Side-Bias and Time-of-Day Influenced Cognition after Minipigs Were Conditioned Using a Novel Tactile Stimulation Device

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Introduction

- Neuroscientists seek to develop tools to detect and track prepathological states of neuro-disfunction

- The least invasive methods are behavior-tests (Figure 1) for humans that use tactile stimulation and responses from the patient to determine the significance of brain pathology and benchmark progress for therapy

- Porcine models of immunohistopathology and diffusion tensor imaging (DTI) were established, but a tactile stimulation test somatosensory system function in pigs needed to be developed.
- Challenges for porcine tests were:
  - Pigs are very cunning, and it is challenging for them to wear devices
- Placement behind the ears may be better for sensitivity, but the side-placement may be more practical
- Pigs may be biased (e.g. right- or left- "handed") in their choices

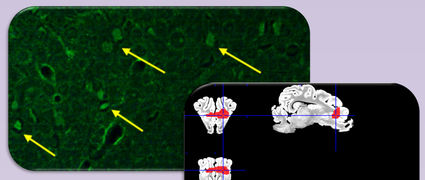


Figure 1. A. Immunohistopathology of a pig brain that was treated with low-level blast overpressure. This sampling requires to euthanize the pig and harvest tissue. B. DTI of the same pig as in A. This requires the pig to undergo anesthesia to gain the images.

C. A human tactile behavior test was developed (Corticalmetrics) and is not invasive. The patient places two finger over the buttons on the mouse and responds with the other hand if the stimuli were on the index finger or the middle finger. The error rate and response times provide information about the functioning of the somatosensory cortex and some cognition (e.g. hippocampus and anterior cingular cortex). This measure allows for repeated tests.

Objectives

- Develop an attachment and conditioning protocol for porcine tactile stimulation devices
- Compare location of placement effectiveness(ear or side), and start-side bias (right or left)
- Explore success rate and time of day influences

Materials and Methods

Animals and Facilities (IACUC #3881)

- Minnesota minipigs (n = 8; boars = 7 gilt = 1; NSRRC, Columbia, MO)
- They were single-housed at the large animal research center (LARC) at Kansas State University in the modular unit.

Training and Testing

- 3M 1522H and Ducttape were used to create pockets to hold the devices only during testing. Four pockets were placed, but each pig was randomly assigned the ear or side testing (Figure 3).
- Training started on day 10 and continued through testing days (days 19-22; Figures 1;and 4).
- Pigs were tested a total of 12 times over 6 sessions in a testing arena (Figure 5).

Video Collection

– Each pen (pigs' and test arena) were recorded continuously with IP cameras (Points North Surveillance, Auburn, ME).

Video Analysis

– Tests were analyzed for correctness by a trained observers using the Observer (Noldus Observer XT 11.5, Leesburg, VA), by using a spatial ethogram (Figure 5)

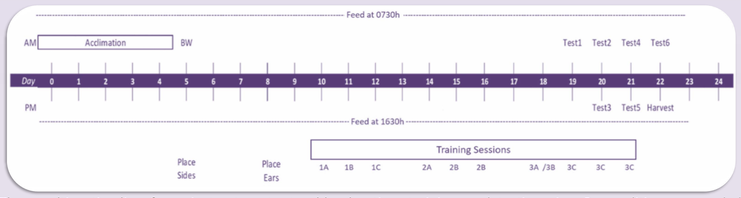


Figure 2. The resulting timeline for pig treatments, trouble shooting, training, and testing pigs. Precondition occurred during the acclimation; pigs were introduced to tubes/toys during feeding, which occurred at 830 and 1430 h. Once trouble-shooting of pockets and devices was complete, pigs began in-pen training sessions to condition them to associate right-stimulation with the right-bowl and red ball and left stimulation with the left bowl and purple toy. During training, pigs were distracted by waterers, therefore, we developed structured a test arena an tested pigs up to four times daily. The tests started on the right side or the left side.

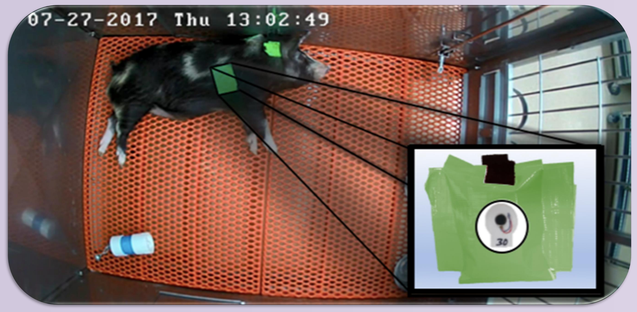


Figure 3. Four pockets for each pig were constructed (green) and were placed behind each ear and on each side (behind each elbow). The devices (#30) were inserted and removed from the pockets before and after each training or testing session. The pig in the figure is resting in his home-pen. Included in each pen were two fixed feed bowls, a water-nipple, and an environmental enrichment device (blue and white toy).

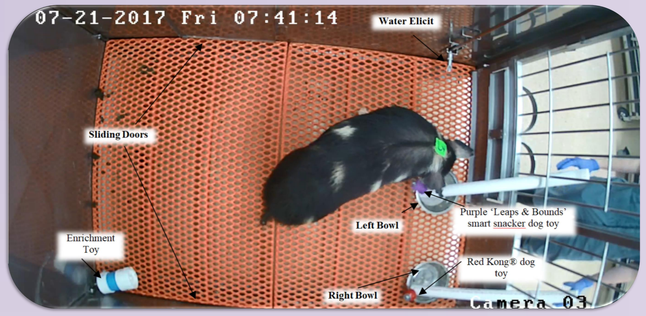


Figure 4. After troubleshooting, in pen training sessions were conducted. Tubes with toys were used during acclimation and training to distributed a feed or treats and condition pigs to associate the right-stimulus with the right bowl (and red ball) and the left-stimulus with the left bowl (and purple toy).

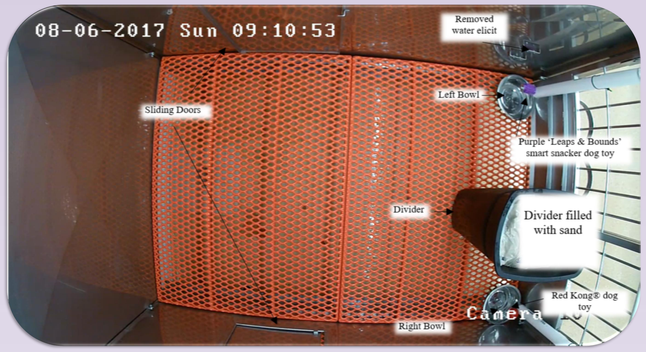
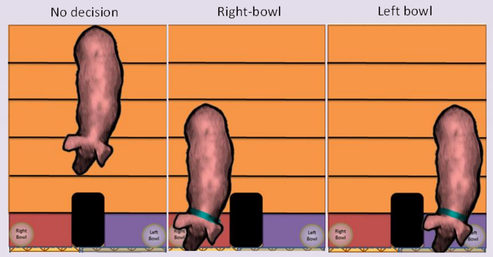


Figure 4. During the in-pen training sessions, pigs added distracting behaviors (e.g. drink water, turns, play with EED), and it was challenging to see if they were conditioned. Therefore, a test arena was constructed that limited distractions and had a short barrier between the bowls to slow down the pigs responses to the stimuli. Up to 2 sessions were conducted in a day. Within each session, pigs were stimulated on both sides. We tested if the start side (Right or left) for each session influenced the correct answer.

Figure 5. Video of each testing session were analyzed using the above spatial-relation criteria. If the pig was in the orange area (physical lines from flooring), then the behavior was time-stamped as "no decision." Then, the pig's head crossed (cyan line) into the right area (red) or left area (purple), then the behaviors were time-stamped as right or left, respectively. The observer was blinded to the stimuli treatments during time-stamping. After time stamping, the correct answer was inserted into the data set.



Results

- Placement (sides or behind ears) did not influence duration in the correct area during tests ($P = 0.11$; Figure 6A).
  - Responses were more variable (Table 1) with ear-placement than side-placement.
- During each session, if the first test was administered on the right side, then pigs spent more time ($P < 0.05$; Figure 6B) in the correct-area.
- Pigs tended ($P = 0.10$) to display greater correct-duration during morning than the evening sessions (Figure 6C).
- Pigs decreased correct-duration each day ($P = 0.009$; Figure 6D).
- Correct-latency was the most variable response (Table 1).

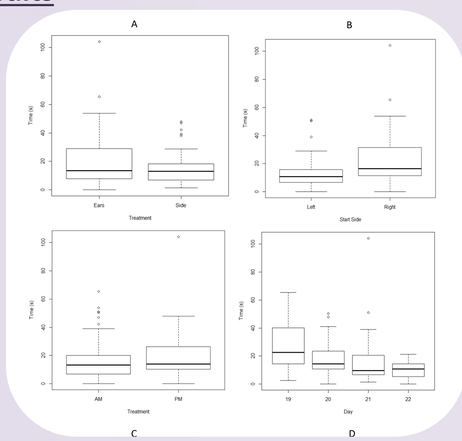


Figure 6. Whisker plots of parameters analyzed. The thick-line in each box represents the mean, the error bars represent the standard error mean, the circles represent data points that differ greatly from the mean. Analyses of interest included: A. Placement of two devices (behind the ears vs. on sides); B. Session start bias (started the 2-test session on the right or left); C. Time of day (morning vs. evening session), and; D. Repeated sessions over time.

Table 1. Correct-variables of descriptive statistics and sample-size calculations for all data points collected. The coefficient of variation (CV%) was used for each parameter in the sample size calculator (Galyean, 2017). The amount of repetitions (Reps) for expected treatment from control differences (Δ) were calculated with α=0.05 ß=0.20.

	Correct % Side	Correct % Ear	Correct % Left	Correct % Right	Correct Latency	Correct Frequency	Incorrect Frequency
n	48	48	48	48	96	96	96
Mean	34.5	39.9	34.4	40.1	10.7	2.4	4.79
Median	32.2	34.4	31.4	38	6.1	2.0	5
±SD	18.3	26.6	21.2	24.3	13.3	1.2	2.43
CV%	52.9	66.8	61.8	60.6	124.7	51.9	50.66
Min	4.4	0.0	0.0	0.0	0.0	0.0	0
Max	82.9	100.0	100.0	100.0	81.3	7.0	14
Shapiro- Wilks	95.8	93.1	88.8	97.3	75.4	91.1	95.74
Reps, 50% Δ	23	37	32	31	131	23	22
Reps, 75% Δ	10	17	14	14	58	10	10

Implications

- Placement on sides will be the preferred method because responses were less variable and some pigs are able to remove the ear patches by pressing the waterer with their ear.
- Pigs may be similar to humans, in that they are right-handed or left-handed, but side-bias among humans does not alter the diagnoses (Favorov et al., 2017). Therefore, more research in pigs is needed to determine if side-biases will influence treatment outcomes.
- Evenings sessions will be eliminated from future protocols and will be reserved for re-conditioning.
- Pigs may get bored or frustrated with the test over time. The tests may need to be altered to include operant-conditioning (e.g. press lever before receive a treat after stimulus) to account for this complexity.

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