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Place and direction learning in a spatial T-maze task by neonatal piglets

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Abstract

Pigs are a valuable animal model for studying neurodevelopment in humans due to similarities in brain structure and growth. The development and validation of behavioral tests to assess learning and memory in neonatal piglets are needed. The present study evaluated the capability of 2-wk old piglets to acquire a novel place and direction learning spatial T-maze task. Validity of the task was assessed by the administration of scopolamine, an anti-cholinergic drug that acts on the hippocampus and other related structures, to impair spatial memory. During acquisition, piglets were trained to locate a milk reward in a constant place in space, as well as direction (east or west), in a plus-shaped maze using extra-maze visual cues. Following acquisition, reward location was reversed and piglets were re-tested to assess learning and working memory. The performance of control piglets in the maze improved over time ($P < 0.0001$), reaching performance criterion (80% correct) on day 5 of acquisition. Correct choices decreased in the reversal phase ($P < 0.0001$), but improved over time. In a separate study, piglets were injected daily with either phosphate buffered saline (PBS; control) or scopolamine prior to testing. Piglets administered scopolamine showed impaired performance in the maze compared to controls ($P = 0.03$), failing to reach performance criterion after 6 days of acquisition testing. Collectively, these data demonstrate that neonatal piglets can be tested in a spatial T-maze task to assess hippocampal-dependent learning and memory.

Keywords

Cognition; hippocampus; memory; scopolamine; swine

Introduction

There is increased interest in using the pig (*Sus scrofa*) as an alternative animal model to rodents and non-human primates for studying brain development and cognition. The porcine brain is gyrencephalic, like the human brain, and is more comparable to human than laboratory rodents in terms of anatomy, growth, and development (see reviews: Lind et al. 2007; Gieling et al. 2011a). Both human and pig brains undergo a major growth spurt from the late prenatal to early postnatal stage, unlike other common animal models (Dobbing and Sands 1979), enhancing their translational value. Compared to non-human primates, pigs are less expensive to house and maintain in research settings, creating a potential intermediate research species between preclinical trials with rodents and clinical trials in humans (Gieling et al. 2011a). For these reasons, pigs are becoming an increasingly popular model to study a

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variety of brain disorders, to investigate the impact of environmental insults on the developing brain, such as nutrition or infection, and to test potential therapeutics (see reviews: Lind et al. 2007; Kornum and Knudsen 2011). In addition to being a precocial species, pigs can be weaned immediately after birth, thrive in artificial rearing systems, and can be trained to perform behavioral tasks using a reward-based system at a young age (e.g., Hagl et al. 2005; Wang et al. 2007; Dilger and Johnson 2010), making them a valuable model to study effects during the perinatal period.

In recent years, several cognitive behavioral tasks for pigs have been developed (Wang et al. 2007; Siegford et al. 2008; Arts et al. 2009; Jansen et al. 2009; Kouwenberg et al. 2009; Dilger and Johnson 2010; Gieling et al. 2011b). However, few of the tests have been applied to piglets in the neonatal period (with the exception of work by Zanella et al.: e.g.,Siegford et al. 2008), when the brain is undergoing rapid growth and development (Dobbing and Sands 1979). Though many of these tasks have been adapted from those found in the rodent, primate, and/or human literature, rarely have they been validated through behavioral or pharmacological means for use in pigs (see review: Kornum and Knudsen 2011).

Therefore, the development and validation of cognitive behavioral tests for neonatal piglets to investigate the impact of perinatal environmental insults on neurodevelopment are needed. A spatial cognitive task was chosen for several reasons. First, spatial cognition is evolutionarily relevant to swine, as free-ranging pigs often live in varied habitats, containing woods, swamps, and grasslands, and must recall the location of resources important for survival, such as food, water, and nest sites (Stolba and Woodgush 1989). Second, the hippocampus has been shown to be sensitive to experimental manipulations, including infection (e.g., Gibertini et al. 1995; Fatemi et al. 1999; Archibald et al. 2004) and dietary treatment (e.g., Lim et al. 2005; Wang et al. 2007; Abraham and Johnson 2009), which are of relevance to future studies in our lab. Last, an abundance of literature in both humans and rodents has demonstrated that the hippocampus plays a pivotal role in spatial learning and memory (see reviews: Jarrard 1995; Maguire 2001; Nakazawa et al. 2004). Therefore, our goal was to develop a hippocampal-dependent cognitive spatial T-maze task for use with neonatal piglets. Validation for this task involved the administration of scopolamine, an anti-cholinergic drug that causes memory dysfunction in a variety of spatial tests in animals and humans (see reviews: Decker and McGaugh 1991; Ebert and Kirch 1998) by acting as a competitive antagonist at muscarinic receptors in brain structures that are important for memory, including the hippocampus (Mesulam et al. 1983; Decker and McGaugh 1991; Nielsen et al. 2009).

Material and methods

Animals, Housing and Feeding

Naturally farrowed Yorkshire piglets (control study: 6 females and 6 intact males; scopolamine study: 5 females and 5 intact males) were obtained from three separate litters from the University of Illinois swine herd. Piglets were brought to the biomedical animal facility 48 h after birth (to allow for colostrum consumption) and housed individually in clear acrylic-sided cages (0.76 m L × 0.58 m W × 0.47 m H) (previously described by Houle et al. 1997; Dilger and Johnson 2010). For enrichment, a toy (plastic jingle ball) and a hand towel were provided to each piglet. Room temperature was maintained at 27 °C and radiant heaters, located directly above the piglets, provided supplemental heat. Piglets were maintained on a 12-h light/dark cycle; however, during the dark cycle minimal lighting was provided. Piglets were maintained on a nutritionally complete commercial piglet milk replacer (Advance Liqui-Wean, Milk Specialties Co., Dundee, IL). Milk was reconstituted fresh each morning to a final concentration of 206 g/L using tap water and supplied at a rate of 285 ml/kg body weight (BW; based on daily recorded weights). This level of feeding

allowed for maintenance and growth, but prevented complete satiation to ensure that the piglets remained motivated for food rewards in the behavioral task. Water was not provided separately from that used in the milk replacer. Milk replacer was delivered from a reservoir to a polyvinylchloride (PVC) bowl secured to the floor of each cage. Using an automatic delivery system (Dilger and Johnson 2010), piglets received their daily allotted milk over 18 meals (once per hour), followed by a 6-hr period where no milk was provided. All animal care and experimental procedures were in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and approved by the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee.

Animal Handling and Habituation

In pilot work for the development of this task, we observed higher levels of non-compliance during testing (i.e., piglets failed to choose a reward arm in the given time) than expected (data not shown). In an effort to reduce non-compliance, handling and habituation procedures were employed prior to behavioral testing. Piglets were handled (i.e., stroked along their bodies, lifted from underneath their bodies to simulate being carried to the maze, etc.) by the experimenters multiple times per day during cleaning/feeding and daily observations. In addition, 1 week prior to the initiation of behavioral testing, piglets were permitted an exploration/exercise period in an adjacent hallway for 5 min each day. The purpose of this period was to allow the piglets to become habituated to a new space in an effort to reduce non-compliance due to fear during behavioral testing.

Design of Spatial T-Maze

To assess spatial learning and memory in neonatal piglets, a clear plastic plus-shaped maze (essentially a double T-maze), positioned over textured black rubber mats, was constructed (Fig. 1). Using a removable barrier, one arm could be blocked off to create a standard T-maze. Therefore, two arms of the maze acted as start arms (north and south, each with a holding area, creating a “start box”), while the other two acted as reward arms (east and west). The plus-shaped design of the maze allowed for the alternation of the start arm during testing (according to a pseudorandom pattern across each 10-trial block, where the same start arm was not used for more than two successive trials), which ensured that the piglet did not solve the task using an egocentric mechanism (i.e., turn body left or right, striatum-dependent), and instead was forced to adopt an allocentric mechanism (i.e., uses extra-maze visual cues, hippocampus-dependent) for solving the task (Fitz et al. 2008). This task is similar to that used to assess ‘place’ and/or ‘direction’ learning within the rodent literature (Tolman et al. 1946; Stringer et al. 2005; Zurkovsky et al. 2006; Walsh et al. 2008).

Extra-maze visual cues (i.e., color posters within the piglets’ dichromatic vision spectrum; Neitz and Jacobs 1989; Lind et al. 2007; Gieling et al. 2011a) were attached with Velcro® to opaque shower curtains that were draped around the perimeter of the testing arena. A stationary PVC bowl, identical to that in the piglet's home cage, was located at the far end of the east and west reward arms. During testing, each bowl contained approximately 3 ml of chocolate milk (i.e., the same milk replacer used for regular feedings with the addition of Nesquic® cocoa powder, supplied according to the manufacturer's directions) to prevent olfactory cues from biasing results. However, one bowl contained a perforated cap that prevented the piglets from accessing the milk reward. Additionally, both reward bowls were covered with an opaque perforated lid to prevent the piglets from choosing the correct arm based on visual cues alone (i.e., perforated cap present or not). In order to access the reward, the piglet used an ethologically relevant rooting motion to flip the lid from the bowl. Chocolate milk was not provided outside of the daily testing session to reduce non-compliance and maintain motivation throughout testing. A video camera was mounted from the ceiling above the arena and used to record piglet movement within the maze. Piglet

movement was tracked live using commercially available software (EthoVision 3.1; Noldus Information Technology Inc., Leesburg, VA).

Behavioral Testing of Control Piglets

Behavioral testing began 14 d after the start of the experiment, when piglets were 16 d of age. For this control study, there was no experimental treatment. Testing was conducted daily between 08:00 h and 12:00 h by one trained experimenter. Piglets completed 10 trials per day, for a total of 13 days. The first 9 days of testing constituted the acquisition phase, where the piglets learned to locate the milk reward in a constant place in space, as well as direction (e.g., west reward arm), using the extra-maze visual cues. A performance criterion of 80% correct was applied, which when reached, would indicate that the piglets had successfully acquired the task. Acquisition was followed by a reversal phase, where the previously incorrect arm (e.g., east), was now rewarded. Baseline performance of control piglets during the reversal phase is important for future studies, where treatment differences in acquisition may not be observed, necessitating the employment of this additional phase of testing.

Following a nightly 6-h food deprivation period, piglets were removed from their home cage, carried to the maze (located in an adjacent room) by the experimenter, and placed in the start arm (either north or south according to the pseudorandom pattern). At the start of each trial, a clear removable barrier was lifted by the experimenter, releasing the piglet into the maze. In pilot work for the development of this task, a “start box” was not employed and piglets were carried into the maze from an adjacent holding container each trial. Piglets appeared to find this procedure aversive (i.e., escape attempts, vocalizations, urination/defecation, and non-compliance during testing); therefore, a start box was employed to reduce stress on the piglet.

Each trial, the piglet was given 60 s to make a choice between the east or west reward arms. If correct, the piglet received a chocolate milk reward. After consuming the reward, the piglet was picked up by the experimenter and placed in the start box in preparation for the next trial. If incorrect on days 1 and 2 of acquisition, the piglet was allowed to retrace its steps and locate the reward in the correct arm of the maze. However, beginning on day 3 of acquisition, and for the remainder of the experiment, if the piglet chose the incorrect reward arm it was not permitted to retrace its steps to find the reward and was returned to the start box. While the piglet was held in the start box, the experimenter refreshed the milk rewards (i.e., mimed replacement for the incorrect reward bowl) and rotated the removable barrier which formed the T-maze (if necessary, depending on start arm alternation schedule). In addition, if any milk drips were present and/or any bouts of urination/defecation had occurred in the previous trial, the entire maze was wiped down with 70% ethanol and a towel. This resulted in an inter-trial interval of approximately 30-60 s. Following the completion of 10 trials each day, the piglet was returned to its home cage. The first piglet to test each morning varied according to a random pattern; thereafter, testing followed a sequential order according to piglet number/home cage. At the end of each testing day, the entire maze was cleaned with a 10% bleach solution and rinsed with water.

Using the Noldus software and a live video image, the dimensions of the maze were defined and a choice parameter established as half the length of the reward arm (~0.45 m). A choice in the maze was recorded by the computer if the piglet crossed this invisible barrier, allowing for an objective and accurate method of data collection. In addition to recording which reward arm was chosen (east or west), the Noldus program also measured the latency to choice (s) and total distance moved (cm). The computer stopped recording all information once a choice was made; therefore, during days 1 and 2 of acquisition, only the initial arm

choice for each trial was recorded, no data were collected while the piglet corrected a wrong choice.

Many attempts were made by the experimenters to control for odor cues/trails in the maze and/or to make odor cues inconsequential and unreliable indicators of reward location. In pilot work for the development of this task, the entire maze was sprayed with a 70% ethanol solution between every trial to eliminate the influence of odor cues on piglet performance. However, we found no difference in piglet performance between the pilot work and the current data set (i.e., both control groups showed similar performance on day 4 of acquisition, approaching the established criterion of 80% correct; pilot: 82.50 ± 8.40; current: 78.33 ± 4.70). If piglets relied on odor cues/trails to locate the milk reward, then piglet performance in the maze should have been impaired in the pilot study, which was not the case. In addition, not all piglets were assigned to the same reward arm; therefore, following odor trails would be an ineffective means to locate the milk reward, as the previous piglet may have been assigned to the opposite arm. Lastly, early in testing, both within a testing session and between piglets, we would expect there to be fewer odor cues, which could impact maze performance. Often piglets, regardless of testing order, performed at 90 to 100% correct once the task was acquired. Combined these data provide evidence that odor cues within the maze were not impacting piglet performance, lending additional support to the use of the visuo-spatial cues by the piglets.

Scopolamine Validation

The effect of scopolamine on task acquisition (6 d total) was determined in an identical, but separate experiment, from that described above. Piglets were obtained, housed, and raised in the same manner as previously described. However, each day of behavioral testing, piglets were injected (intramuscularly; i.m.) with scopolamine (scopolamine hydrobromide, SIGMA) or phosphate buffered saline (PBS) 15-30 min prior to the initiation of testing (similar to that described by Nielsen et al. 2009). To reduce soreness or other adverse effects due to repeated injections, the site of drug administration varied each day, alternating between neck and ham for all piglets. Following the second injection of scopolamine in the neck on Day 4 of testing, piglets demonstrated signs of aversion (i.e., increased vocalizations during injection, rolling head/neck within the home cage immediately after injection) compared to drug administration in the ham; thereafter, injections were restricted to the ham. Scopolamine was provided at a dose of 0.04 mg/kg BW, the optimum level determined by Nielsen et al. (2009) to observe cognitive behavioral changes without sedation, and at a concentration of 0.67 mg/ml of solution. Scopolamine and PBS were mixed once at the start of behavioral testing, aliquoted into individual tubes, and stored at -80°C. Each morning prior to testing, the necessary number of tubes of scopolamine and PBS were thawed, stored on ice throughout testing, and administered as necessary.

Statistical Analysis

Data analysis was conducted using the MIXED procedure of the Statistical Analysis Systems software (SAS Institute Inc., Cary, NC). Data were transformed as necessary to meet the assumptions of the test (e.g., homogeneity of variance). Transformations for the control data set include: proportion correct/total trials (angular), latency to choice and total distance moved (log). Transformations for the scopolamine data set include: latency to choice (log) and total distance moved (log). Control data were analyzed as a two-way (sex × day) repeated measures ANOVA, while the scopolamine data were analyzed as a three-way (treatment × sex × day) repeated measures ANOVA. Sex was not significant for any measures in the scopolamine data set and was removed from the model, leaving two factors (treatment and day) in the repeated measures model. Post-hoc paired contrasts were used to further examine interactive effects for the validation study (control vs. scopolamine on each

day of acquisition training). For the control data set, contrasts included A1 vs. A9 (change in performance during acquisition), R1 vs. R3 (change in performance during reversal), and A9 vs. R1 (change in performance when reward location was initially reversed). Statistical significance was accepted at $P < 0.05$, statistical trends at $P < 0.10$. Unless otherwise stated, data are presented as untransformed Least Squares Means (LSM) ± Standard Errors of the Means (SEM). Graphs were created in GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Results

Cognitive Performance of Control Piglets

Proportion Correct/Total Trials—Repeated measures ANOVA revealed a day effect on correct choices in the maze (ANOVA: $F_{11, 110} = 30.18$; $P < 0.0001$; Fig. 2a), where piglet performance improved throughout acquisition (A1 vs. A9; $P < 0.0001$) and reversal testing (R1 vs. R3; $P < 0.0001$). As expected, the proportion of correct choices was lower on the first day of reversal testing compared to the last day of acquisition (R1 vs. A9; $P < 0.0001$). Neither sex (ANOVA: $F_{1, 10} = 0.07$; $P = 0.7906$) nor a sex × day interaction (ANOVA: $F_{11, 110} = 0.84$; $P = 0.5998$) were observed.

Proportion of Trials Non-Compliant—Non-compliance was low for this task (0.45% ± 0.21); though the main effect of day (ANOVA: $F_{11, 110} = 3.38$; $P = 0.0005$) and a sex × day interaction (ANOVA: $F_{11, 110} = 3.38$; $P = 0.0005$) were observed (Fig. 2b). Compared to the last day of acquisition (A9), piglets showed higher non-compliance on the first day of reversal testing (R1; $P < 0.0001$). However, non-compliance decreased throughout reversal testing (R1 vs. R3; $P < 0.0001$). Additionally, we observed a trend for males to show higher non-compliance than females (ANOVA: $F_{1, 10} = 4.44$; $P = 0.0612$).

Latency to Choice (s)—Latency to make a choice in the maze was affected by day (ANOVA: $F_{11, 110} = 11.68$; $P < 0.0001$; Fig. 2c), where latency decreased throughout acquisition (A1 vs. A9; $P < 0.0001$) and reversal testing (R1 vs. R3; both $P < 0.0001$). However, latency to choice increased when piglets began reversal testing (A9 vs. R1; $P < 0.0001$). There was no effect of sex (ANOVA: $F_{1, 10} = 0.05$; $P = 0.8314$) or a sex × day interaction (ANOVA: $F_{11, 110} = 0.66$; $P = 0.7747$).

Total Distance Moved (cm)—Similar to latency to choice, the total distance that piglets moved in the maze was affected by day (ANOVA: $F_{11, 110} = 6.09$; $P < 0.0001$; Fig. 2d). Piglets moved a lower total distance as testing progressed during both acquisition (A1 vs. A9; $P = 0.0272$) and reversal testing (R1 vs. R3; $P < 0.0001$), but increased at the start of reversal (A9 vs. R1; $P < 0.0001$). Conversely, neither sex (ANOVA: $F_{1, 10} = 1.91$; $P = 0.1969$) nor a sex × day interaction (ANOVA: $F_{11, 110} = 0.75$; $P = 0.6902$) were observed.

Cognitive Performance of Scopolamine Piglets

Proportion Correct/Total Trials—Repeated measures ANOVA revealed an effect of treatment (ANOVA: $F_{1, 8} = 6.07$; $P = 0.0391$) and day (ANOVA: $F_{5, 40} = 7.25$; $P < 0.0001$) on correct choices in the maze (Fig. 3a). Additionally, there was a significant treatment × day interaction (ANOVA: $F_{5, 40} = 2.77$; $P = 0.0307$), where piglets treated with scopolamine showed deficits in learning the spatial task compared to controls. On Day 5 of acquisition, when control piglets exceeded the performance criterion (90.0 [± 6.06]% correct), piglets treated with scopolamine had significantly fewer correct choices in the maze (64.0 [± 6.06]% correct; $P = 0.0032$). When provided an additional day of testing, scopolamine treated piglets still failed to reach the performance criterion compared to controls (Day 6: 62.0 [± 6.06]% correct; $P = 0.0014$).

Latency to Choice (s)—Latency to make a choice in the maze was affected by treatment (ANOVA: $F_{1, 8} = 6.45$; $P = 0.0348$; Fig. 3b). In addition, the main effect of day was significant (ANOVA: $F_{5, 40} = 13.98$; $P < 0.0001$), with piglets taking less time to make a choice throughout the course of acquisition. However, a treatment × day interaction was not found (ANOVA: $F_{5, 40} = 0.86$; $P = 0.5153$). Post-hoc contrasts revealed that piglets treated with scopolamine showed a longer latency to make a choice in the maze on days 1 ($P = 0.0091$) and 2 ($P = 0.0087$) of acquisition compared to controls.

Total Distance Moved (cm)—The distance travelled in the maze was affected by day (ANOVA: $F_{5, 40} = 3.05$; $P = 0.02$; Fig. 3c), where total distance moved decreased throughout the course of acquisition. However, neither a treatment (ANOVA: $F_{1, 8} = 1.35$; $P = 0.2784$) nor a treatment × day interaction were observed (ANOVA: $F_{5, 40} = 1.78$; $P = 0.1384$). Post-hoc contrasts revealed that piglets treated with scopolamine moved a higher total distance in the maze on days 1 ($P = 0.0390$) and 2 ($P = 0.0264$) of acquisition compared to controls.

Discussion

The porcine brain is more comparable in terms of anatomy, growth, and development to the human brain than laboratory rodents (Lind et al. 2007; Gieling et al. 2011a). In addition, due to their precocial nature, piglets can be tested at a young age, mimicking the neonatal period of human infants (Siegford et al. 2008; Dilger and Johnson 2010). Due to ethical concerns, investigating early-life insults, such as infection or nutritional deficiencies, on neurodevelopment and cognitive function is not possible in humans. Therefore, neonatal piglets provide a unique translational animal model to address some of these concerns.

The cognitive behavioral task we developed for neonatal piglets was based on rodent literature for a ‘place’ and/or ‘direction’ learning T-maze task (e.g., Tolman et al. 1946; Stringer et al. 2005; Zurkovsky et al. 2006; Walsh et al. 2008), and has not been previously applied to swine. For this type of task, animals are trained to locate a food reward in a constant place in space, as well as direction (either east or west), in a plus-shaped maze with two start arms (north or south, pseudo-randomized) based on extra-maze visual cues. The plus-shaped design of the maze allows for alternation of the start arm, unlike a standard T-maze, which ensures that the animals cannot solve the task using an egocentric mechanism (i.e., turn body left or right each trial, striatum-dependent), and instead are forced to adopt an allocentric mechanism (i.e., uses visual cues, hippocampus-dependent) for solving the task (Fitz et al. 2008). It was found that rats with hippocampal lesions were impaired on a direction learning task compared to controls, lending additional evidence that a task of this design is hippocampal-dependent (Stringer et al. 2005).

In our task, the ability of control piglets to locate the food reward improved over time, as evidenced by reduced latencies to make a choice and distance moved in the maze, and with piglets surpassing our performance criterion of 80% correct (a criterion used in previous learning and memory experiments with pigs; e.g., Croney et al. 2003; Nielsen et al. 2009) by day 5 of acquisition. When the correct reward arm was reversed, performance was significantly reduced compared to the last day of acquisition, as piglets displayed a strong preference for the previously rewarded arm. Anecdotally, we observed piglets showing signs of ‘frustration’, including vocalizations, pawing at the perforated lid in an attempt to access the chocolate milk, and repeatedly looking at visual cues after making an incorrect choice. However, throughout reversal testing, piglet performance in all measures (i.e., latency, distance, and proportion correct) improved as they were able to extinguish their previous memory of the correct reward arm location and learn the new location based on the extra-maze visual cues.

In future experiments, where piglets will be exposed to environmental insults, it is likely that additional acquisition days (more than 5 days required for controls) will be needed for all piglets to reach similar levels of performance in the maze. However, it is also possible that certain experimental treatments (e.g., nutritional deficiencies or infection) may prevent piglets from being able to acquire the task and reach performance criterion at all. In this case, reversal testing may not be necessary and/or interpretations of any findings should be made with caution, as a failure to reach performance criterion during acquisition will bias performance during reversal testing as well. If cognitive differences between treatments are not detected during acquisition, it is probable that reversal testing to assess working memory, which is likely more difficult for animals as they must unlearn an old strategy for solving the task and learn a new one, will be a more sensitive measure (Vorhees and Williams 2006; Dilger and Johnson 2010).

Non-compliance, defined as a piglet failing to make a reward arm choice within the 60-s time limit, was low for this task (less than 0.5% of trials). Interestingly, control males showed a trend to be more non-compliant than females. However, we believe that this is due to personality differences within that group of piglets, and is not specific to the spatial T-maze task, as the control piglets in the scopolamine experiment did not show any sex biases. However, in previous pilot work for the development of this task, we did find much higher levels of non-compliance than expected (up to 8% of trials across both sexes). In an effort to reduce the incidence of piglets not responding during testing, we familiarized them to handling procedures and provided opportunities for exploration outside of the home cage prior to the initiation of testing. In addition, handling immediately prior to testing was minimized by placing the piglets in a “start box” located within the start arms of the maze between trials. Lastly, the reward in the maze was the same milk replacer used for regular feedings with the addition of cocoa powder (previous experiments have provided chocolate rewards to pigs with success; e.g., Moustgaard et al. 2004; Moustgaard et al. 2005; Arts et al. 2009). Piglets could only receive the chocolate milk reward during testing, not within their home cage at regular feedings, increasing incentive for piglets to perform the task. We were unable to find discussion in the literature on the incidence of non-compliance in other swine cognitive behavioral tasks; therefore, it is unknown if these issues were due to the young age of the piglet or as a side effect of working with swine in land maze (i.e., tasks where the animals are not forced to respond, unlike the Morris water maze; Morris 1984).

When scopolamine, an anti-cholinergic drug that can impact learning and memory, was administered prior to testing, piglets showed longer latencies to make a choice and a higher total distance moved in the maze on the first two days of acquisition compared to controls. Additionally, scopolamine-treated piglets appeared unable to acquire the task based on set performance criteria, showing a significantly lower proportion of correct reward arm choices in the maze on days 5 and 6 of acquisition, with only 64% and 62% correct, respectively. As performance in the maze did not improve from day 5 to day 6 in scopolamine treated pigs, it appears that the provision of an additional testing day, beyond that required for control pigs, was still insufficient to allow piglets to reach a performance criterion of 80% correct. Similarly, piglet performance in a Delayed Non-Match to Sample (DNMS) task was impaired when treated with scopolamine (Nielsen et al. 2009).

Although muscarinic receptors for acetylcholine are located throughout the brain (Mesulam et al. 1983; Decker and McGaugh 1991), our data suggest that the impact of scopolamine on other brain structures than the hippocampus, including the striatum, anterior cingulate cortex, and amygdala, are unlikely to be the primary mode of action for this drug in our study. First, motor function, as an indicator of striatal function (Devinsky et al. 1995), was not disabled in this study, as confirmed by similar distances moved in the maze between scopolamine-treated and control piglets on days 3-6 of acquisition. Secondly, motivational

effects, as confirmed by the ready consumption of milk rewards by both treatments throughout testing, as well as similar latencies to make a choice in the maze compared to controls on days 3-6 of acquisition, demonstrate that the striatum and the anterior cingulate cortex were not affected by scopolamine treatment as well (Schultz et al. 1992; Devinsky et al. 1995). The reduced latency to choice and decreased total distance moved by scopolamine-treated piglets during days 1 and 2 of acquisition could be interpreted as anxiety-like behaviors (involving the amygdala; Pellow et al. 1985; Davis 1997; Graeff et al. 1998); however, as there was no impact on maze performance during this time point, piglets still readily consumed the milk rewards (Merali et al. 2003), and the effects were not long-lasting, it is unlikely that scopolamine's effects on the amygdala could account for the differences in task acquisition observed on days 5 and 6 of testing. Collectively, these data, combined with the measures taken to control for the impact of confounding cues within the maze (e.g., odor) and/or prevent the use of alternative strategies to solve the task (i.e., egocentric mechanism), demonstrate that scopolamine is likely acting through the hippocampus to cause impairments in learning, as seen in many rodent studies of spatial learning (see reviews: Decker and McGaugh 1991; Ebert and Kirch 1998). With the exception of Nielsen et al. (Nielsen et al. 2009), no swine cognition studies have validated performance in behavioral tasks with a pharmacological challenge to inhibit learning and memory (Kornum and Knudsen 2011).

Conclusions

In summary, neonatal piglets were able to acquire a novel place and direction learning spatial T-maze task using extra-maze visual cues. Based on data from a validation study using scopolamine, an anti-cholinergic drug that affects the hippocampus and other related structures to cause memory dysfunction, piglets appear to be solving the task using the visuo-spatial cues provided, which is mediated (in part) by the hippocampus. This task will be a valuable tool in future work investigating the impact of environmental insults, such as nutritional deficiencies or infection, on neurodevelopment and cognition during the critical brain growth period in neonatal swine as a model for human infants.

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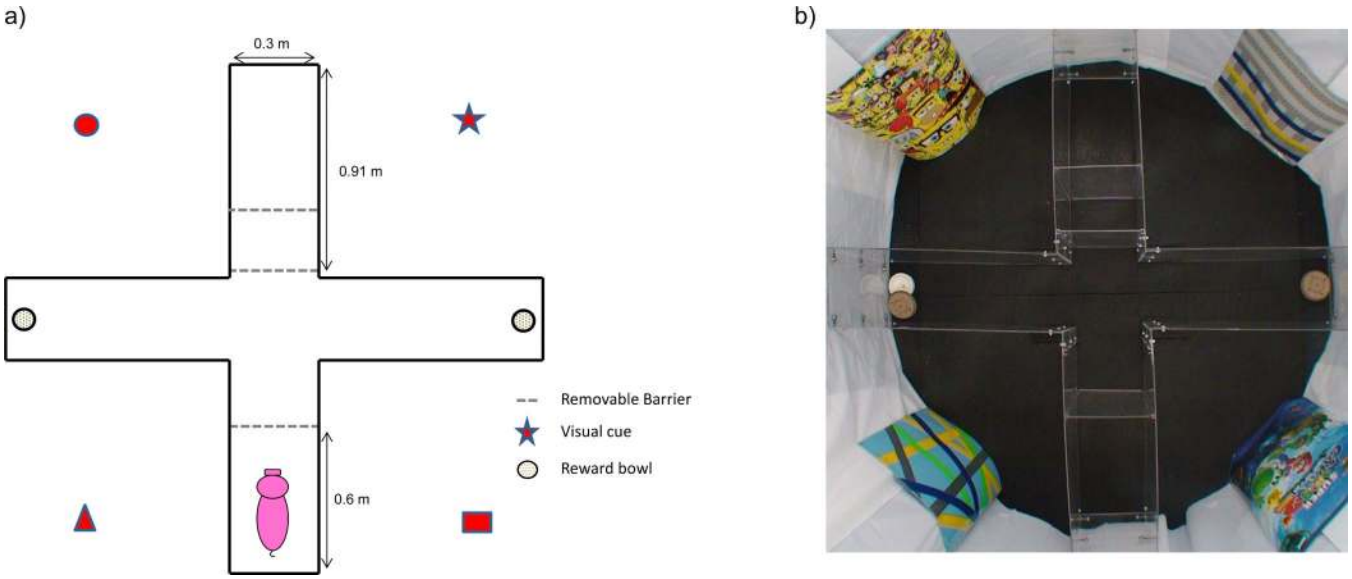

Fig. 1.

Schematic (a) and photograph (b) of T-maze used in cognitive behavioral experiment. Piglets were trained (acquisition phase: 60-s trials, 10 trials per day for 6 to 8 days) to locate a chocolate milk reward in a constant place in space, as well as direction (east or west), using extra-maze visual cues in a clear plus-shaped maze with four arms; two start arms (north and south, start location randomly alternated each trial) and two reward arms (east and west, each piglet assigned to one correct reward arm at the start of the experiment). During the reversal phase (60-s trials, 10 trials per day for 3 days), the correct reward arm for each piglet was reversed and the piglets were re-tested to asses learning and working memory. b) Shown in the picture: South start arm configuration; barriers to create start boxes, which were lifted to release the piglet into the maze; reward bowls with lids (East – lid intact; West – lid removed by piglet via a rooting motion); and visual cues surrounding the maze

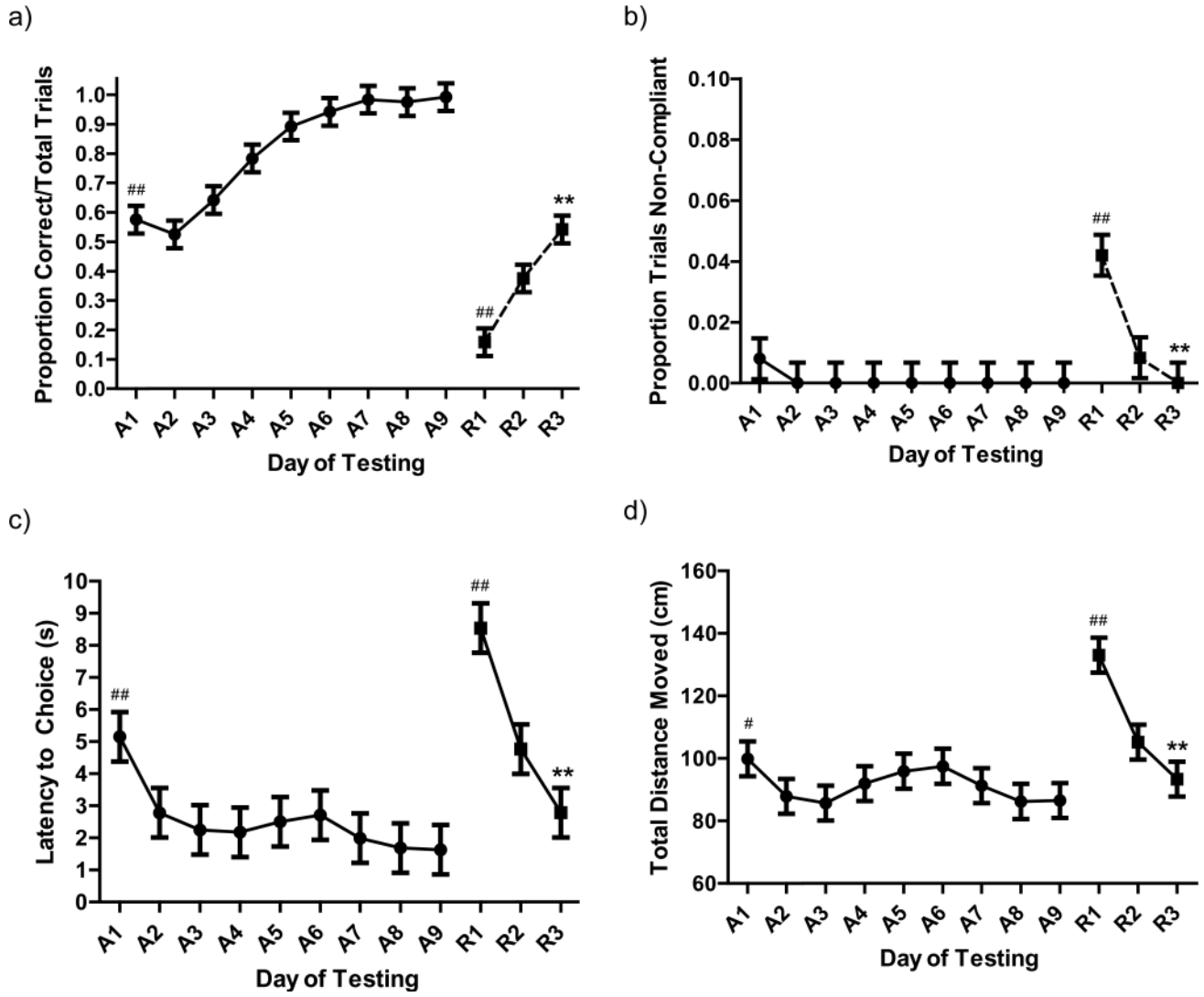

Fig. 2.

Cognitive behavioral performance (a: proportion of correct reward arm choices/total trials), non-compliance (b: proportion of trials with no choice within the 60-s time limit), and locomotor readouts (c: latency to choice; d: total distance moved) in the place and direction learning spatial T-maze task by neonatal piglets in the absence of an experimental treatment. Each data point represents the average (± SEM) performance of 12 piglets (2 female and 2 intact male piglets from 3 separate litters) subjected to 10 daily trials during both acquisition (A1-A9) and reversal (R1-R3) testing. Paired contrasts were used to separate means during acquisition (A1) and reversal (R1) from the last day of acquisition testing ($^{\#}P < 0.05$; $^{\#\#}P < 0.0001$ different from A9). Additionally, contrasts were used to separate means R1 vs. R3 ($^{**}P < 0.0001$)

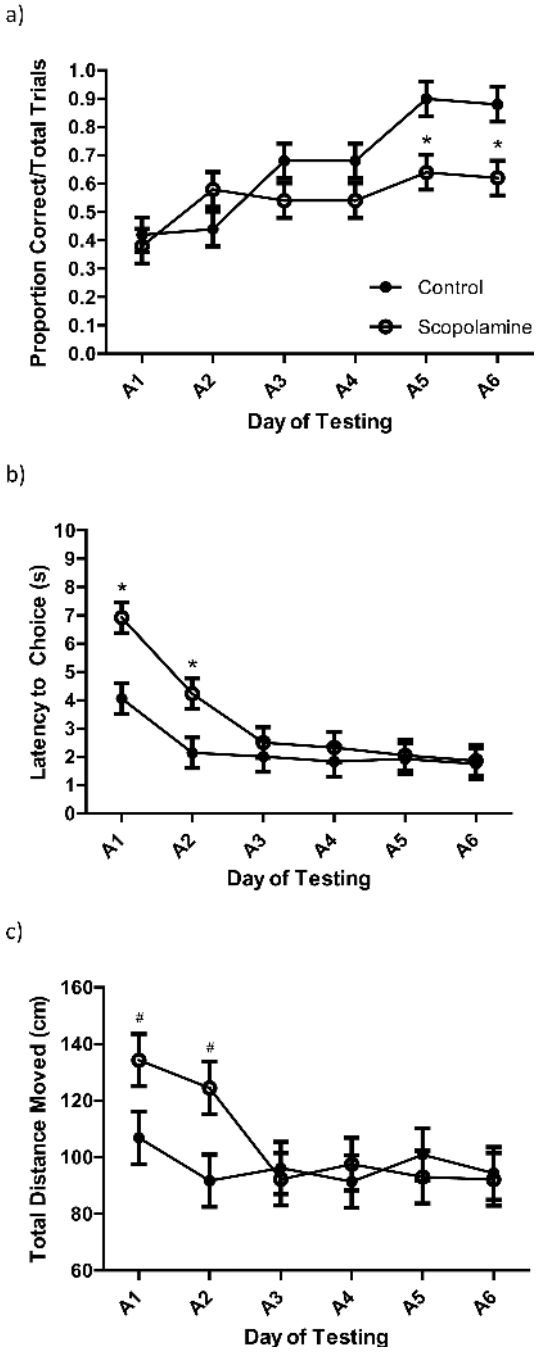

Fig. 3.

Cognitive behavioral performance (a: proportion of correct reward arm choices/total trials) and locomotor readouts (b: latency to choice; c: total distance moved) in the place and direction learning spatial T-maze task by neonatal piglets administered (i.m.) scopolamine (n = 5) or vehicle (PBS, n = 5). Each data point represents the average (LSM± SEM) performance of 5 piglets (n = 2 or 3 males and n = 2 or 3 females per treatment) subjected to 10 daily trials during acquisition (A1-A6). Contrasts were used to separate treatment means during each day of acquisition ($^{\#}P < 0.05$; $^{*}P < 0.01$)