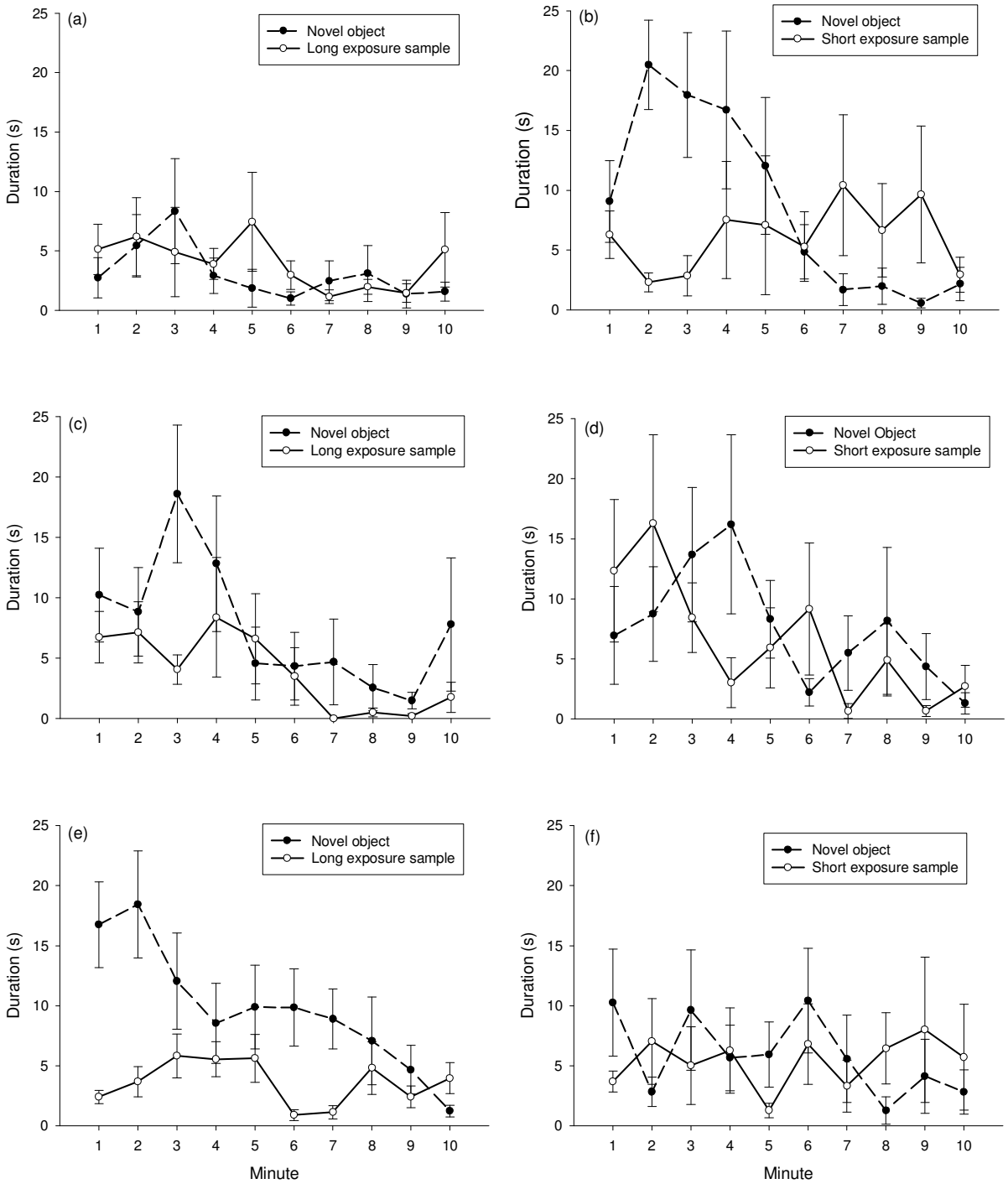

Figure 1.3: Mean ± S.E. total duration of contact with the novel (dashed line) and sample (solid line) objects for each minute during the 10-minute test session, separated by delay. Delay 1 – (a) and (b); Delay 3 – (c) and (d); Delay 5 – (e); Delay 6 – (f).

4. Discussion

Our results show that pigs failed to display a novelty preference when tested with the short exposure sample object at any delay interval. One possible reason for this result might be because a 10-minute exposure time was not enough time for pigs to habituate and become familiar with the object. Studies of object recognition using novelty preference in rodents and infants have generally used exposure times of a few minutes, with reliable novelty or familiarity preferences at delays up to 24 hours in rodents (Ennaceur and Delacour, 1988; Mitchell and Laiacona, 1998) and 3 months in infants (Courage and Howe, 1998). However, in these studies, subjects were tested alone. Pigs in this experiment received the sample objects in their home pen with their littermates, which may have affected how much access individuals had to the objects. Since we did not record pigs' behaviour towards the sample objects in the home pen, it is impossible to say how much of the 10-minute exposure time the pigs spent in contact with the objects. Some pigs may have spent the whole time in contact, while others may have spent only seconds. Measurement of initial contact would be necessary to know if 10 minutes was sufficient for pigs to habituate to the sample objects.

However, by looking at the pigs' responses to the novel object over the 10 minutes of the test phase, it may be possible to ascertain if this response decreases over time, indicating habituation. At the 1-hour delay, when pigs were tested for the first time, duration of contact with the novel object seems to show a pattern of

initial increase over the first few minutes, a peak, and then a decrease in the last minutes of the test. This is especially clear for the pairs tested with the short exposure sample object (Figure 1.3). Wood-Gush and Vestergaard (1991) found a similar decrease in exploration of the novel object by pigs over a 5-minute test period. Although the pattern is not as clear for all the delays and sample object exposure times, and it is not possible to draw concrete conclusions from these data, the patterns suggest that pigs could have habituated to the sample objects in the 10 minute exposure time.

While pigs failed to indicate recognition of the short exposure sample object at any delay by a novelty preference, they did show a significant preference for the novel object when tested using the long exposure sample object at the 3-hour and 5-day delays, indicating recognition of the sample object. These results fit our prediction that pigs would be able to recognize a sample object, previously experienced for 2 days, after long delays. However, pigs showed no preference for the novel object over the long exposure sample object at the shortest delay interval, 1 hour. In most of the rodent, non-human primate, and human infant literature, this null result would be interpreted as a failure to recognize the sample object. This interpretation seems implausible for this experiment due to the success of the pigs in recognizing the same long exposure sample object at later delays.

One possible reason why pigs might have failed to show a novelty preference at the 1-hour delay, but clearly preferred the novel object at the 3-hour

and 5-day delay when tested with the long exposure sample object, is the discrepancy between the exposure environment and the testing environment. Human adults exposed to visual stimuli in one environment and tested in another showed impaired performance on a visual recognition task (Richmond et al., 2004). Also, relocating a familiar object to a novel location causes it to regain some novelty (Galani et al., 1998; Platt and Novak, 1999). Pigs in our experiment were exposed to the sample objects in their home pens, and tested in a different test environment, which could have impaired performance at the 1-hour delay. Since pigs were re-exposed to the sample object during the test phase in the test environment at the 1-hour delay, this may have prevented impairment at later delays. On the other hand, pigs receiving the long exposure sample object at the 3-hour delay had not experienced this sample object in the test environment before, because they were tested with the short exposure sample object at the 1-hour delay; yet they showed a novelty preference.

Another explanation for lack of novelty preference over the long exposure sample object at the 1-hour delay could be retroactive interference, due to the presentation of the short exposure sample object in the home pen 1 hour prior to testing. Due to the similar nature of the stimuli, pigs may have been impaired in their retrieval of the information about the long exposure sample object in the test situation. In tests of spatial memory, pigs were susceptible to retroactive interference when the interfering stimulus was similar to the task stimulus (Laughlin et al., 1999). Also, in a test of short-term social memory in rats,

disturbances during the delay interval, including the introduction of a new juvenile rat, disrupted recognition of the original juvenile stimulus rat (Burman and Mendl, 2000). As the effects of retroactive interference can be transient and fade with time (Fagan, 1977), this may explain why pigs were able to show a novelty preference at the 3-hour and 5-day delays.

It is interesting to note that pigs receiving the long exposure sample object at the 1-hour delay explored the objects for significantly less time than their counterparts receiving the short exposure sample object at the same delay. They also explored less at the 1-hour delay than they did when tested at later delays of 3 hours and 5 days. It is possible that this reduction in exploratory behaviour is related to the lack of discrimination found in the first delay but not later delays with the long exposure sample object. Why pigs decreased their response in this delay and no others is unclear.

In conclusion, our hypothesis that increasing exposure time would result in novelty preference at longer delays was supported. We found that pigs preferred a novel object over a sample object that they had previously encountered over a two-day period, at delays up to 5 days; but not one they had previously encountered for only 10 minutes, even when tested only one hour later. This implies that rotational programs of environmental enrichment using objects should be most effective when the exposure time to the objects is less than 2 days and the delay before re-exposure is greater than 1 week, although we were unable to

determine from our experiment when recognition of the long exposure sample object would have faded.

Our second hypothesis, that we should see decreasing novelty preference over increasing delays, was unsupported by our results. The lack of a novelty preference at the earliest delay with the 2-day sample object sheds doubt on the interpretation that the null results found for the 10-minute sample object indicated a failure to recognize the object. However, with regard to the implications for an environmental enrichment system, our results still indicate that shorter exposure times should be sought, because whether or not the pigs recognized the sample object, they still explored it to the same extent as a novel one.

EXPERIMENT TWO

The Use of Spontaneous Novelty Preference to Test Object Recognition Memory in the Domestic Pig

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Abstract

In a previous experiment, we used a spontaneous object recognition task to study the effects of initial object exposure time and delay interval on subsequent preference of domestic pigs for a novel object versus a previously encountered object. However, the results were inconsistent and hard to interpret. For this experiment, we attempted to match our methodology as closely as possible to that described for studies of rodent object recognition memory. To this end, we exposed pairs of pigs to sample objects in a test pen for 5 minutes, then divided the pairs into three delay interval treatments. These groups were tested 1 hour, 24 hours, or 5 days after initial exposure to the sample object, with a copy of the sample object and a novel object. We predicted a decline in novelty preference as the delay interval increased, with novelty preference indicating recognition of the sample object, and no preference a failure in recognition. Two trials were conducted, differing in habituation technique and location of the objects in the pen. We analysed pair data for the total duration of interaction with the objects in the sample and test phases, as well as indices of discrimination and location preference, with trial and delay as fixed effects. Wilcoxon signed-Ranks tests were carried out on the difference scores to determine if pigs preferred the novel to the sample object. We found no significant effects of trial or delay on discrimination score, and no preference for either object at any delay. These

results may indicate that the spontaneous object recognition test is unsuitable for testing memory for objects in young pigs, although efforts to reduce or account for individual variability in object response should be made before dismissing this test.

Keywords: Pig; Memory; Novelty Preference; Object Recognition

1. Introduction

Interest in the cognitive abilities of farm animals has become more prominent in recent years. Fueling this interest is the belief that only by understanding an animal's cognitive capabilities and limitations can we make objective judgments about an animal's potential to "suffer" (Nicol, 1996). Also, studies of cognitive abilities may help to facilitate learning in the production environment, or discover how susceptible learning and memory are to disruption from common husbandry practices (Mendl et al., 1997).

Pigs are excellent subjects for cognitive studies. They are adapted to living in variable and complex environments, and show great behavioural diversity when observed in the wild or a semi-natural environment (Newberry and Wood-Gush, 1988). They also form complex stable social relationships with group members (Graves, 1984), which may necessitate the use of individual recognition (Ewbank et al., 1974; Gheusi et al., 1997).

Memory is one type of cognitive ability that has not been extensively tested in pigs. Although several studies have looked at spatial memory in pigs (e.g. Mendl et al., 1997; Laughlin et al., 1999), not many studies have looked at non-spatial memory abilities like recognition. Object recognition is one form of recognition memory. How long a pig can remember an object seen previously and what factors affect this memory might be important to know when providing objects as environmental enrichment. Also, measuring this ability in pigs may be used as part of an overall analysis of the effects of environmental and pharmacological manipulations on cognitive capability.

Measuring an animal's behaviour towards a novel and a previously encountered object is one way to assess object recognition memory. Preference for the novel object serves to indicate recognition of the previously encountered object, although preference for the previously encountered object would also indicate recognition. In studies with rodents and non-human primates, it has been found that, as the memory load is increased by increasing the delay between exposure and testing, novelty preference decreases (Ennaceur and Delacour, 1988; Platt and Novak, 1999).

In a previous experiment (Gifford et al., Experiment 1), we found that the response of pairs of pigs to a novel object simultaneously presented with a previously encountered sample object was not consistent over delay intervals. In other words, pigs performed differently on this object recognition task than rats and non-human primates. One possible explanation for this difference could be

the difference in methods used in our previous experiment, as compared with the standard test of object recognition found in rodent studies (Ennaceur and Delacour, 1988).

In the rodent spontaneous object recognition test, individuals are tested at only one delay interval, and all groups receive exposure to only one sample object, in the test arena. In contrast, we exposed all groups of pigs to two different sample objects for different exposure times in their home pen, then tested all groups at three to four separate delay intervals in a test pen. Exposing subjects to sample stimuli in one environment and then testing in another has been found to impair visual discrimination in human adults (Richmond et al., 2004). Also, the order the pairs were tested with the two sample objects varied.

The purpose of this experiment was to examine if pigs respond similarly to rodents in a spontaneous object recognition test at short delays, and to obtain a baseline measure of object recognition abilities in the pig. We conducted two trials, with the second trial differing in the habituation procedure used and the location of the objects during testing. After familiarization with the test pen, all pigs were exposed in pairs in the test pen to two copies of a sample object for a duration of 5 minutes, then pairs of pigs were assigned to one of three experimental delays: 1 hour, 24 hours, and 5 days. After their respective delays, pairs were tested in the test pen with a copy of the sample object and a novel object, and all snout contact with each object was recorded for 5 minutes. If pigs behave similarly to rats on this task, we hypothesized that, as the delay interval

increases following initial exploration of the sample object, preference for the novel stimulus over the sample object should decline until no preference for the novel or sample object is shown. Specifically, we predicted a novelty preference at the earliest delay, 1 hour, and possibly at 24 hours, but no preference for either object at the 5-day delay.

2. Methods

2.1 Subjects, Housing and Management - Trial 1

Subjects were crossbred domestic pigs (sows of Large White x Yorkshire x Landrace lineage, boars of Duroc x Hampshire lineage) housed at the Washington State University Swine Center. After farrowing, sows and litters were kept in fully slatted farrowing crates (2.1 m long x 1.8 m wide). According to standard practices in the United States, piglets were weighed at birth, and had their teeth clipped, tails docked, and ears notched on day 1. Males were castrated at 7 days of age. Litters were weighed and weaned at approximately 21 days of age, and sorted by weight and sex before being placed into nursery pens (3 x 4.8 m). On Day 4 after weaning, 64 pigs were moved into one of four pens of 18 pigs in a separate nursery room. Two of the pens housed male pigs, while the other two housed females. In the nursery room, the average light intensity from the

fluorescent bulbs was 416 lux, and the photoperiod was 8L:16D, with lights on at 6 am. All pigs received a standard starter diet (19.4% crude protein, 1.42% lysine, 3405 kcal DE/kg) and water *ad libitum*. We assigned pigs in each pen into same-sex littermate pairs, and randomly chose 6 pairs of pigs per pen for study (n=24 pairs). Pairs were divided into three treatment groups as follows: Delay 1 hour (n=8), Delay 24 hours (n=8), Delay 5 days (n=8). Within each delay treatment, half of the pairs were female and the other half male. No litter appeared more than once in any delay treatment. All pigs were weighed at the end of the experiment (approx. 32 days of age) and remained with the herd until reaching market weight.

2.2 Test Pens and Habituation Procedure – Trial 1

All tests of recognition were conducted in one of two test pens (1.5 x 1.7m) made of plywood, painted black, with metal joiners in the corners. Test pens were located in empty pens in the same room as the nursery pens. The floors of the pens were the fully slatted plastic floors found in the home nursery pens. One wall of each test pen was slanted outwards to allow for a clear view of the entire pen by a video camera mounted to a tripod and suspended from the ceiling directly above the slanted wall. The camera was connected to a video-recorder and monitor housed in a moveable wooden cart placed to the side of the test pen.

When pigs were approximately 26 days of age, they were habituated to the test pens in the following way: 4 pairs of pigs from the same nursery pen were

placed in the test pen where three of the pairs would later be tested, while another 4 pairs from the same nursery pen were placed in the other test pen. They were allowed to explore the test pen for 10 minutes, then were returned to their nursery pen. A pair of blue work coveralls was hung over one wall of the test pen to encourage interaction with objects while in this environment. The same procedure was repeated for the other three nursery pens. Once pigs from all nursery pens had been run through, we placed one pair at a time into the test pens and allowed a second exploration of the test pen for 5 minutes. Pairs were placed back in the nursery pen at the end of the 5 minutes.

2.3 Object Recognition Memory Test – Trial 1

On Day 6 after weaning, when pigs were about 27 days of age, we began the object recognition test. In the initial sample phase, a pair of pigs was placed in the test pen with two identical copies of a sample object attached to the sides of the test pen, one on each side. Both test pens were used simultaneously. For this experiment, we used four different sample objects: a black rubber hose, a white cotton rope, a clear vinyl hose, and a brown manila rope balanced across delay treatments and sex of pairs. All objects were 1.75 cm in diameter and 30.5 cm long. Pairs were placed in the test pens simultaneously, with one pig facing the front wall and the other facing the back wall. Then the video recorders were started and the experimenters left the room. Pairs were allowed to explore the pen

for 5 minutes before they were placed back in their home pen. Sample objects were removed, and new sample objects attached with plastic electrician's ties. Then another set of pairs was placed into the test pens and the procedure was repeated. A pre-determined order was used so that the time that pairs from the different treatment groups were placed in the test pens for the sample phase was balanced across time of day.

The test phase occurred after the required amount of time had passed for each delay treatment group. The three treatment groups were tested at delays of 1 hour, 24 hours, or 5 days after initial exposure to the sample objects, respectively. The procedure used in the testing phase was identical for all treatment groups. Pairs were again placed into the test pens facing opposite walls of the pen. On one side, a copy of the sample object was attached. On the other side, a different sample object unfamiliar to the pair was attached. Pairs were allowed 5 minutes to explore the objects and the test was recorded on videotape before pigs were removed to their home pens.

During both the sample and test phases, clean gloves were used to handle objects when placing them in the test pens to avoid contamination of objects with human and pig odours. For each pair, a duplicate sample object was used in the test phase rather than one of the original samples so that relative attraction to the sample and novel objects was not confounded by pig odours deposited on the sample objects in the sample phase. Average room temperature during the sample and test phases in Trial 1 was 20 ± 1°C.

2.4 Trial 2 Procedures

All procedures used in the second trial were identical to the first trial, except in the following areas: immediately after weaning, 64 pigs were sorted by weight and sex and placed into one of four nursery pens in groups of 18 pigs, instead of four days after weaning; habituation occurred over 2 days, starting on Day 4 after weaning, and included two 5-minute habituation sessions using individual pairs on the first day, and one 5-minute habituation session with individual pairs on the second day; instead of coveralls, two 1-gallon plastic orange juice bottles were attached to the front of the test pen during habituation, approximately 1 m apart and at snout height. Objects were attached in these positions on the front of the pen, instead of the sides, for both the sample phase and the test phase. These measures were changed for this trial to try to reduce anxiety in the testing environment, and to increase the likelihood of pigs noticing that two different objects were present in the pen, facilitating comparison. Average room temperature during the sample and test phases of Trial 2 was 24 ± 2°C.

2.5 Video Analysis

Videotapes of each pair during the sample and test phases were analysed by a single observer (AKG) to determine the duration of exploration of the two sample objects in the sample phase, and the sample and novel objects in the test phase. Each video session was analysed in real time using a hand-held Psion (Psion PLC Inc., UK) data logger. The behaviour of each pig in a pair was analysed separately. Contact (i.e. object interaction) was defined as touching the object with any part of the snout in front of the eyes, and the bout criterion interval was set at 5 seconds as established by Gifford et al. (Experiment 1).

2.6 Statistical Analysis

In both trials, pairs of pigs that failed to explore any object in either the sample or the test phase were removed from the analysis. For trial 1, the remaining pairs were distributed as follows: Delay 1 (n=7), Delay 24 (n=7), Delay 5 (n=6). For trial 2, the pairs were as follows: Delay 1 (n=5), Delay 24 (n=3), Delay 5 (n=6).

Data were compiled for all pigs concerning birth weight, weaning weight, and growth rate from weaning to the end of the experiment. Behaviour towards the objects in the tests was averaged for pairs of pigs. From each pairs' behavioural data, several variables were calculated from measurements of contact

with the sample objects in the sample phase and contact with the novel and sample objects in the test phase. These are described in Table 2.1.

Variable	Description
S1	Sum of contact with both sample objects in sample phase
D1	(Total duration of contact with novel object) – (Total duration of contact with sample object in test phase)
S2	Sum of contact with the novel and sample objects in test phase
D2	(D1/S2) - to control for differences in overall exploratory activity
A1	(Contact with sample object on left side of pen) – (Contact with sample object on right side) – to assess possible side bias in sample phase
A2	(Contact with object on left side of pen) – (Contact with object on right side) – to assess possible side bias in the test phase
B1	(Contact with object nearest entry door to room) – (Contact with object nearest back of room) – to assess possible room orientation bias in the sample phase
B2	(Contact with object nearest entry door to room) – (Contact with object nearest back of room) – to assess possible room orientation bias in the test phase

Table 2.1: Variables calculated from contact with the two sample objects in the sample phase and the novel and sample objects in the test phase in Experiment 2.

Both D1 and D2 are measures of discrimination between the novel and sample objects, as commonly reported in the rodent literature. S1 and S2 were calculated to determine if there were treatment differences in total exploration time in either phase. Since the two test pens were on opposite sides of the room, two different measures of location preference were measured: A1 and A2 measured a preference for the object on the left versus the right side of the test pens in each phase, respectively, while B1 and B2 measured a preference for the

object on the side of the test pen closest to the door of the nursery room versus the side closest to the back of the room.

To test the hypothesis that preference for the novel object would decline over time, differences between the delay treatment groups for the variables D1 and D2 were compared using the General Linear Model (Proc GLM) option in SAS (SAS Institute, 1996). Delay and trial were the main factors in the model, with pair as the unit of replication. The other variables were also tested for delay and trial effects using the GLM. Wilcoxon signed-ranks tests were conducted for the variables D1, A1, A2, B1, and B2 to determine if these variables were significantly different than zero for each delay treatment group. This test is more sensitive than the paired Student's t-test for small numbers (SAS Institute, 1996). Values of D1 and D2 differing from zero would indicate a preference for the novel or sample object, while values of A1, A2, B1, or B2 differing from zero would indicate a side or room orientation bias.

Finally, to examine whether initial birth weight or the time spent exploring the sample objects in the sample phase (S1) was correlated with subsequent performance on the object recognition task (D1 and D2), a Pearson's product moment correlation was conducted on data from the individual pigs and the averaged pairs.

3. Results

There were no significant main or interaction effects of delay and trial on any of the variables tested (Table 2.2). Wilcoxon signed-ranks tests revealed no significant side bias or room orientation bias in either the sample phase or the test phase for any delay, although room orientation bias in the sample phase (B1) was approaching significance for pairs in the 24-hour delay treatment group (S=-18.5, N=10, P=0.065). During the test phase, there was no preference shown for either the novel or the sample object at any delay (Figure 2.1).

	Delay			Probability		
Variable	1	24	5	Delay	Trial	Delay x Trial
D1	5.14 ± 10.54	25.44 ± 12.37	2.74 ± 6.78	0.32	0.29	0.74
D2	0.14 ± 0.16	0.13 ± 0.20	-0.09 ± 0.13	0.57	0.55	0.56
S1	57.85 ± 15.52	77.94 ± 19.25	50.13 ± 10.62	0.40	0.11	0.80
S2	54.45 ± 10.76	68.06 ± 11.51	46.30 ± 6.30	0.35	0.51	0.21
A1	-4.70 ± 17.00	20.49 ± 12.39	7.55 ± 9.69	0.54	0.32	0.98
A2	-2.15 ± 10.63	11.53 ± 14.50	-3.68 ± 6.74	0.66	0.35	0.88
B1	3.51 ± 17.03	-24.57 ± 11.54	-10.67 ± 9.42	0.39	0.20	0.80
B2	-11.85 ± 10.03	7.85 ± 14.77	7.73 ± 6.42	0.30	0.11	0.48

Table 2.2: Effect of delay between initial exposure to sample objects and re-exposure in test phase on variables calculated for Experiment 2. Data averaged for pairs of pigs from both trials (means ± S.E.).

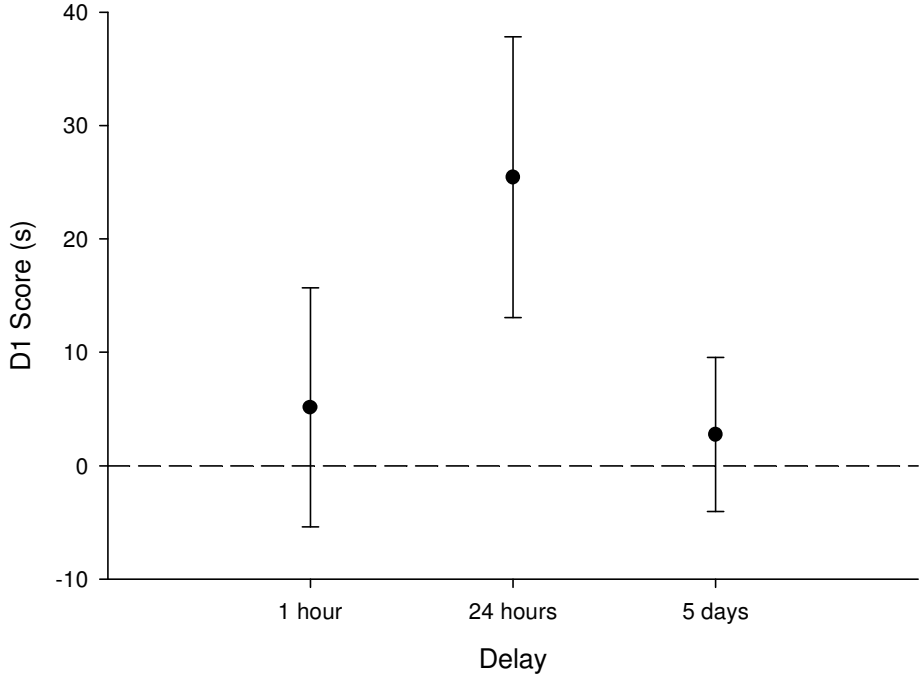

Figure 2.1: Mean ± S.E. discrimination scores (D1) for each delay interval. Values greater than zero indicate that more time was spent with the novel object. Wilcoxon signed-ranks tests failed to show that any D1 score was significantly greater than zero (P>0.05).

Birth weight averaged 1.62 ± 0.03 kg, weaning weight averaged 7.04 ± 0.12 kg, and growth rate from weaning to the end of the experiment (at approx. 32 days of age) averaged 0.19 ± 0.01 kg/d for pigs tested in Trials 1 and 2. There was no correlation found between birth weight and D1 or D2 score (measures of discrimination in the test phase), nor was there a correlation between total exploration time of the sample objects during the sample phase (S1) and D1 or D2 score (P>0.05).

To examine the behaviour of the pairs of pigs towards the objects over time during the test phase, we plotted the duration of contact with the novel and sample objects for each minute of the 5-minute test, separated by delay (Figure 2.2). We also plotted the total amount of exploration of both sample objects in the sample phase (Figure 2.3), and the novel and sample objects in the test phase (Figure 2.4), by minute and delay, and the average over all delays.

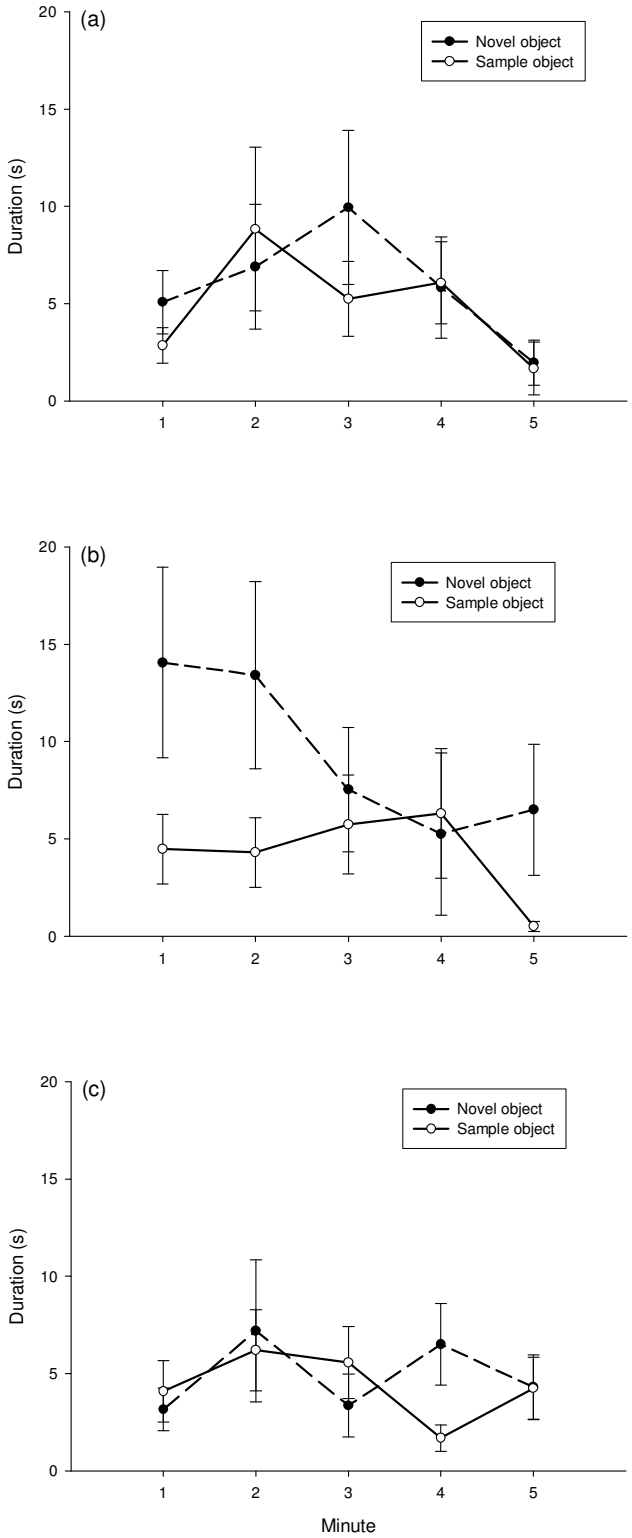

Figure 2.2: Duration of contact (mean ± S.E.) with the novel (solid) and sample (dashed) objects for each minute of the 5-minute test phase in Delay 1 (a), Delay 24 (b), and Delay 5 (c).

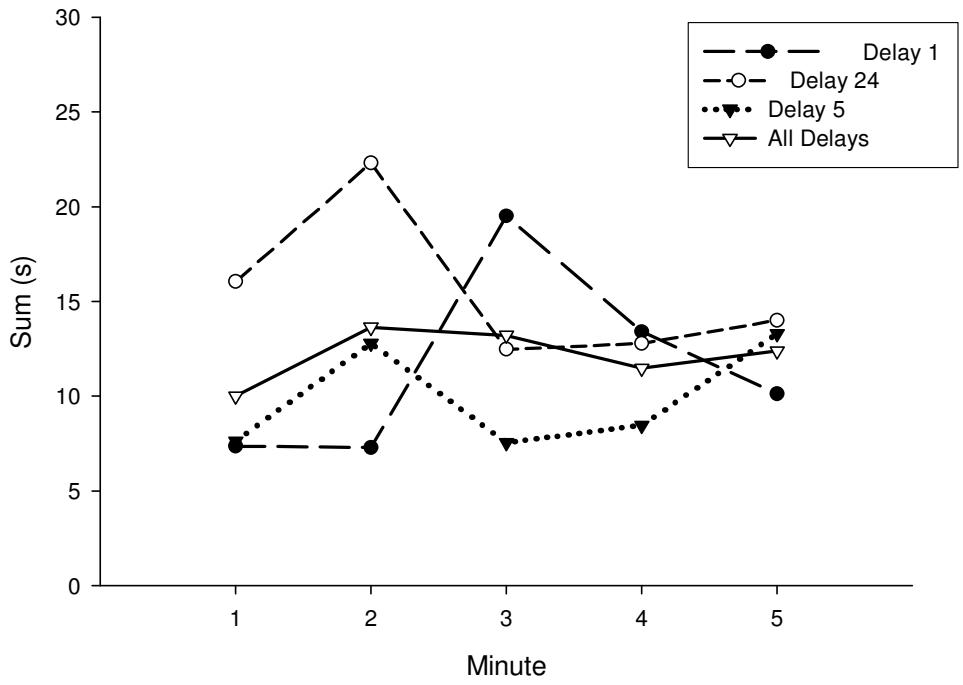

Figure 2.3: Total duration of contact (mean) with both sample objects in the sample phase by minute, for the Delay 1, Delay 24, and Delay 5 treatment groups, and the averaged total over all three delay groups.

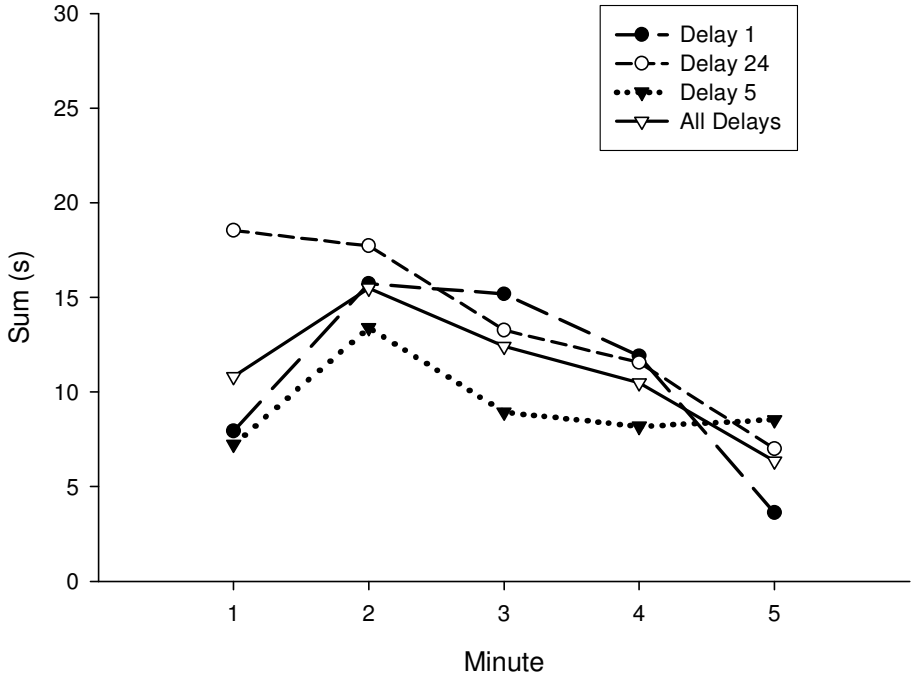

Figure 2.4: Total duration of contact (mean) with the novel and sample objects in the test phase by minute, for the Delay 1, Delay 24, and Delay 5 treatment groups, and the averaged total over all three delay groups.

4. Discussion

Contrary to our predictions, we found that the delay treatment had no effect on the preference of the pigs for the novel object. Pigs showed no preference for the novel object at any of the delays, indicating a possible failure to recognize the sample object.

It is possible that pigs were unable to become familiar with the sample objects with only a 5-minute exposure time. Looking at the behaviour of the pigs

towards the sample objects over the entire sample phase, there does not seem to be a marked decrease in exploration of the samples by the end of the 5-minute period. This may be an indication that pigs had not fully habituated. Failure to allow subjects to completely habituate to the sample stimulus has resulted in findings of no preference and even familiarity preference in infants tested immediately on a paired visual comparison task (Hunter et al., 1982; Hunter et al., 1983). However, considering the short exposure times used in studies of rodent and human infant object recognition memory (generally 2-3 minutes), it seems surprising that pigs would be unable to show recognition of the sample object at even a 1-hour delay when given 5 minutes to explore the samples.

Fear of the testing environment could have resulted in the observed failure to show a novelty preference at any delay. We habituated pigs to the testing environment before conducting tests, by placing them in the test pen in groups or pairs on separate occasions with objects. However, we still observed signs of fear among the piglets when they were placed in the test pens. We had to eliminate 14 of our 48 pairs due to failure to interact with any objects. Most pairs in these cases stayed relatively immobile throughout the whole test, and did not explore the test pen. If some pigs in the remaining pairs were experiencing fear while in the test pen, or still considered it novel, they may have preferred the less novel sample object to some extent as seen in rats (Sheldon, 1969), balancing out any novelty preference of more relaxed pigs. We tried to increase habituation to the testing environment in Trial 2 by placing pairs in the test pen for a total of 15 minutes on

three separate occasions over two days. The lack of any trial effects on discrimination variables (D1 and D2), and the fact that most of the pairs eliminated came from the Trial 2 group (4 pairs from Trial 1 vs. 10 pairs from Trial 2), indicates that rather than increasing habituation to the testing environment relative to Trial 1, the procedures in Trial 2 may have resulted in less habituation to the test pen.

There was a considerable amount of variability among the pairs in discrimination scores. Individual differences in motivation to explore the objects could have played a factor. Because the amount of exposure to the sample object has a large effect on later novelty preference (Ennaceur and Delacour, 1988), we predicted that variability in discrimination scores could result from variability in exploration of the sample objects amongst the pairs. Pigs that spent little time with the sample objects would not be able to habituate as fully to the objects as pigs spending more time with the samples. We expected low S1 scores to be associated with lower D1 and D2 scores, and higher S1 scores to be associated with higher discrimination scores. Therefore, the lack of a significant correlation between total exploration of the sample objects in the sample phase and later discrimination score, as measured by the variables D1 and D2, was surprising.

The inability of pigs to show a novelty preference at any delay suggests that this test may not be suitable for testing object recognition abilities in young pigs. However, attempts should be made to reduce or account for individual variability

on this task, and provide greater habituation to the test environment, before a final verdict is reached.

GENERAL DISCUSSION

We initially predicted that pigs would respond similarly to other species in the spontaneous object recognition test, both in their innate spontaneous preference for novelty, and their behavior towards the novel object as exposure time to the sample object, and the delay interval, were increased. It has become clear after analysis of the results of both experiments that pigs do not seem to perform like rodents, non-human primates, or human infants in this task.

The results from our first experiment do not appear to support the gradual trace decay hypothesis (Roberts and Grant, 1976), or the modified four-phase memory hypothesis proposed for human infants (Bahrick and Pickens, 1995). If the first were operating, we should have seen a gradual decrease in novelty preference over time, with novelty preference at the earliest delays and no preference at later delays. Instead, we observed an increase in novelty preference from the 1-hour delay to the 5-day delay for the long exposure sample object. The second hypothesis states that as the delay interval increases, accessibility of the memory trace for the object decreases, and an increasing interest in the sample object is displayed (Bahrick and Pickens, 1995). This causes performance on the spontaneous recognition task to shift from novelty preference at the earliest delays, to no preference at intermediate delays, familiarity preference at longer delays, and no preference at the longest delays. Infants have shown this pattern when

tested at long delays (Courage and Howe, 1998; Rose et al., 2004). Conversely, our pigs showed no preference for the novel object over the long exposure sample object at the earliest delay, followed by a clear novelty preference at later delays, not a familiarity preference.

In defense of the proposed theories, it is possible that other factors besides the state of the memory trace or recognition affected responses towards the objects in the 1-hour delay, and extension of the delay past 5 days would show either a gradual decrease in novelty preference or a four-phase shift.

The results from our second experiment also did not match results found for other species or support our predictions. Although studies testing object recognition memory in rodents, non-human primates, and human infants have found novelty preferences at various delays, our pigs showed no preference for novelty at any delay, even though the exposure time and delays were comparable to those used with other species.

One way in which both Experiment 1 and Experiment 2 were not comparable to studies of object recognition in other species involves the length of the test phase analyzed for results. In most studies using rodents or human infants as subjects, the time given for subjects to explore the novel and sample stimuli in the test phase is relatively short (2-3 min.). One study of spontaneous object recognition in rats by Dix and Aggleton (1999) showed that the first two minutes of the test phase were the most sensitive measures of novelty preference, as discrimination scores dropped and were no longer significantly greater than zero

in the third minute. The authors suggested that increasing the test phase could act to add noise to the data and decrease the task sensitivity. This is because subjects habituate to the novel object over the course of the test phase, and differential exploration would be expected to decrease once the novel object was similar in familiarity to the sample object. In our first experiment, we gave pigs 10 minutes to explore objects in the test phase, and in our second experiment we gave them 5 minutes. It is possible that this extra time added noise to our data, obscuring results. Looking at the results by minute for both experiments, this seems especially likely for the 1-hour delay with the short exposure sample object in Experiment 1, and the 24-hour delay in Experiment 2. Shortening the test phase may help to increase the test's sensitivity to detect novelty preference.

In both our studies, pigs were tested in pairs as opposed to individually, which also differed from traditional studies found in the rodent literature. This could have influenced the exploratory behavior in ways unforeseen, affecting our results. Investigation of novel stimuli is highly influenced by the presence of social companions, with social facilitation occurring (Birke and Archer, 1983). In a test of memory, this would be a problem, since it would be unclear whether an animal was truly recognizing an object as familiar or novel or simply copying the other animal(s). However, we were constrained to using pairs in our experiment due to the high anxiety exhibited by pigs of this age when placed into the test pen alone. We found that when pigs were placed into the test pen alone, even after being habituated to the test pen in pairs or groups, they either ran around the pen

and attempted to escape, or stood motionless in the middle of the pen emitting low, frequent grunts. We observed no contact with objects in the pen when individuals were left alone in the test pen for 5 minutes.

To combat this problem statistically we used pair as the unit and averaged a pairs' responses to the objects. It might be possible to test young pigs alone if they are habituated to the test pens extensively, and/or are trained to associate a reward with time in the pen. We noticed that providing pigs with a straw floor in the first experiment seemed to facilitate exploration of the pen, as all pairs interacted with the objects, while 14 pairs did not in the second experiment where no straw was provided. On the other hand, greater habituation to the test pens could also have been a factor in the first experiment. Another option for testing pigs alone might be to separate a pair of pigs by a barrier in the test pen or home pen, where only one of the pigs has access to the objects (Mendl et al., 2002). The presence of the other pig might calm the observed pig, but would not interfere with the choices made by the observed pig.

It is important to consider the question being asked when deciding on testing alone or in groups. If the only interest were in a test of pure memory with the intention of comparing between species, testing animals alone would be preferred. On the other hand, studies of the effect of novelty and memory for objects being used in an enrichment program would be better studied in groups of animals, since this is how the enrichment would be implemented. Animals in

groups may have a very different response toward a novel object and a previously encountered one than a pig alone.

In both experiments we observed high levels of individual and pair variation in exploratory behavior. This phenomenon could have hindered finding any treatment differences, especially for Experiment 2. Differences between pigs in their response to the objects could have been due to a variety of causes. For instance, pigs may have varied in the level of fear they were experiencing in the test pen. On a similar note, differences in risk aversion, or the willingness to approach novelty, could have caused the variation we observed in discrimination scores. Observations of differences in exploratory behavior between pigs classified as having "reactive" and "non-reactive" personalities seem to support this theory (Erhard and Schouten, 2001, p. 347). Alternatively, although we balanced for the use of objects as sample or novel stimuli, it is possible that some pigs were more attracted to one object type than another, regardless of its novelty or familiarity. If this were the case, we would expect to observe high levels of individual and pair variability in contact with the novel and the sample objects. In order to determine between these separate hypotheses, further analysis of the data, including measures of latency to approach the two stimuli and other behaviors performed by pigs in the test pen, would be necessary.

Studies of personality classification in pigs may offer suggestions to control the problem of individual variation. Pigs that were classified as "reactive" by their response to manual restraint in a backtest showed decreased latencies to approach

and interact with novel stimuli in a novel environment (Erhard and Schouten, 2001, p. 347). However, they also seemed to respond superficially to the objects, exploring them for short bouts. "Non-reactive" pigs, on the other hand, took more time to contact the objects, but investigated them more thoroughly. These results indicate different exploratory "styles" between reactive and non-reactive pigs. It may be possible to group pigs by reactive score on a pre-weaning backtest before assigning treatments. In this way the individual variability within groups may be reduced. Other ways to reduce the effects of individual variability on the outcome of results would be either to establish a stable performance of novelty preference before testing (Sik et al., 2003), or analyze the consistency of an individual pig's responses over time as well as averaging group responses (Kristensen et al., 2001).

In studies of object recognition using novelty preference, the main assumption is that no preference equals no recognition of the sample object. Although this assumption has been challenged (Sophian, 1980), it is still a highly evoked explanation, especially in studies with rodents. Our first experiment provides some evidence that the assumption might indeed be faulty. Pigs explored the long exposure sample object for the same amount of time as the novel object at the 1-hour delay, but explored the novel object for longer when tested at the 3-hour and 5-day delays. If the lack of novelty preference at the 1-hour delay meant pigs could no longer remember having seen the sample object before, they should not have been able to discriminate between the novel and sample objects at the

later delays. It is feasible that organisms could have no preference for one object over the other, even though they recognize one of the objects.

There is the possibility that the null results in our studies were not due to recognition failure, but a failure by the pigs to prefer to explore the novel object more than the previously encountered one. Although spontaneous novelty preference is a robust, widespread phenomenon, it can be affected by environmental factors such as familiarization time (Hunter et al., 1982; Hunter et al., 1983) and environmental novelty (Sheldon, 1969). Kristensen and colleagues (2001) conducted a study of social recognition of familiar and unfamiliar pigs, and showed that the preference of pigs for the familiar or unfamiliar pig was opposite for two different treatment groups. They were unable to explain why the pigs raised in an ammoniated atmosphere spent more time near the familiar pig, while those in a "normal" atmosphere preferred the unfamiliar pig. However, their study does indicate that spontaneous preference for novelty by pigs can be influenced by environmental factors. In support of the existence of spontaneous novelty preference in pigs, Wood-Gush and Vestergaard (1991) showed that pigs would prefer a novel object to a previously encountered one if given the choice. Pigs spent more time with the novel object in the 5-minute test, and sought to contact it first, even from the very first trial before pigs were familiar with the procedure. Our first experiment also showed that pigs displayed a significant novelty preference when the sample object was highly familiar. Unfortunately, because the preference for novelty was not consistent, we cannot say for certain that our

null results were not due to a failure by the pigs to show spontaneous novelty preference.

Overall our results indicate that the spontaneous novelty preference method may not be a feasible option for testing object recognition in pigs, unless ways are found to decrease or account for individual variability in response to objects varying in degree of novelty, or pigs can be shown to have a stable preference for novelty at immediate delays before any treatments are imposed.

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