

LEARNING AND MEMORY IN MATERNALLY-REARED  
AND MATERNALLY-DEPRIVED PIGS (*Sus scrofa*)

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Learning and Memory in Maternally-Reared and Maternally-Deprived Pigs (*Sus scrofa*)

by

© Riana Mian, B.A. (Honours)

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## Abstract

Four sibling groups, each consisting of three Yucatan miniature pigs (*Sus scrofa*), were tested on the effects of rearing condition on learning and memory. Within each group of siblings, one piglet was sow-reared (SR), one piglet was a maternally-deprived runt (MD runt), and one piglet was a maternally-deprived large littermate (MD large). In Experiments 1 and 2, the pigs were trained to open tray lids and to shuttle from the entrance of the training room to the first pen and open a tray lid. All twelve pigs were able to learn both tasks. The SR pigs had longer latencies than the MD runt and MD large pigs in the shuttle task. These longer latencies in SR pigs may be explained by this group being more predisposed than MD pigs to explore their environment. Experiment 3 examined whether the MD and SR differed in their performance on discrimination and reversal tasks and whether experiencing a context change would facilitate reversal learning. There was no difference between the three rearing groups on these tasks. The pigs that experienced a context change learned a reversal in fewer errors to criterion than those that remained in the same context. This finding supports the hypothesis that context change serves as a conditional cue in reversal learning. Experiment 4 tested pigs' memory for the tray they visited less recently. Four pigs were exposed to four different trays with particular odors and locations in sequence. The pigs were then given a choice between the first and last items in the sequence. All four pigs chose correctly at an above chance level. The results suggest that pigs are sensitive to relative recency. These findings may lead to further episodic-like memory tests with pigs.



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## CHAPTER 1: OVERVIEW

Young mammals often require a significant amount of parental care for an extended period of development. This care is crucial to their survival and well-being. In fact, adequate maternal care is undoubtedly necessary for the healthy emotional, physiological and behavioral development of offspring. Since the 1950s we have learned just how devastating the consequences of being reared without this adequate maternal care can be. Harlow, Harlow & Soumi (1971) demonstrated with rhesus monkeys that motherless infants became emotionally disturbed, hypersensitive to stressors and unable to console themselves. As mothers, they were neglectful and even abusive to their own offspring; unable to provide the comfort they never experienced themselves (Harlow et al., 1971). Currently, research has demonstrated that maternal deprivation can cause brain and neuroendocrine alterations as well as impairments which include learning and memory deficits that last well into adulthood (Anderson & Teicher, 2004; Lyons & Schatzburg, 2002).

### *1.1. Maternal Deprivation Affects Brain Development.*

*A. Alteration of HPA Axis Functioning.* The hypothalamic-pituitary-adrenal (HPA) axis is a system that responds to stress by triggering physiological changes that prepare an animal to defend itself or flee from danger (Rose, 1989). The amygdala, a brain region implicated in fear and aggression, can trigger activation of the HPA axis. Efferents from the amygdala impinge on the ventromedial nucleus of the hypothalamus and cause it to synthesize corticotrophin-releasing hormone (CRF) and arginine vasopressin (AVP). These hormones stimulate the anterior pituitary to secrete adrenocorticotropic hormone which in turn stimulates the adrenal cortex to release glucocorticoids. The quick release

of glucocorticoids regulates the HPA system via a negative feedback loop. As glucocorticoid levels raise, receptors in the hippocampus, septum and amygdala are activated and turn off the HPA axis activity, resulting in a shutting down of glucocorticoid production. However, during chronic stress the system does not regulate properly and fails to terminate its action (Stuart, 1996).

Glucocorticoids, or so-called “stress hormones”, signal the body’s “fight or flight” response. They benefit the organism by increasing vigilance, attention and alertness, thus aiding survival (DeKloet, Joels & Holsboer, 2005). Their short term function may also boost fear-related learning and memory (Joseph, 1998; McEwan, 2000). However, long-term effects include reduced immune response, anxiety disorders and hippocampal changes (Glaser, 2005; Lupien & Lepage, 2001; McEwan, 2004; Reiche, Vissoci, Morimoto & Nures, 2005). Chronic stress, and thus, prolonged activity of the HPA axis, may cause the system to become over active or to remain activated long after a stressor has been removed (Stuart, 1996). Indeed, persons suffering from depression and other affective disorders typically show malfunction of the HPA axis (Barden, 2003). Furthermore, stress early in development has been demonstrated to cause such abnormalities of the HPA axis. The effects may last well into adulthood (Beatson & Taryon, 2003; Bjorntor, 1996; Fletcher & Brewer, 2001; Heit & Graham, 1999; Holden & Holahan, 2005; Marti, Garcia, Velles, Harbuz & Armario, 2001; Mirescu, Peters & Gould, 2004; Penke, Felszeghy, Fernette, Sage, Nyakas & Arlette, 2001; Saltzman, King, Mandonsky, King,; Teicher, Anderson, Polcari, Anderson, Navalta & Kim, 2003).

Maternal deprivation is perhaps the most severe stressor that a young organism can experience. Maternal deprivation leads to exaggerated HPA activity that persists often

into adulthood (Bremner & Vermetten, 2001; Ladd et al, 2000; Macri, Mason & Wurbel, 2004; Plotsky, Thirivikraman, Nemeroff, Caldji, Sharma & Meaney, 2005). Penke et al. (2001) found that even one period of 24-hour maternal separation of rat neonates at an early critical period can affect the HPA axis long into adulthood. Long-term behavioral changes that resulted from this separation included a heightened stress response to restraint and permanently altered feeding behavior, whereby the deprived rats consumed less food than non-deprived rats.

*B. Effects on the Hippocampus.* The hippocampus functions as a center for spatial learning and memory (Burgess & O'Keefe, 1996). The hippocampus also plays a role in deactivating the HPA response. Early stress during hippocampal development can cause long-term HPA alterations (Bremnar & Vermetten, 2001). The hippocampus is especially vulnerable to early stress as it undergoes a considerable amount of development in the postnatal period (Bremnar & Vermetten, 2001). This is true for rodents and other mammals, including humans (Barker, Wojtowicz & Boonstra, 2005; Gould, 1999; Gould, Tanapat, Rydel, Hastings, 2000; Guidi, Ciani, Severi, Contestabile & Bartesaghi, 2005; Montero-Pedrazuela, Venero, Lavado-Autric, Fernandez-Lamo, Garcia-Verdugo, Bernal & Guadano-Ferraz, 2006). Maternal deprivation has been shown to induce hippocampal alterations such as reduced glucocorticoid receptor binding and reduced mossy fiber density (Anderson & Teicher, 2004; Huot, Plotsky, Lenox & McNamara, 2002). In contrast, high quality maternal care can support enriched hippocampal development (Bredy, Grant, Champagne & Meaney, 2003). Bredy et al. (2003) found that rat pups that received a greater frequency of maternal grooming had superior hippocampal development compared to those who were groomed less often.

## *1.2. Maternal Deprivation Affects Cognition and Emotionality.*

*A. Effects on Emotionality.* Early maternal deprivation can lead to long-lasting emotional instability. Because the nervous system, including the HPA axis and hippocampus, is adversely affected by maternal deprivation (Bremner & Vermetten, 2001; Ladd et al, 2000; Plotsky et al, 2005), it follows that emotional responses may be affected. Some animals intermittently separated from the mother show anxiety at the time of separation (Weweres, Kaiser & Sachser, 2003). As discussed, they may also be more fearful as adults (Penke et al., 2001). Indeed, animal models of depression have been developed using maternal deprivation procedures, suggesting that early stress may well be one of the root causes of depression. Symptoms of depression-like syndrome induced by early maternal deprivation include anxiety, anhedonia and vulnerability to substance addiction (e.g., Huot, Thirvikraman, Meaney & Plotsky, 2001; Matthews & Robbins, 2003).

*B. Effects on Learning and Memory.* Early stress that alters the HPA axis and hippocampus, can, in turn, influence learning and memory processes. Just one instance of 24-hour maternal deprivation can lead to lasting cognitive impairments (Sibug, Oitzl, Workel & Kloet, 2001). In their study, maternally-deprived rats made more errors on a spatial task in a Morris water maze than non-deprived animals. However, it is not only full maternal separation that can lead to spatial learning deficits. Disrupted maternal presence in the form of intermittent absence also impaired performance on reversal learning tasks (Lyons & Schatzberg, 2002). These tasks involve switching to a new response when an old response is no longer reinforced and are a measure of mental flexibility. Overall, it appears that an adequate level of maternal care may be necessary to

maximize cognitive development in young animals.

### *1.3. Maternal Separation: Positive Effects?*

Long maternal separations have been shown to be detrimental to offspring. There is some evidence, however, that brief absences increase maternal attention upon the mother's return and may have positive effects on offspring (Pryce & Feldon, 2003). Under natural and real world conditions, mothers with young in a nest would have to leave their young for short periods to search for food. Therefore, young of such species would be expected to have evolved mechanisms to cope with such absences. Briefly separating rat neonates from the mother for 15 minutes, a procedure called "handling", can, in fact, reduce the offspring's anxiety in later life via changes in glucocorticoid receptors (Ladd et al, 2000; Pryce & Feldon, 2003). The mechanism which appears to induce this effect is increased maternal care upon reunion of the mother and pups (Ladd et al, 2000; Macri, Mason & Wurbel, 2004). Following brief separations, mother rats were observed providing their young with a burst of attention, including licking, grooming and a greater frequency of nursing. This was not the case after long (3-hour) separations. Thus, mothers may compensate for brief absences by increasing the level of care they provide while long absences may be disruptive to normal mother-offspring interactions.

### *1.4. Maternal Deprivation in the Rat versus Other Mammals.*

Maternal deprivation has been studied extensively and almost exclusively in rats, which may make one wonder if the effects that have been observed are generalizable to other species. An important consideration is whether other animals demonstrate the period of hyporesponsiveness to early stress that has been observed in rats. Rats

experience a period early in the first two weeks of development when they show very limited adrenal responses to stress (Douglas, 2005; Levine, 2001). Healthy neuronal development in glucocorticoid-receptor rich brain areas benefits from this low-stress period when cortisol levels are kept steady and low (Sapolsky & Meaney, 1986). The mother rat regulates the stress response in the infant with a number of behaviors that may include grooming, passive contact and feeding (Levine, 2001). Thus, neonatal rats may be especially sensitive in terms of impairments of the HPA axis and behavioral alterations as a result of maternal separation or deprivation. It is possible that early sensitivity to stress is a normal function of infant attachment, allowing for rapid attachment to form between rat neonates and mother (Moriceau & Sullivan, 2005). Though it is likely other rodents experience this hyporesponsiveness, it is not known whether many other mammals undergo a similar period. Primates studied to date have not shown this hyposensitive period (Gunner & Donzella, 2002; Levine & Mody, 2003). It is therefore important to examine the effects of maternal deprivation in non-rodent mammalian species.

Pigs are a suitable mammal in which to study maternal deprivation. They are known not to have an early hyporesponsive period (Kanitz, Tuchsherer, Puppe, Tuchscherer & Stabenow, 2003). In commercial animal production, animals may be removed from the mother after birth or separated intermittently for routine procedures during daily animal care or farming routines. Knowledge of animals' tolerance for these separations may have beneficial applications for the well-being of domesticated animals as well as to maximize the efficiency of farming practices and animal husbandry. Pigs may suffer substantially from social deprivation, as they appear to be very social animals,

establishing group dominance hierarchies (Beilharz & Cox, 1967). As well, pigs can discriminate familiar and unfamiliar conspecifics (Kristensen, Jones, Schofield, White & Wathes, 2001; McLeman, Mendl, Jones, White & Wathes, 2005) as well as familiar and unfamiliar handlers (Koba & Tanida, 1999).

Kanitz et al. (2003) found that 2-hour daily maternal separation produces some effects in pig offspring that are similar to those found in rats. For example, the maternally-separated pigs demonstrated significantly less activity in an open-field test during isolation. They also demonstrated a long-term suppressed immune response. Brain differences included changes in glucocorticoid receptor binding in the hippocampus, hypothalamus and amygdala, implicating HPA-axis modification as a result of the maternal separation.

Kanitz, Manteuffel & Otten (1998) found that weaning and restraint stress significantly decreased glucocorticoid binding in the hippocampus and amygdala in maternally-deprived pigs. While rats appear to benefit from brief handling in early life, pig neonates responded with permanent HPA axis impairment and reduced body weight (Weaver, Aherne, Meaney, Schaefer & Dixon, 2000). There is also some evidence that social stress experienced by a sow can cause an increased cortisol response in her female offspring (Jarvis, Moinard, Robson, Baxter, Ormandy, Douglas, Seckl, Russell & Lawrence, 2006).

### *1.5. What Do We Know about Pig Cognition?*

*A. Discrimination Abilities in Pigs.* Pigs, like most animals ever tested, are capable of discrimination learning. They can solve discriminations using cues from various sensory modalities and with a combination of cues from different modalities. Croney,

Adams, Washington & Striklin (2003) found that pigs could solve discriminations with olfactory, visual and spatial cues. Pigs can also discriminate between familiar and unfamiliar objects in their environment (Gifford, 2005). There is also evidence pigs can make intra- and extra-dimensional shifts on visual and spatial discrimination tasks (Moustgaard, Arnfred, Lind, Hansen & Hemmingsen, 2004).

*B. Spatial Memory in Pigs.* Pigs can use spatial memory to return to food sites. In addition, they appear to use their memory to select the site yielding the greatest amount of food. Held, Baumgartner, Kilbride, Byrne & Mendl (2005) found that pigs could remember the locations of food sites and selectively return to the one yielding the greater amount of food. Pigs are capable of remembering the locations of food sites even after some time has elapsed. Mendl, Laughlin & Hitchcock (1997) trained pigs to relocate food sites after intervals of ten minutes and two hours. The pigs successfully learned to locate the food after both the short and longer intervals.

*C. Factors Affecting Learning in Pigs.* Learning and memory in pigs and other animals can be affected by a number of factors. Interference, in the form of disturbances that appear to distract the animals, may affect their ability to retain learning. Laughlin & Mendl (2004) found, however, that incorporating "costs" into a memory task protected against interference effects by causing the animal to attend more to the correct choice to avoid the costs. Another factor that appears to affect learning in pigs is whether they are being placed in either a win-shift or win-stay contingency. In a win-stay condition, making the same choice as the previous choice is rewarded. In win-shift, making a relatively novel choice is rewarded. Pigs on a win-shift condition learned a spatial memory task more quickly than those on win-stay, as is typical of other animals (Gaffan



& Davies, 1981; Olton & Schlosberg, 1978; Timberlake & White, 1990). Pigs may have a natural tendency to prefer non-searched (or less recently searched) food sites (Laughlin & Mendl, 2000). Pigs' emotional stability may also play a role in learning. Bolhuis, Schouten, Leeuw, Schrama & Wiegant (2004) found that pigs with poor coping characteristics, defined as less tolerance for a restraining procedure, had more difficulty on a reversal task than better coping pigs. This finding may have implications for MD pigs, which might be expected to have altered emotionality.

We are interested in whether MD pigs differ from SR pigs on tests of learning and memory performance. As described, pigs are capable of using visual and spatial information to solve problems. In the following experiments, we tested pigs for discrimination learning abilities, reversal learning and spatial memory. We compared pigs from different rearing groups to determine if maternal deprivation leads to changes in learning and memory performance.

## CHAPTER 2

### Experiments 1 and 2: Pre-training

#### *Overview*

In order for pigs to perform the discrimination, reversal and memory tasks, they must first be capable of performing the basic components of these tasks; in my studies, the task was opening the tray lids and shuttling from the entrance of the training room to the first pen. Shuttling refers to the animal walking back and forth between two areas, in this case, between the entrance and the first pen. Experiments 1 and 2 were carried out for the purposes of pre-training, to first teach the animals to open tray lids and shuttle so that later tasks could build on this learning. These tasks also made it possible to examine whether SR and MD pigs differed on the pre-training tasks. It is possible that MD pigs would be less active in open field tests, for example, if they are experiencing a depression-like state (Pryce & Feldon, 2003) and, thus, they may have longer latencies on these tasks. Rats that were maternally-separated were less active in open field tests (Kaneko, Riley & Ehlers, 1997). Kanitz, Tuchscherer, Puppe, Tuchscherer & Stabenow (2003) also found that pigs that were intermittently separated from the sow early in development were less active in open-field tests.

#### Method

##### *Subjects*

Subjects were 12 (6 male, 6 female) Yucatan miniature pigs (*Sus scrofa*) with a mean age of 63 days (SE = 8.58, range 33-102) at the onset of training (Appendix A). Each of the litters was born of different sows and paternity was unknown (the breeding was not

monitored so it is possible some pigs were sired by the same male). The pigs had equal handling experience and were involved in a concurrent study on nutrition for which they were placed in different rearing conditions. In total, there were three groups of piglets from four litters in the following rearing conditions: MD runt (n = 4), MD large (n = 4) and SR (n = 4). For each litter group, one runt piglet (MD runt) was selected from the litter (weight <800 g) and paired with the largest same sex sibling (MD large) from that litter (weight >1100 g). The two were removed from the sow at age 3 days and housed together in one pen. For four weeks, the pigs were fed milk replacer on an ad libitum basis ten times per day at the same times each day. Pigs were separated for feeding. Beginning at 4 weeks of age, they were fed standard pig chow (Co-op Pig Grower). This ad libitum feeding took place for 5 hours per day beginning at approximately 12 pm. A third piglet from each litter, reared with and fed by the sow, was then placed with the MD runt and MD large sibling at 30 days of age. This sibling was of the same sex as the two siblings for three of the four sibling groups used in this study. Once removed from the sow, the SR pig was fed the same pig chow diet as the MD runt and MD large siblings. The three pigs were separated only for feeding using the partitions in each pen. The animals could self-administer water from a nozzle in the pen ad libitum. Weekly blood samples were taken from the pigs for the nutrition study. On sampling days, this took place before training. Animals were cared for in accordance with the Canadian Council on Animal Care.

### *Apparatus*

#### *Home pens*

Training took place in the home pen before daily feeding. The room containing the

pens measured 15.82 x 3.95 m. There were eight parallel pens on each side of the room (Figure 2.1). Pens consisted of wire-fenced front walls with latching doors, brick back walls and metal side partitions. The floors consisted of rubber matting over concrete. Each pen measured 1.45 x 1.17 m. Pens were sectioned into two equal halves by a 1.29 m high metal divider that could be raised. The divider was used to separate the pigs at various times. Toys for the pigs to play with, such as balls, were available. Two bricks were placed in the left corner of each pen to keep the pigs' food dishes from being tipped over during feeding. On side B (Figure 2.1), pens also had swinging door flaps that allowed access into pens in an adjacent room. The door flaps on side A were not in use during the study. The pens in the adjacent room were similar to the home pens. Pigs were moved to the pens in the adjacent room once a day while their pens were being cleaned. Some pigs switched from being housed in the pens on side A to those in side B during the course of the experiment.

### *Materials*

#### *Trays*

Stainless steel (21 x 21 x 2.5 cm) trays were used to hold the food during training (Figure 2.2). A square metal lid covered each tray and could easily be slid off with a small amount of effort. The bottom half of each tray, which was covered by a perforated divider, was filled with approximately 200 g of pig chow. The pigs could not access this food; its purpose was to control for odor cues emitted by the food that was used as a reward during training. Approximately 5 g of pig chow was used for each reward during training for Experiments 1- 4. The food was placed on top of the divider inside the tray. A detachable perforated metal cube (5 x 5 x 3 cm) was centered on top of the lid; this

could be filled with an odor to create distinct odor cues. Tray lids were cleaned once a day, after training (odors on trays between trials should not have influenced performance as the trays were periodically switched between trials).

### *Data Analysis*

The means and standard error of the mean (M, SE) are reported. One-way and two-way Analyses of Variance (ANOVAs) were used for statistical analyses. All reported probabilities are two-tailed ( $\alpha = 0.05$ ), unless otherwise stated.

### *Design and Procedure*

Pigs were individually separated for training and were always trained before daily feeding in all experiments. Trays were placed inside the pen in the same location on each trial whenever possible; tray placement location was varied between pigs. Placing the tray in front of the pig signaled the beginning of a trial. The experimenter and tray remained in the pen with the pig until the pig opened the tray. Pigs were allowed unlimited time to open the tray. For this and the subsequent experiments, the lid was determined to be open when the pig's snout could fit inside the tray. Once open, the pig was permitted to eat the pig chow for 15 seconds. The tray was then picked up by the experimenter and more food added to replace the food the pig had eaten. The next trial began immediately, using the same tray. Eight training trials per day for two days were carried out for all pigs. Length of training sessions varied depending on the latency to open the tray. Training sessions occurred a mean of 2.92 (SE =1.12) days apart.

## Results

A 3 x 16 ANOVA (Rearing condition x Trials) was conducted on latency scores (Table 2.1). Mean latencies for each group to open the tray lid are shown in Table 2.2

and are graphed in Figure 2.3. There was no effect of rearing condition on latency scores ( $F(2, 9) = 0.29, p > 0.05$ ). There was a significant effect of trials on latency ( $F(15, 135) = 2.08, p < 0.05$ ) such that as trials progressed, latencies became significantly shorter. There was no interaction between rearing condition and trials ( $F(30, 135) = 0.84, p > 0.05$ ).

## Experiment 2: Shuttle training

### *Method*

#### *Subjects*

Subjects were the same animals used in Experiment 1. Mean age of subjects at the onset of training was 75.6 days (SE = 6.43, range 52-105 days).

#### *Apparatus*

##### *Training room*

Training took place in a room adjacent to the home pen room. It consisted of a long corridor with one row of five similar pens on one side and a brick wall on the other (Figure 2.4). The pens were constructed of wire fence with latching doors and concrete floors. There was a low concrete divider in the center of each pen. In each pen, a tire swing was tied up against the back wall, out of reach. The entire room measured 13.41 x 10.36 m. Each pen was 3.29 x 2.37 x 2.44 m. The doorway to the room opened to a shorter corridor (1.37 m) that turned at a right angle into the longer corridor. Doors to the pens not in use were kept closed during training or testing.

## *Materials*

### *Trays*

The trays were those used in Experiment 1. Two trays per subject were used for this Experiment.

### *Design and Procedure*

The pig entered the doorway of the training room, where a tray was placed on the floor next to the door. The area from the door of the training room to the corner of the first pen consisted of the start area (Figure 2.4). The short corridor between Pen 1 and the start area contained a floor-ceiling-wall that blocked the view from one area to the other.

Pigs were required to remove the lid of the tray at the door and eat for 15 seconds before the tray was removed. Once the first tray was removed by the experimenter, a second tray was immediately placed on the floor in the center of Pen 1 by an assistant. Pigs were required to find the food tray in Pen 1 and open it. The pigs were then required to return to the start area and open that tray before the tray was again put down in Pen 1. After all pigs completed at least seven initial trials, the latency to open the pen tray was recorded. Timing began at the point when pigs turned the corner from the start area to Pen 1.

Training continued until pigs achieved a latency of less than 60 seconds to open the tray. The mean number of trials for all pigs was 20.4 (SE =1.67, range 14-32 trials). The mean number of training days to criterion was 2.02 (SE=0.14, range 2-3 days).

## Results

A 3 X 14 ANOVA (Rearing condition x Trials) was conducted on latency scores for each of the three rearing conditions (Table 2.3). Means for each rearing group to shuttle are shown in Table 2.4 and graphed in Figure 2.5. There was a significant effect of rearing condition on latencies ( $F(2, 9) = 14.54, p < 0.05$ ). The SR group had significantly longer latencies to shuttle to the pen and open the tray contained in the pen than the MD runt and MD large groups. There was a significant effect of trials ( $F(13, 117) = 4.29, p < 0.05$ ), with latencies declining over trials. There was no interaction between rearing condition and trials ( $F(26, 117) = 0.91, p > 0.05$ ).

## Discussion

In Experiment 1, there were no differences among groups for latencies to open the tray lid. The pigs improved significantly over trials and were thus prepared for the next phase of training.

Previous studies with rats revealed that maternal deprivation resulted in less activity in an open field (Kaneko et al., 1997; Kanitz et al., 2003). We predicted that if the same pattern of behaviour would be exhibited by pigs, the reduced activity of the MD pigs would result in longer latencies to open lids. The findings, however, were opposite to this prediction: it was the SR pigs that had longer latencies to shuttle to the pen. Observation of the pig's behavior during the trials may explain this unexpected finding; the SR pigs spent more time exploring the training room than the MD pigs. Their increased exploration behaviour produced higher latencies to open the tray lid.

It is possible that SR pigs were less motivated to obtain the food reward. Given that their developmental history involves "on demand" nursing, as compared to the formula-



fed (every 2-hour) MD piglets, the SR pigs may have been more self-driven and less regimented, or may have developed different environmental associations with food stimuli. Secondly, it is also possible that maternal deprivation, like more brief maternal separations in rats, imparted some benefits to the MD runt and MD large pigs that enabled them to learn the task more quickly. Newel (1967) found that maternally-separated rats learned an avoidance task more quickly than maternally-reared rats. Similarly, Pryce & Feldon. (2003) demonstrated that rats briefly separated from the dam were better than non-handled rats at avoidance and spatial learning tasks. Finally, it is also possible that SR pigs are intrinsically more motivated by exploration behaviour than are MD pigs. This could involve decreased attentional processes and/or increased anxiety. Although the explanation for these results is not yet understood, it was demonstrated that pigs can be trained to open tray lids and to shuttle between two locations to open the tray lids.

## CHAPTER 3

### Experiment 3: Reversal

#### *Overview*

In a discrimination reversal task, an animal must learn to reverse its responses to two stimuli. The previously rewarded stimulus is no longer reinforced. Rather, the animal must now direct its response to the other stimulus in order to obtain reinforcement (Macintosh, 1974).

*Importance of reversal learning.* Reversal learning tasks provide insight into cognitive abilities such as mental flexibility and the capacity to shift attention. Animals may use reversal learning for survival in the natural environment. For example, they may switch their foraging location when food becomes less available in a particular area. Day, Crews and Wilczynski (1999) found that lizards have adapted different behavioral strategies to maximize foraging, with active foragers learning reversal tasks more quickly than less active foragers.

*Reversal learning and stress.* Reversal learning may be affected in animals that experience early stress. Sibug et al. (2001) found that rats that were maternally-deprived for one 24-hour period early in the postnatal period were impaired on reversal tasks. Lyons & Schatzberg (2002) found poor reversal learning in squirrel monkeys that experienced disrupted maternal presence. There is also evidence that maternally-separated animals have difficulty shifting attention in attentional-set shifting tasks (Lovic & Fleming, 2004).

*Reversal learning strategies.*

*Learning set formation.* To learn reversals, an animal may apply a learning set.

According to the learning set theory (Levine, Levinson, & Harlow, 1959), an animal learns the underlying heuristic or rule that applies to a series of related tasks. If an animal is using a learning set, it should show improved performance on new reversals after experiencing past reversals. In such a case, the animal would have learned the underlying rule that applies to reversal tasks (when responding to one stimulus that no longer pays off, switch to the other stimulus). The learning set may only apply when the stimuli used change along the same dimensions as the previous stimuli (Bitterman, Wodinsky, Candland, 1958, as cited in Macintosh, 1974).

Macintosh, McGonigle, Holgate & Vanderver (1968) found that, with rats, after several reversals, a reversal can be learned with only a single error. Komischke, Giurfa, Lachnit and Malun (2002) found that honeybees with reversal experience were superior to honeybees without this training in solving new reversals. Though the groups were comparable on the initial discrimination, having experienced even one reversal task improved honeybees' performance on the new reversal situation.

*Win stay, lose shift.* Animals may solve reversals by using a win-stay, lose-shift strategy (Restle, 1958). During the initial discrimination, an animal chooses the stimulus associated with reward. If it is no longer rewarded during the reversal stage, the animal may switch its responding to the other stimulus. Each time the animal finds one choice no longer rewarded, it abruptly shifts to the other. Therefore, reversals may be learned very quickly. Rats, and perhaps other species, may have a tendency to follow a win-shift pattern, preferring to seek out more novel choices (Gaffan & Davies, 1981; Olton & Schlosberg, 1978; Timberlake & White, 1990). Schusterman (1962) found chimpanzees solved successive discrimination reversals in a win-shift, lose-stay pattern.

*Factors affecting reversal learning.* Many factors affect reversal learning. The over-training reversal effect, age effects and context all contribute to variability in animals' performance on reversal learning tasks.

*Over-training reversal effect.* Ishida & Papini (1997) trained turtles on a left-right spatial discrimination. Half the turtles were trained to criterion of 19 out of 20 consecutive trials correct and half were over-trained an additional 100 trials. The turtles in both groups were reversed on the original discrimination. The over-trained turtles learned the reversal in significantly fewer trials. Reid (1953) trained rats on a black-white discrimination. One group was reversed immediately, one after an extra 50 trials and one after 150 additional trials. The greater the number of training trials, the faster the reversal was learned (Reid, 1953). It appears that over-training works to decrease an animal's resistance to extinction perhaps by increasing attention to the relevant stimulus.

*Reversal learning and age.* Reversal learning is a cognitive capacity that may decline with advanced age. Tsuchida, Kubo and Kojima (2002) found that aged Japanese macaques performed more poorly on a simple position reversal than their younger counterparts. The aged macaques were similar in ability to the adult macaques but inferior to young macaques, indicating that there may be a slow decline of reversal learning with age. The ability to solve position reversals is thought to be linked to the medial orbital cortex. Aged macaques have decreased functioning in this area and lesions to the area inhibit the ability to solve reversal learning tasks.

Anderson, Monte and Kemf (1996) studied multiple reversals in young adult and older adult stumptailed monkeys. The older adult monkeys had more perseverative errors.

Their performance improved over a number of reversals, suggesting practice can attenuate age-related deterioration in performance.

*The role of context in reversal learning.* Context may serve as a cue in discrimination learning. During reversal learning, contextual cues associated with the first response may interfere with this new learning. Switching animals to a new context for reversal learning may attenuate this effect. In addition, context change may signal to the animal that a new contingency is in place. Two theories help explain the challenge in learning a reversal in the same context as the discrimination.

*Stimulus fluctuation theory.* Stimulus-fluctuation theory (Estes, 1955a, as cited in Bjork & Bjork, 1992) states that when a response is conditioned, available elements (features that are present in the environment during conditioning) are conditioned and unavailable elements (those not present) are not. During extinction, unavailable stimulus elements become conditioned while the available elements are being unconditioned. Over many learning trials or extinction trials, the set of available and unavailable elements will fluctuate. As some are conditioned with the response, the result is to slow the forgetting process. In reversal learning, the first response is extinguished and a new response conditioned. According to stimulus fluctuation theory, spontaneous recovery of the response from the first discrimination could occur during reversal learning due to the presence of fluctuating conditioned stimulus elements. This could explain the difficulty in forgetting the first response and why moving to a new context between discrimination and reversal could aid reversal learning by reducing elements conditioned to the first response.

*Interference theory.* Interference theory (Melton & Irwin, 1940, as cited in Postman & Underwood, 1973) dictates that when an old response is extinguished and a new one is reinforced, interference comes in the form of unlearning and competition. Unlearning involves the weakening of the original association between a stimulus and the response. Competition takes place when the old response persists unreinforced along with the new response that is being learned. This could take place during reversal learning; the learning from the original discrimination is being unlearned while the reversal is being learned. However, the original response is still elicited by various contextual cues, resulting in interference. Changing the context may reduce interference.

Mcdonald, King and Hong (2001) trained rats on a reversal of a stimulus-response task. The rats were trained to discriminate between lit and unlit arms of an eight-arm radial maze to find food rewards. Half the rats were then trained on a reversal in a novel but similar maze with different extra-maze cues. The other half was reversed in the original maze. The rats that were trained in a new context learned the reversal more quickly than those in the original maze.

Changing the context between two successive tasks with competing responses may reduce interference. Cheng & Wignall (2006) trained honeybees on two tasks in succession that involved competing responses. They were then tested on Task 1 followed by a test of Task 2. The honeybees' learning on the tests appeared to have been affected by retroactive interference (Cheng, 2005). However, a 60-minute delay before the second test attenuated this effect. Cheng & Wignall (2006) concluded that new memories do not erase older ones but rather honeybees hold onto old memories (thus these memories can

interfere with newer ones). Similarly, Chittka (1998) found that bumblebees suppress rather than lose their initial training when learning a series of reversals.

Insects such as honeybees may use contextual cues to navigate within their environments (Collett, Fauria & Dale, 2003). To investigate whether context change reduces the retroactive interference effect, Cheng (2005) trained honeybees on two landmark-locating tasks. When context remained the same for both tasks, learning was impaired. However, when the context was changed, learning was unaffected by interference. This suggests that context is used as a retrieval cue and that switching context aids in the learning of two different responses (Cheng, 2005).

Walsh, Skinner & Martin (in press) found that harp seals that learned a reversal of a visual discrimination in a novel context made more correct choices and required fewer trials to criterion than those reversed in the original context. All six seals were trained on a visual discrimination. Half the seals experienced a reversal of reward contingencies in the same tank while the other half were reversed in a second tank (not previously used in training). Seals reversed in the novel tank learned the reversal more quickly and with fewer errors than the seals reversed in the original tank.

Context cues from one sensory modality may be more important than another. Thomas, McKelvie & Mah (1985) used a context change from no-light/white noise to light/tone. Pigeons learned a discrimination and then a reversal in one of these contexts. They found that changes in visual but not auditory cues aided reversal learning. Similarly, Pagani, Brown & Stanton (2005) found that discrimination reversals that involved a maze change or texture change alone did not facilitate learning but a maze plus texture change facilitated learning. Thomas & Empedocles (1992) found that when

pigeons learned a discrimination and reversal of two key colours in the presence of particular odors (isoamyl acetate and eucalyptus oil), the odors worked as facilitators to aid reversal learning. The switch in odors may have drawn attention to the context change.

Chiszar & Spear (1969) found that context change aided reversal learning and increased long term retention when long inter-trial intervals (spaced practice) were used. Rats were divided into two groups and trained on a visual / spatial discrimination in one of two T-mazes: one with a black left arm and white right arm and one with a black left arm and half-black, half-white right arm. Half the rats were given reversal training in their original maze and half in a new maze. In these contexts, half the rats were trained with massed practice while the other half had spaced practice (long inter-trial interval). Spaced practice commonly results in spontaneous recovery of the extinguished response (the first discrimination in this case). The context change seemed to attenuate this effect and facilitate reversal learning in the rats given spaced practice.

In the present experiment, we trained pigs on a left-right spatial discrimination and a discrimination reversal. Half of the pigs remained in the same context for the reversal and half were trained in a new context. Given that early maternal deprivation can induce learning deficits in rats, we predicted that the MD runt and MD large groups should make more errors on the task than SR pigs. In light of the literature suggesting context change facilitates reversal learning, we also predicted that pigs in the new context would make fewer errors than pigs in the same context.



## Method

### *Subjects*

The same animals used in the previous two experiments were tested in Experiment 3. There were four pigs in each rearing group (SR, MD runt, and MD large) for a total of twelve subjects. Mean age at the start of this experiment was 80.8 days (SE = 6.45, range = 56-113).

### *Apparatus*

#### *Training room*

The same training room used in Experiment 2 was used for the present experiment.

### *Materials*

#### *Trays*

The trays used were those from the previous experiment, however, three trays per subject were used during this experiment.

### *Design and Procedure*

The pig entered the start area. Two trays were then set down in the center of Pen 1 parallel to each other and approximately 50 cm apart. For half of the pigs, the tray on the right was the correct choice and for the rest, left was correct (Appendix B). The correct tray was loaded with pig food. When the pig chose a tray, the other tray was picked up and pigs had to return to the start area. If the tray contained food, the pig was allowed to eat. The start area tray was not used after 4 weeks and was replaced with only the pig chow on the floor in its place. Choice on the left / right test was also recorded. This continued until each pig had reached a criterion of 18 out of 20 consecutive trials correct.

After criterion was reached, the discriminations were reversed. If right had been correct, it was now left and vice versa. For half of the pigs, the reversal also involved a context change (Appendix B). Two pigs in each rearing condition had a context change and two stayed in the same context. For the context-change group, the pen adjacent to the original pen (farther from the start area) was used for the discrimination. Ten trials a day were carried out until the criterion of 18 of 20 trials correct was reached.

### Results

A 3 x 2 ANOVA (Groups x Context change) was performed to determine whether the rearing-condition groups differed on their performance of the initial left-right discrimination, using the number of errors to criterion (Table 3.1). Whether a pig was in the Same or Different group was included in the analysis though no context change had yet taken place. This was done to determine that pigs placed in the groups of Same and Different context did not differ on the discrimination before context change was implemented. Not surprisingly, there was no context effect ( $F(1, 6) = 0.86$ ) since at this point there was no context change. There was no significant difference between groups, ( $F(2, 9) = 1.21, p > 0.05$ ). A 3 x 2 ANOVA analyzed performance on the discrimination reversal; using errors to criterion for all three groups (Table 3.2). There was no effect of rearing condition, ( $F(2, 6) = 1.94, p > 0.05$ ) on errors to criterion. As predicted, there was an effect of same versus different context, albeit marginally significant ( $F(1, 6) = 4.46, p < 0.10$ , one-tailed; Figure 3.2). The power for the same versus different context was approximately 50% with six subjects per group. It would take an increase to 13 subjects per group to have a power of 80% (Keppel & Wickens, 2004). There was no

interaction between rearing condition and same versus different context ( $F(2, 6) = 0.16, p > 0.05$ ).

### Discussion

Our data suggest an effect of context on reversal discrimination learning. This is consistent with the literature that states that changing the context between discrimination training and reversal facilitates reversal learning. However, our results must be interpreted cautiously due to the small number of subjects in this study.

We did not find the expected effect of rearing condition on reversal learning or an interaction of rearing condition with context. This may indicate that early rearing condition has no effect on these particular learning processes in the miniature pig. However, it should be noted that the MD pigs were reared in sibling pairs. Thus, the presence of this familiar conspecific may have attenuated the effects of maternal separation. In addition, the pigs' environment was somewhat enriched; they had access to more than one room and were given toys such as soccer balls. Enriched environments have been suggested to attenuate maternal separation effects (Hellemans, K., Benge, L., Olmstead, M., 2004). Another mitigating factor could be early handling of the pigs. The subjects in this experiment experienced interaction with their human handlers several times per day for feeding, pen cleaning and during experimental training. This could have attenuated the effects of being separated from the sow, as handling may reduce anxiety.

In the present experiment, the context was changed along a spatial dimension. Other sensory cues were kept as constant as possible. While we do not know which sensory cues are most important to discrimination and reversal learning in this species, our data

suggest that changes in spatial context affect reversal learning in a manner similar to that seen in other species.

## CHAPTER 4

### Experiment 4: Memory test

#### *Overview*

*Episodic memory defined.* Tulving (1997) characterizes episodic memory as unique from other memory systems. It is the only one not oriented in the present. “Retrieval in episodic memory means ‘mental time travel’ through and to one’s past” (Tulving, 1997, p.11). It is also directed toward our inner experiences rather than more general world knowledge. To disclose any chapter from your personal history, you would be required to draw on episodic memory. Each episodic memory must be discrete from any another and may include the integrated what, where and when of an event, i.e., "I was in the classroom learning grammar when I heard the first bombs drop". These memories are explicitly available for conscious recall.

*Episodic-like memory in animals.* The lack of language in non-humans and the confusion of episodic memory with semantic memory and conditioning make demonstrating episodic memory in animals a challenge. Researchers, including Clayton & Dickinson (1999), have suggested that animals may be capable of episodic-like memory since such memory could have adaptive significance for procuring food, avoiding known danger zones and so forth. It is possible that these memories exist in animals but not in a form equivalent to that in humans. In an attempt to circumvent these problems, Clayton and colleagues have re-defined episodic memory. They suggest a flexibly integrated “what, where and when criteria”. This definition excludes the need for an animal to consciously remember an event from its past, as it is not currently possible to directly test for such an ability.

*Episodic-like memory in scrub jays.* Clayton & Dickinson (1999) demonstrated what appeared to be episodic-like memory in western scrub jays, a food-caching corvid. Birds in one group (“degrade group”) were given information about the differential decay rate of two food types. They cached perishable worms and non-perishable peanuts and recovered them after intervals of four hours, one day or four days. On Day One, when the worms were still fresh, the birds preferentially searched the worm sites, as these were the preferred food. After four days, only peanuts were fresh, and the birds searched preferentially for those. Birds in the second group (“replenish”) found only fresh worms in their cache sites at any interval and always preferred to look for worms. These findings suggest that the birds remembered the unique caching episode: what was stored, where and when it was stored, and were able to separate one episode from other similar episodes of caching. This was the case even when only one tray was used for all caching episodes, ruling out the relative familiarity of one tray over another (Clayton, Yu & Dickinson, 2001). Clayton, Dickinson & Yu (2003) found further support that scrub jays have episodic-like memory by demonstrating: 1) they did not use the differential rate of forgetting one food and 2) the birds could update their information about cache sites and change their behavior accordingly. These are two factors that are important to the definition of episodic-like memory. Clayton et al. (2003) tested the birds trained on this procedure at times that were intermediate to the established one and four day tests. While the replenish group remained steady in their perishable food item (cricket) searching over the five days, birds in the degrade group searched for crickets on Day One and then switched their searching completely to non-perishable peanuts. It is unlikely, then, that the jays were forgetting one food type more rapidly than the other. When birds were

given new information about the state of the caches, i.e. that they were fresh after three days, they switched their preference accordingly. Thus, they updated their memory for the caches.

*Episodic-like memory in pigeons.* Pigeons can remember the specific details of their past episodes according to Zentall, Clement, Bhatt, & Allen (2001). In this study, pigeons were autoshaped to peck disks in response to a vertical line and refrain from pecking when the line was horizontal. Pigeons then chose between two coloured lights depending on whether or not they just pecked. Pigeons were able to remember the action they had just performed and responded accordingly.

*Episodic-like memory in mice.* Mice can remember the unique what-where and when of an experience, according to Dere, Huston & De Souza Silva (2005). The mice encountered a familiar and a novel object in different locations and in temporal order. Dere et al. (2005) suggested that mice could remember the object, location and relative recency of that object.

*Episodic-like memory in rats.* Eacott & Norman (2004) found evidence of episodic-like memory in rats. Eacott & Norman (2004) trained rats to learn four unique combinations of object, context and place. Rats showed recognition for objects in familiar configurations with retention interval delays up to one hour. Lesions to the hippocampal system severely impaired object recognition.

Ergorul & Eichenbaum (2004) have demonstrated episodic-like memory in rats. Rats were taught a sequence involving both spatial and odor cues. Food cups filled with spice-scented sand were placed in a fixed spatial location and a reward was buried in the sand within each cup. Each location was paired with one particular odor. The rats were

presented with one cup each trial, in sequence, A-D. The rats were then tested with two different cups from the sequence. By choosing the earlier-appearing cup, the rat was rewarded. The rats were able to learn the sequence and correctly chose the cup found earlier in the sequence. Lesions to the hippocampus caused rats to return to the most recent rather than the least recent place. It may be that without the hippocampus an animal can remember what and where, but its associated memory for "when" is impaired (Ergorul & Eichenbaum, 2004).

*Criticisms of episodic-like memory experiments in non-humans.* Hampton & Schwartz (2004) raise some important considerations that underscore how tests of episodic-like memory in animals fall short of our definition of episodic memory as it relates to humans. They point out that while episodic memory is a form of explicit memory that must be accessible through free recall, tests with non-humans rely on recognition memory. Another problem is that episodic memory is oriented to the past. In the scrub jay experiments by Clayton & Dickinson (1999), the status of the birds' cache sites was oriented more to the current state of the caches rather than past. Attempts to replicate the model used by Clayton & Dickinson (1999) with other species have so far failed (Hampton & Schwartz, 2004). Finally, tests for episodic memory in animals cannot be distinguished from semantic memory, a type of memory for rule-based information rather than memory for personal experiences.

Roberts (2002) cites a number of alternative explanations for what appears to be episodic memory in animals. These include relative strength of memory traces and circadian oscillators that set animals up for time-behaviour associations.



However, Zentall (2005) points out that animals cannot only "report" about their recent experiences or behaviour but can use anticipation of some future event to guide their behaviour. He argues that animals may have some representation for these anticipated future events in memory. Zentall (2005) acknowledges the limitations of testing for episodic memory in animals, including the problem of distinguishing between semantic and episodic memory and the difficulties with Clayton & Dickinson's (1999) what-where-when definition of episodic memory. In humans, episodic memories do not always have all three criteria but nevertheless resemble personal accounts of unique events. However, because animals appear able to judge time duration, plan for the future and "answer" questions based on their own recent responses, there may be a basis for establishing that they are capable of answering more elaborate questions about their past experiences than was previously thought (Zentall, 2005).

In the current study, we replicated the first step of Ergorul & Eichenbaum's (2004) study of episodic-like memory by training pigs on a sequence of tray and odor combinations in distinct locations. We then tested whether pigs could return to the least recent tray when tested with the most recent and least recent trays. We examined whether the distance between trays affected the pigs' performance on tests.

## Method

### *Subjects*

Four female pigs from the previous experiments, with a mean age of 164 days (SE = 3.53, range 153-176) were used in the odor/spatial component of this experiment. Of the four, two were from the SR group, one was from the MD runt group and one was from

the MD large group. The remaining 8 pigs were not included in this experiment because of a conflict with a concurrent nutritional study that the pigs were involved in.

### *Apparatus*

#### *Training room*

The training room used in Experiments 1-3 was used for the present experiment. Only the first of the five pens was used.

### *Materials*

#### *Trays*

Four trays from the previous experiment were used.

#### *Odors*

Eight spice mixes and a liquid essence purchased from a local grocery store were each mixed with tap water (2g spice to 150 ml water or 10ml essence to 150ml water). An odor mix was soaked into a small piece of paper towel and placed in the perforated cube on the tray lid. The odors used were cocoa, ginger, mint, thyme, cumin, garlic, parsley and lemon.

### *Design and Procedure*

#### *Odor discrimination training*

Six pigs were given odor training to determine their olfactory discrimination abilities with these odors. Two pigs (Val and Dora) would not continue on to the memory experiment due to their relative lack of cooperation and slow performance on the reversal and odor discrimination task. The procedure followed that of the left-right discrimination, however one tray contained an odor, while the other contained "no odor" (plain tap water). The trays were switched randomly from the left to right position to reduce

reliance on spatial cues. Pigs had 10 trials a day until they were choosing correctly for 9 out of 10 consecutive trials. Appendix C lists the odors each pig was tested on and the number of trials needed to reach criterion for each odor. The particular odors used were not important. What was relevant was whether pigs could distinguish between a variety of odors. In order to solve the memory task, pigs must be able to tell several odors from each other (if they are using odor as a cue) so whether they were capable of smelling each of these odors on their own was of interest. Once a pig learned the odor discrimination, it was trained on another odor in order to get as much of this data as possible. Thus, some pigs were trained on more discriminations than others. It is of some concern that the trials to criterion for each odor were variable. It may be important that the odors be equally easy to distinguish before being used in the memory task. However, some odors are simply more potent and detectable than others, and this may be the reason for the discrepancy.

#### *Memory experiment*

Eight spatial positions on the pen floor (Figure 4.1) were chosen and numbered as were eight spice odors. Each day, four positions and four odors were used. Odors were placed in the tray lid as described above. The locations and odors were quasi-randomly selected (using number tables generated from numbers in the phone book) and listed in order, four location-odor pairs per training day. No odor or location was used more than once in a training session. No particular odor/location pair was used more than three times throughout the experiment and individual locations appeared no more than ten times during the experiment.

Trays were given a number from 1-4 to correspond to the location-odor pair that appeared first, second, third and fourth each training day. Pigs began in the start area while a tray was placed in Pen 1. As before, they had to return to the start area after each trial. This procedure was repeated for the remaining three trays. After four trials, pigs immediately received a test in which Tray 1 and Tray 4 were placed in their respective locations and pigs had to choose one tray. Tray 1, the first tray that pigs were trained on each day, was always correct and contained the food reward. Choice on test (correct or incorrect) was recorded. The pigs received a mean of 22.2 tests each (SE =1.93, range 18-26). Appendix D shows the number of tests each pig received and their correct or incorrect choice on the test.

## Results

Choice on tests (correct or incorrect) for each pig is shown in Appendix D. A chi-square analysis was performed to determine whether the pigs choose Tray 1 more often than chance. Pigs returned to the first tray 72% of the time, ( $\chi^2 (1) = 9.68, p < 0.01$ ; Figure 4.2). Percentage correct on tests was analyzed in blocks of four. Percentage correct for each pig can be seen in Appendix E. There was no evidence of a linear trend, ( $t (3) = 1.06, p > 0.05$ ). That is, performance did not improve across blocks.

To rule out that choice on test did not depend on how close or far the correct tray was in relation to the other tray, a Pearson Correlation determined that there was no significant correlation between choice on test and distance between trays ( $r (87) = -0.044, p > 0.05$ ). This indicates that distance between trays (Appendix F) did not predict performance, (see Appendix F).

## Discussion

Pigs could distinguish between two objects they encountered in different locations and temporal order. Interestingly, they did not show acquisition but had a preference for the least recent tray. These data are consistent with an interpretation based on the pigs being sensitive to relative recency. The memory trace for one episode or object is stronger the more recently it occurred. Pigs may have been employing a heuristic such as "choose the least recent food tray" (the one with a weaker memory trace) in order to be rewarded. Olton, Collison & Werz (1977) found that rats do not use recency or other serial-order effects to choose previously unvisited arms in a radial maze. The rats could chose a new arm over 14 times in an 17-arm radial maze before making an error and did not seem to repeat the most recent choices during those errors. Further study is needed to determine if pigs have a similar tendency or if they are indeed remembering a response pattern that depends on recency. This tendency to respond to the least recent tray is similar to the win-shift pattern that has been demonstrated in rats (Olton, Collison & Werz, 1977) and in pigs (Laughlin & Mendl, 2004).

The distance between trays did not affect pigs' ability to make the correct choice. Even when the incorrect tray was much closer to the door where pigs entered the pen, they returned to the least recent (and farther away) tray. In addition, two trays close together did not seem to confuse the pigs, as they still chose correctly. That they appeared highly motivated to choose correctly is interesting given that the pigs would be fed (at the start area immediately after choosing and after the test during regular feeding time) regardless of choice.

Ergorul & Eichenbaum (2004), Eacott & Norman (2004) and Olton & Papas (1979) found that damage to the hippocampus impaired performance on tests on memory for objects with distinct odors and spatial locations. Maternal deprivation is linked to hippocampal impairment (Anderson & Teicher, 2004; Huot, Plotsky, Lennox & McNamara, 2002; Sibug et al., 2001). In the present study, SR pigs did not perform differently than MD pigs. However, we do not have the data to consider this outcome statistically.

Although the mechanism guiding their choices is not clear, the observation that pigs return to the least recent location indicates that pigs are sensitive to temporal order. This observation may provide the basis for developing tests of episodic memory in pigs in the future when the effects of maternal deprivation can be examined.

## CHAPTER 5: GENERAL DISCUSSION

The previous experiments provided insight into the learning and memory performance of pigs. They also expanded what is known about the effects of rearing condition on learning and memory. It was found that pigs are capable of making discriminations, reversals and using relative recency to solve a memory task. Pigs that were moved to a new context learned the reversal in fewer errors to criterion than pigs that remained in the same context. Rearing condition did not appear to interact with performance on these learning tasks with the exception of the shuttle training experiment. On this measure, SR pigs had longer latencies to shuttle than MD runt and MD large pigs.

*Maternal deprivation.* Studies have found that maternal deprivation produces detrimental outcomes in offspring. These effects include HPA axis and emotional disturbances (Bremnar & Vermetten, 2001; Ladd et al., 2000; Marci et al., 2004; Plotsky et al., 2005). Maternally-deprived animals may also demonstrate brain and learning impairments. For example, they may demonstrate reduced spatial abilities (Sibug et al., 2001) and impaired reversal learning (Lyons & Schatzberg, 2002).

The hippocampus, a brain structure involved in various types of learning and memory, such as navigational abilities (Burgess & O'Keefe, 1996), is thought to be affected by variations in maternal care. Bredy et al. (2003) found that maternally-deprived rats had less surviving cells in the hippocampus than non-maternally deprived rats. This may provide some clues as to why maternally-deprived animals show a deficit in learning and memory abilities. Other possible explanations for the effects of maternal deprivation on learning and memory in offspring include anxiety and changes in attentional abilities (Bolhuis et al., 2004).

The literature on maternal deprivation has focused mainly on rats. However, rats may have different outcomes from maternal deprivation compared to other mammals. This is because rats undergo a period early in their development when they are hyporesponsive to stress. It appears that the rat dam regulates the HPA axis with the result that rat neonates are protected from the deleterious effects of stressors. This may be a mechanism that helps to foster attachment of infants to the mother, thus aiding in their survival. Humans are not known to undergo this hyporesponsive period (Gunner & Donzella, 2002; Levine & Mody, 2003) and it is not known whether other mammals experience a similar period.

*Maternal deprivation in the current studies.* Overall, there is no evidence to suggest that maternal deprivation affects hippocampal-based learning performance in MD pigs. Instead, performance was equivalent to SR pigs, with the exception of Experiment 2. There were no differences between SR and MD pigs on discrimination, reversal or memory tasks.

The finding that SR pigs had longer latencies on the shuttle task was in contrast to the literature showing that maternally-deprived animals are less active and so have longer latencies on some learning tasks (Kaneko et al., 1997; Kanitz et al., 2003). There are alternative explanations for our lack of a maternal deprivation effect. In Experiment 2, SR, not MD, pigs had longer latencies to shuttle. MD pigs appeared to explore the room much less than the SR pigs. The MD pigs may have been more fearful, and therefore less comfortable with exploring a novel environment. Alternatively, they may have been familiar with a regimented feeding schedule (as they were formula fed on a set schedule) while SR pigs were accustomed to being fed on demand. Because food was readily available from the sow, they may have become less motivated to procure food and



therefore, less motivated to solve a task with a food reward. It would have been instructive to have obtained growth rates for the pigs, so as to compare SR and MD pigs on this and its relationship to motivational differences in feeding behaviour. Future studies on maternal deprivation in pigs should take this into consideration.

Pigs may differ from other species in the effects of maternal deprivation on learning. As discussed, maternal deprivation has been primarily studied in rats, which have a hyporesponsive period not seen in other species. Pigs may have other coping mechanisms that are not yet understood. Each MD pig in this study was paired and raised with a sibling partner. The pair could be observed to engage in close contact (such as nestling together to sleep) as well as playing together, especially earlier in their development. The presence of this littermate may have attenuated the negative effects of maternal deprivation. In addition, these pigs were handled by humans for brief periods each day. Handling has been shown to reduce the negative impact of maternal deprivation (Ladd et al, 2000; Pryce & Feldon, 2003). It is also possible that with a larger number of subjects, an effect of maternal deprivation on pigs can be detected.

Future studies should investigate maternal deprivation further in non-rodent mammal species. This may help to uncover whether mammals that may not undergo a hyporesponsive period are similarly affected by maternal deprivation.

*Learning in pigs.* In recent years, attention to the cognitive abilities of pigs and other livestock animals has increased. Partly, this may be due to the heightened awareness of animal welfare. The purpose of the preceding experiments was, in part, to extend what is known about the cognitive abilities of pigs.

It is known that pigs can learn discriminations (Croney et al., 2003; Moustgaard et al., 2004). However, very few studies have examined reversal learning in pigs. In Experiment 3, it was found that pigs can learn a left-right discrimination and a reversal. Macintosh (1974) found that rats may make only a single error with successive reversal learning. This was also the case for some pigs in the current study. It was also found that context change had an effect on pigs' ability to learn a reversal and that this effect occurred in a single reversal. This is in agreement with several studies that show that context change helps to reduce retroactive inference and serves as a retrieval cue that a new response contingency is in effect (Cheng, 2005; Chiszar & Spear 1969; Thomas et al., 1985; Walsh et al, (in press)).

*Olfactory discrimination.* Pigs can recognize conspecifics from the scent of their urine ( Meese, Connor & Baldwin, 1975). Croney et al., (2003) found that pigs can use odor to find hidden food rewards. In Experiment 4, it was demonstrated that pigs can make two-choice discriminations between two odors (scented and unscented trays), learn new odor discriminations in succession and switch their responses when the reward contingency is switched (i.e. when the correct tray was switched from being the scented to the unscented tray or vice versa).

*The question of episodic-like memory in animals.* Pigs have impressive memory abilities as has been demonstrated on various spatial learning tasks (Held et al., 2005; Laughlin & Mendl, 2000). What is known about memory in pigs was extended by Experiment 4. We found that pigs are capable of detecting the relative recency of two items in distinct spatial locations. This may represent a starting point for developing tests of episodic-like memory in animals. This will be discussed in greater detail later.

Clayton & Dickinson (1999) and others have made an interesting case that episodic memory can be demonstrated in animals (Dere et al, 2005; Ergorul & Eichenbaum, 2004). For example, food-storing corvids appear to use their memory for cache status and location to direct their future cache retrieval (Clayton & Dickinson, 1999). Rats trained on a sequence of location-odor pairs can return to the pair that appeared earlier in the sequence (Ergorul & Eichenbaum, 2004).

*Criticisms of episodic-like memory studies in animals.* Hampton & Schwartz (2004) argue that the birds in the Clayton & Dickinson (1999) experiments are actually applying knowledge of the current state of the caches, not mentally traveling back in time. Roberts (2002) asserts that animals that appear to remember a sequence of events in order are using a memory trace in working memory, not episodic-like memory. The memory for these events is held in working memory as the animal makes its choice. Accuracy on such tests tends to decline as the interval before the test lengthens (Roberts, 2002).

Another possible alternative explanation for accounts of episodic-like memory in animals is that they are displaying a win-shift tendency. In a win-shift spatial memory task, animals are rewarded for choosing the less recently visited place. Gaffan & Davies (1981) found that rats had a tendency to avoid the most recent place they visited on a radial maze and that this tendency was stronger after non-reward than after reward. When the choice was between a more familiar versus more novel place, rats again learned a win-shift strategy better than win-stay (Gaffan & Davies, 1981). This is in accordance with the findings of Olton & Schlosberg (1978) in a similar radial arm experiment. They found that rats learned a win-shift strategy more rapidly than they did a win-stay strategy. When every choice was rewarded, rats followed win-shift, not win-stay pattern. There

may be a natural tendency that once found in one place searches are directed to a different place (Olton & Schlosberg, 1978). Timberlake & White (1990) found that both food-deprived and non-deprived rats that were rewarded (food was placed at the end of each arm of a radial maze) all tended to follow a win-shift pattern. However, non-deprived, non-rewarded rats tended to search previously visited arms instead (Timberlake & White, 1990). In the current experiment, pigs were required to choose between two places, one more recent than the other. The pigs, like rats, may have a tendency to avoid more recent places in favor of less recent in accordance with the win-shift strategy. This could explain their choice of the less recent tray on tests.

*Are there better ways to test for episodic-like memory?* Some authors have suggested ways to improve upon the methods used in tests of episodic-like memory. Zentall (2005) argued that it is perhaps important to ask animals questions about their own past behaviour in order to make their answers more in line with our conceptions of human episodic memory. It has been demonstrated thus far that animals can indeed make responses based on their own earlier responses (Zentall et al., 2001). There are several persistent problems that have surfaced when researchers have tried to demonstrate that animals have episodic memory. One such problem is that when a person expects to be asked a question about their personal history or when they deduce their past experience based on general information they are actually using their semantic memory, not episodic memory. In animal studies, it is difficult to create an environment where the subjects do not expect to be "asked" the question. For examples, in the study by Ergorul & Eichenbaum (2004) rats were trained to be presented with cups A through D followed by a choice test with two of the cups. It could be argued that the animals thus "expected" to

be tested on their memory for two of the cups. Randomizing the order of cups presented may not completely eliminate the potential that the animals are expecting the test and relying on semantic memory to respond accordingly. Zentall et al. (2001) made an attempt to reduce this problem by training pigeons to peck "yes" and "no" keys in response to lights that prompt them to answer whether or not they just pecked to the previous lights. Attempts were made to make these questions as unanticipated as possible. Pigeons were shown yellow and blue lights and were not required to peck in order to be reinforced. They were then unexpectedly prompted with the lights associated with the answers yes and no to reveal whether or not they had just pecked. However, the birds still had been previously trained to peck in response to the yellow light in an earlier experiment. They may have therefore associated it with rule-based, semantic memory.

*Future directions.* In conclusion, our data showed that the pigs had a preference for the least recent tray in the sequence. What is not clear is which cue or cues pigs were using to make their choices. Odor probes and spatial probes (where the odor and spatial cues of each tray are switched during the test) could help answer this question. Future studies should extend this experiment in order to investigate whether pigs and other species are capable of episodic-like memory.

If there is evidence for the existence of episodic-like memory in the pig and other domesticated and commercially-used animals, this could dramatically change the way we view and treat them. The knowledge that animals can remember their personal experiences may place a greater emphasis on animal welfare and care. The discovery of a method for uncovering episodic memory that does not require language could also be

applied to tests for memory deficits in humans lacking verbal ability. Therefore, this is a worthwhile problem that deserves further study

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Table 2.1: Two-way Analysis of Variance on latency to open lid for SR, MD runt  
and MD large pigs

Source	DF	SS	MS	F
Group	2	232.82	116.41	0.29
Between-Ss error	9	3654.84	406.09	
Trials	15	5540.25	369.35	2.08*
Group x Trials	30	4462.34	148.74	0.84
Within-Ss error	135	23993.66	177.73	
Total	191	37883.91		

\* significant at .05 alpha level

Table 2.2: Mean ( $\pm$  SE) latencies in seconds to open the lid for the SR, MD runt and MD large pigs

Trial	Rearing-condition Group					
	SR		MD runt		MD large	
	M	SE	M	SE	M	SE
1	20.50	7.79	22.75	3.09	17.00	8.49
2	10.50	3.57	17.75	8.84	8.75	2.78
3	10.50	4.29	29.50	12.58	10.25	5.30
4	13.50	4.84	14.25	5.28	22.00	17.67
5	11.50	4.29	9.75	1.75	15.00	7.53
6	7.00	.58	37.75	25.56	22.75	17.44
7	7.50	2.10	7.00	1.08	15.50	10.05
8	9.00	1.22	6.00	2.04	4.50	1.55
9	7.75	2.32	9.00	3.58	5.75	3.09
10	6.75	1.80	12.25	6.42	5.50	2.22
11	15.75	11.93	4.25	1.03	3.25	1.31
12	3.25	1.31	2.50	0.50	2.00	0.41
13	15.75	3.71	6.25	1.97	3.25	0.85
14	14.75	6.61	6.25	1.44	5.00	1.22
15	8.00	2.48	7.00	4.34	4.50	1.94
16	6.50	1.85	3.50	1.19	8.25	4.57

Table 2.3: Two-Way Analysis of Variance on latency to shuttle for pigs in the SR, MD  
runt and MD large groups

Source	DF	SS	MS	F
Group	2	104394.62	52197.31	14.54*
Between-Ss error	9	32303.50	3589.28	
Trials	13	195974.79	15074.98	4.29*
Within-Ss error	117	411588.50	3517.85	
Interaction	26	83535.71	3212.91	.91
Total	167	823797.12		

\*significant at the .05 alpha level

Table 2.4: Mean (+SE) latencies in seconds to shuttle for SR, MD runt and MD large pigs

Trial	Rearing condition group					
	SR		MD runt		MD large	
	Mean	SE	Mean	SE	Mean	SE
1	169.75	66.57	127.75	58.17	115.00	29.75
2	181.75	66.26	40.25	11.74	73.75	17.86
3	141.25	45.04	49.75	32.03	44.00	6.84
4	81.75	37.11	67.00	21.61	173.75	99.29
5	100.50	28.25	56.50	16.83	29.50	8.57
6	88.50	32.33	31.75	12.49	55.00	32.57
7	119.25	26.93	21.75	5.76	22.50	6.17
8	92.25	30.54	21.50	8.97	11.25	3.50
9	63.25	8.42	16.25	5.20	16.00	1.22
10	89.75	27.75	29.75	23.09	16.25	3.12
11	40.00	23.93	30.50	16.55	25.00	9.39
12	57.00	18.25	6.25	0.63	10.25	2.02
13	49.25	22.45	18.50	9.70	12.75	5.17
14	33.75	7.82	9.50	4.99	11.50	2.60

Table 3.1: Two-Way Analysis of Variance on errors to criterion on the left-right  
 Discrimination for pigs in Same and Different context in SR, MD runt and MD large  
 Groups during training of the reversal

Source	DF	SS	MS	F
Group	2	44.67	22.33	1.02
Context	1	18.75	18.75	0.86
Interaction	2	26.00	13.00	0.59
Error	6	131.50	21.92	
Total	11	220.92		

Table 3.2: Two-way Analysis of Variance on errors to criterion on the discrimination reversal for pigs in the SR, MD runt and MD large groups in the same and different context conditions during the initial training

Source	DF	SS	MS	F
Group	2	26.17	13.08	1.94
Between Ss error	6	40.50	6.75	
Same / Different	1	30.08	30.08	4.46
Interaction	2	2.17	1.08	0.16
Total	11	98.92		

Figure 2.1: Diagram of home pens

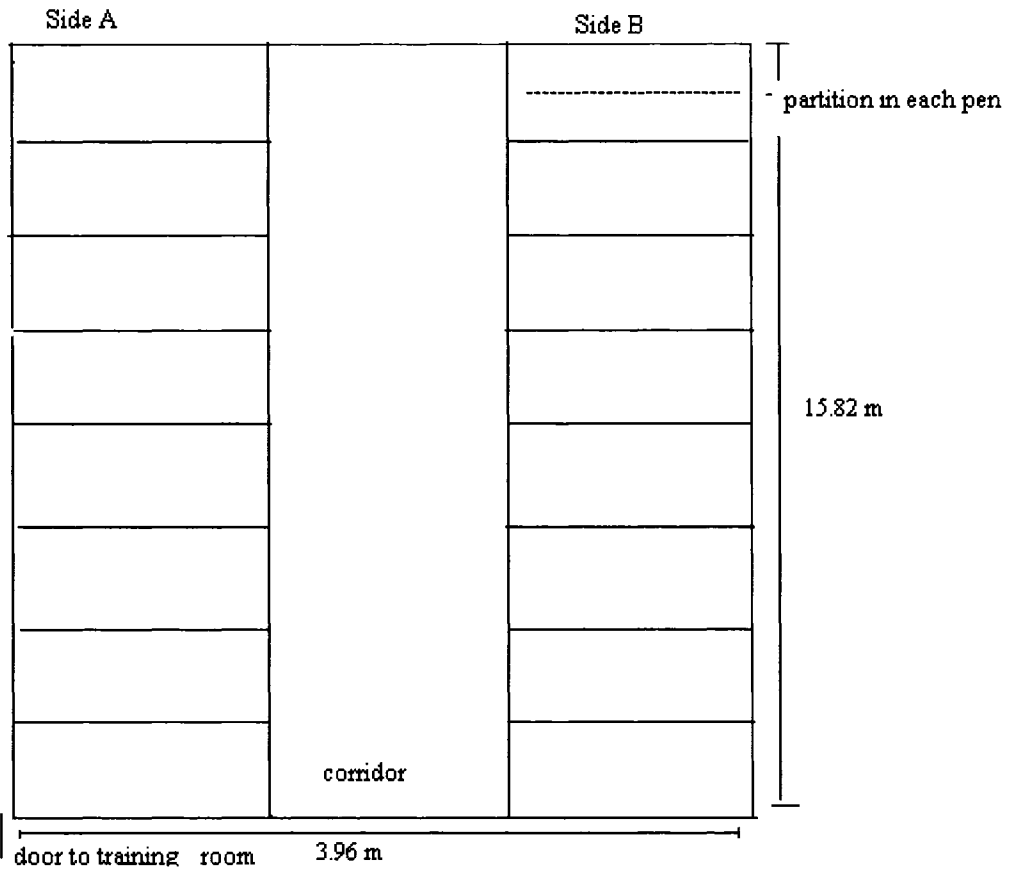


Figure 2.2: Diagram of Tray

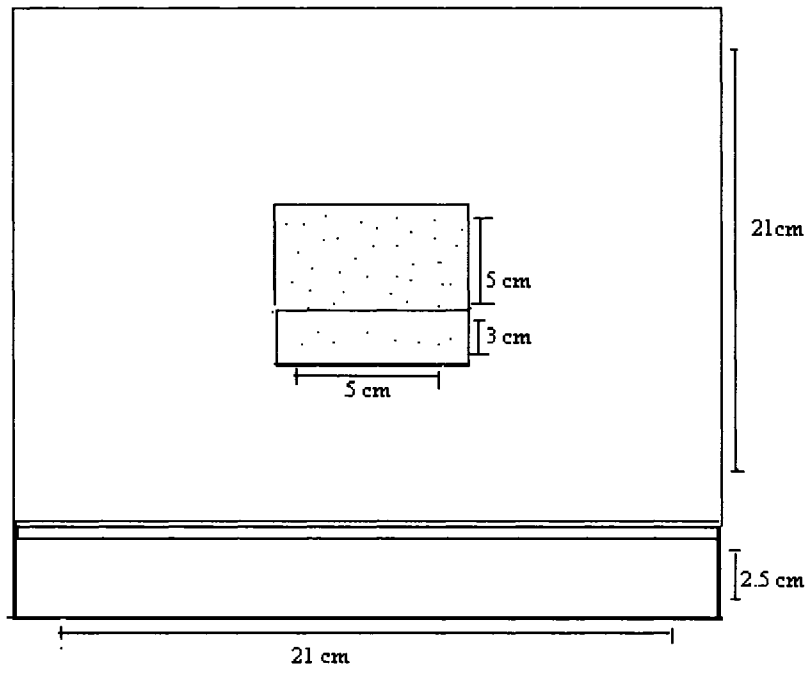




Figure 2.3: Graph of mean group latencies to open tray lid

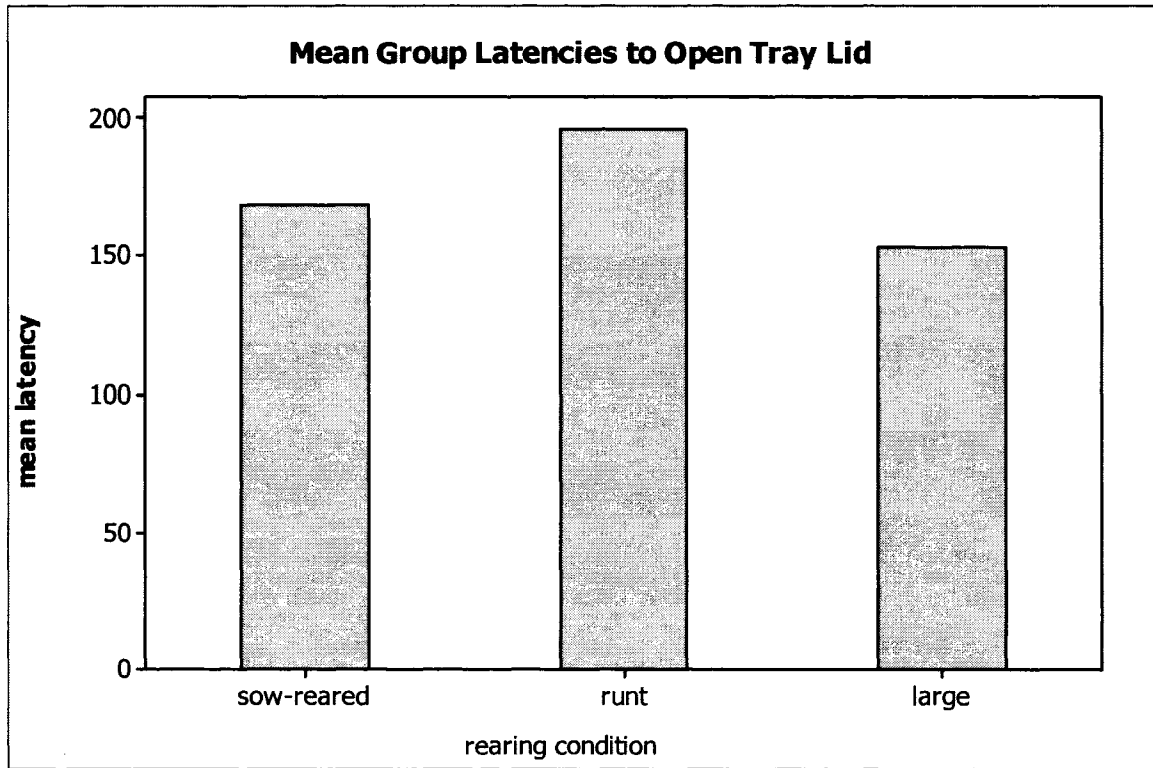


Figure 2.4: Diagram of training room

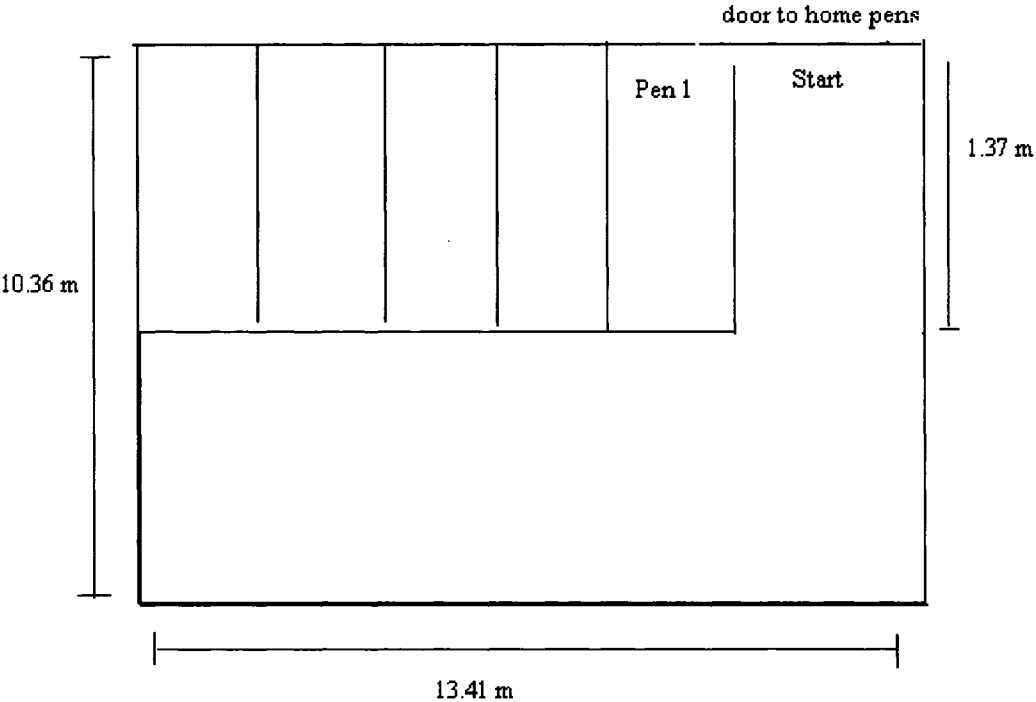


Figure 2.5: Graph of mean group latencies to shuttle

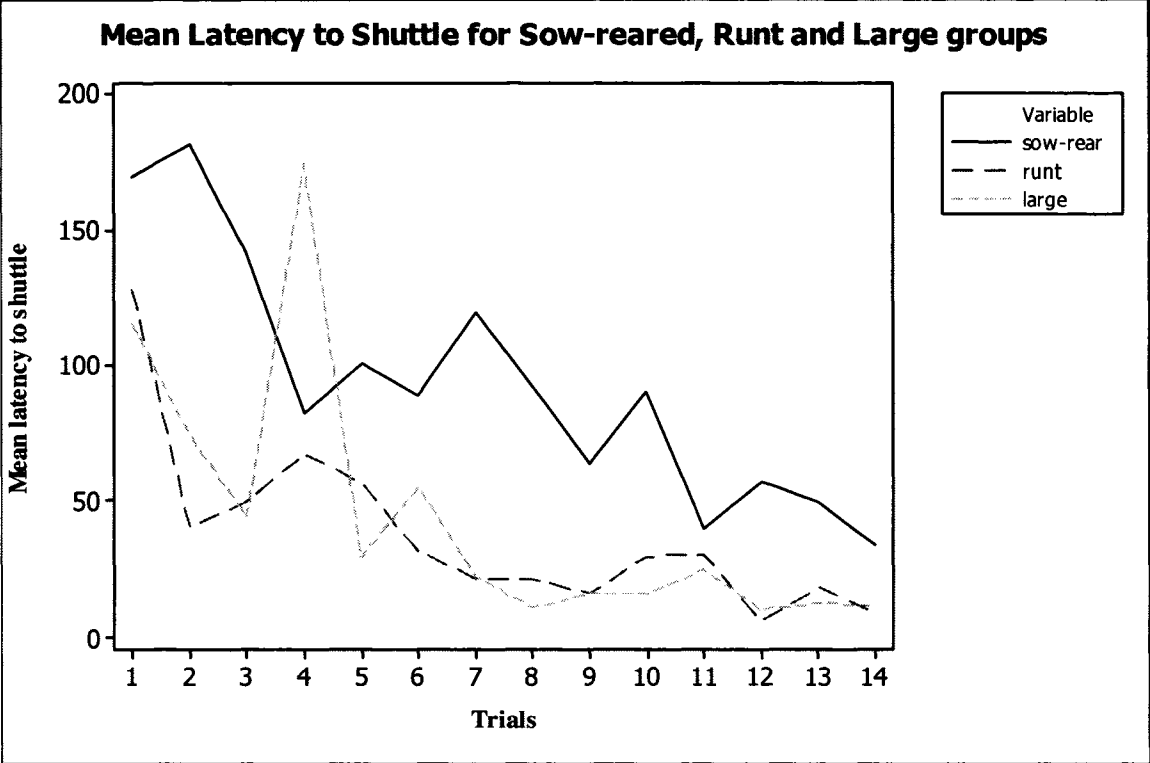


Figure 3.1: Mean group errors to criterion for the left-right discrimination

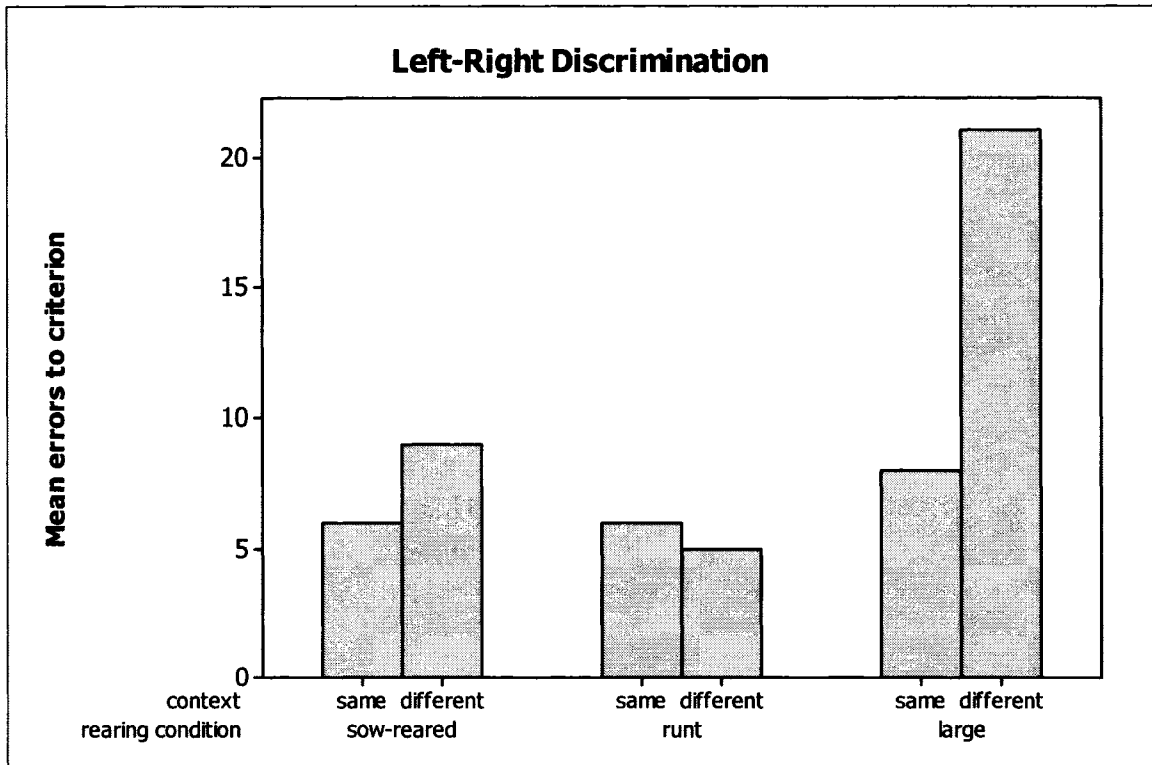


Figure 3.2: Mean group errors to criterion on the discrimination reversal

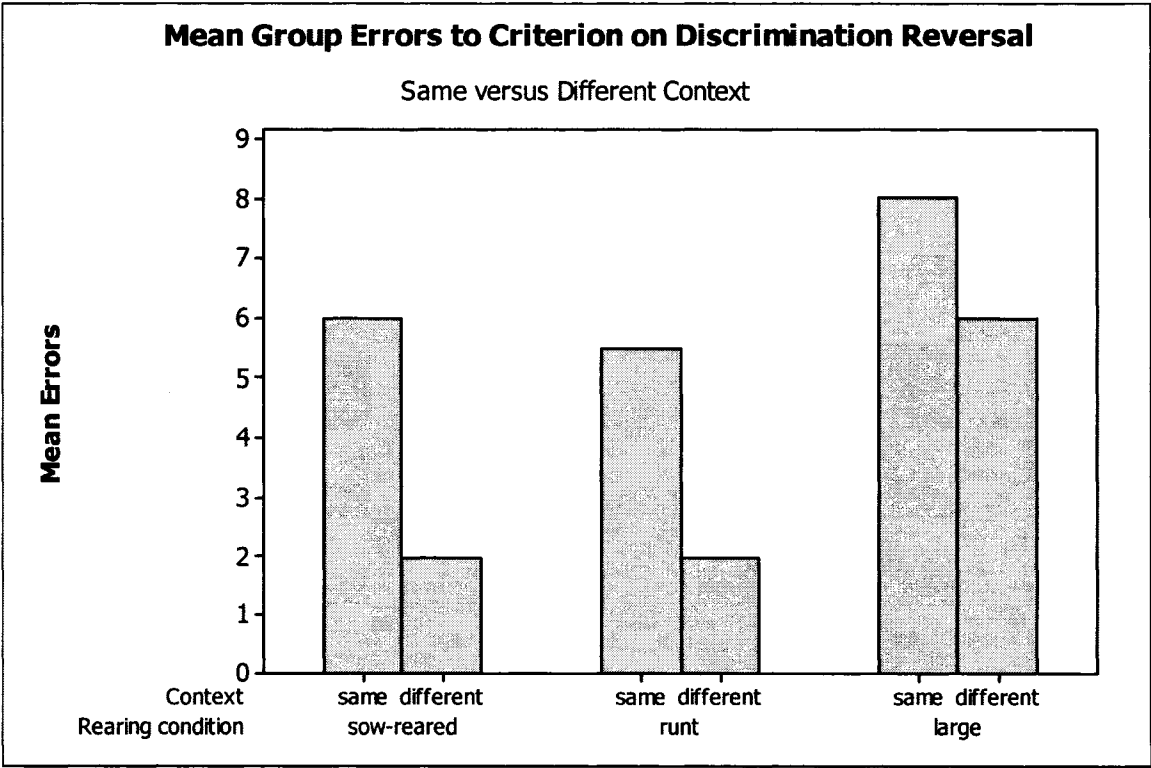


Figure 4.1: Diagram of tray locations

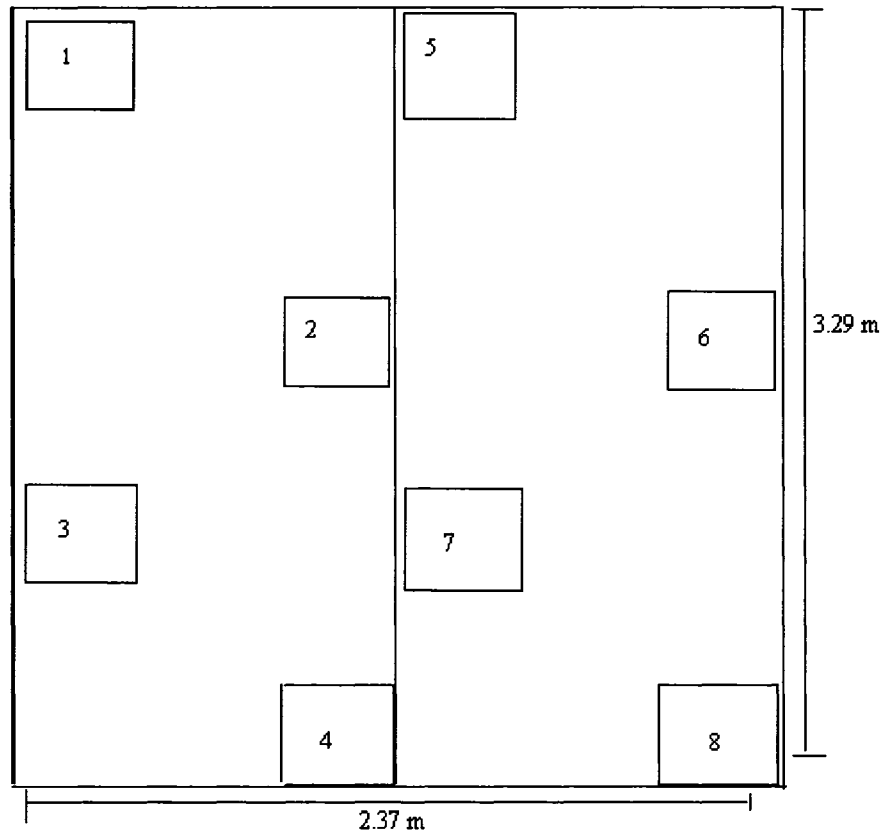
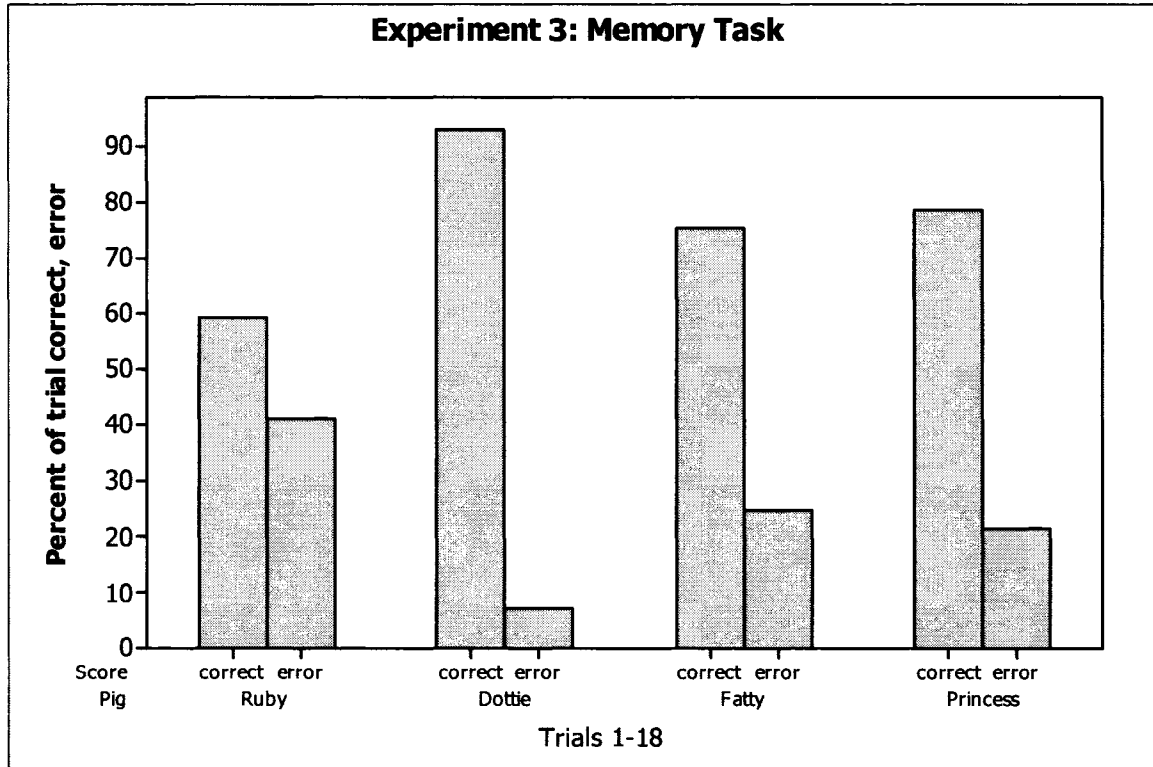


Figure 4.2: Percentage of correct choices and errors for individual pigs on the memory task



Appendix A: Age of pigs in days at onset of experiments

Subject	Rearing	Experiment				
		1	2	3 (L/R)	3 (Reversal)	4
Dora		102	105	110	114	
Ruby		102	105	113	114	172
Fatty		102	105	112	119	176
Val		78	86	89	99	
P.T.		78	81	89	96	153
Dottie		78	86	89	96	153
Harvey		41	58	63	78	
Chopper		38	61	65	87	
Rosie		38	61	65	77	
Doug		33	52	56	59	
Chevy		33	55	60	62	
Quincy		33	52	58	60	



Appendix B: Errors to criterion on left-right discrimination and reversal task for pigs in the SR, MD runt and MD large groups in Same and Different context

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Pig	Rearing Group	Context	Errors L-R	Errors Reversal
P.T.	SR	Same	4	5
Ruby	SR	Different	8	3
Rosie	SR	Same	2	7
Chevy	SR	Different	1	1
Dottie	MD runt	Different	3	3
Dora	MD runt	Same	2	5
Harvey	MD runt	Same	4	6
Quincy	MD runt	Different	2	1
Val	MD large	Same	7	12
Fatty	MD large	Different	4	7
Chopper	MD large	Different	17	5
Doug	MD large	Same	1	4

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Appendix C: Odor training

Trials to Criterion

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P

Pig	Odors tested	Number of trials
Dottie	cocoa +	47
	oregano -	9
Dora	orange -	50
	garlic -	11
	vinegar +	30
Fatty	cocoa -	26
	thyme +	16
Ruby	ginger +	30
	mint -	40
Val	orange +	36
	cumin -	60
P.T.	mint +	20
	parsley -	26

white pepper - 11

lemon + 10

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*Note.* Plus sign indicates that the odor was the correct choice. Minus sign indicates water (no odor) was the correct choice. Number of trials indicates number of trials to reach criterion of 9 out of 10 trials correct.

Appendix D: Scores on memory test for individual pigs

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Correct choice (C) or error (X)

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	Dottie	P.T.	Fatty	Ruby
Trial				
1	C	X	C	C
2	C	C	C	C
3	C	C	X	C
4	C	X	X	X
5	C	C	C	X
6	C	C	C	X
7	C	C	X	X
8	C	C	C	C
9	C	X	C	X
10	C	C	C	C
11	X	X	X	X
12	C	X	C	X
13	C	C	C	C
14	C	C	C	X
15	C	C	C	C
16	C	C	C	C
17	C	C	X	C

18	C	X	X
19	C	C	X
20	C		C
21	C		C
22	C		C
23	C		C
24	C		C
25	C		C
26			C
27			
28			

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*Note.* Correct choice indicates that the pig chose tray 1 when given a choice between tray 1 and tray 4. Error indicates that the pig chose tray 4 instead.

Appendix E: Percentage correct on memory test in blocks of four trials

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Pig	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
Ruby	75%	25%	25%	75%	50%	100%
Dottie	100%	100%	75%	100%	—	—
Fatty	50%	75%	75%	100%	25%	—
P.T.	50%	100%	25%	100%	100%	100%

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*Note.* Blocks 5-6 were excluded from analysis because not all pigs had completed them

Appendix F: Distance between trays in each pair of locations in centimeters

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Locations	Distance	Locations	Distance
1-2	137.20	4-5	332.70
1-3	256.50	4-6	218.40
1-4	198.10	4-7	119.40
1-5	137.20	4-8	137.20
1-6	274.30	5-6	137.20
1-7	218.40	5-7	256.50
1-8	396.20	5-8	198.10
2-3	119.40	6-7	119.40
2-4	256.50	6-8	256.50
2-5	119.40	7-8	119.40
2-6	137.20		
2-7	78.70		
2-8	218.40		
3-4	119.40		
3-5	218.40		
3-6	274.30		
3-7	137.20		
3-8	218.4		









