Development of an eyeblink conditioning paradigm in young pigs

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Introduction
- Eyeblink conditioning (EBC) is an associative learning paradigm commonly used in rodent, rabbit, sheep, dog, and human research.¹
- The neural circuitry necessary for acquisition of a conditioned eyeblink response has been extensively described, allowing for precise characterization of behavioral changes observed using this paradigm.²
- The magnetic distance measurement technique (MDMT) utilizes a static magneto-sensitive chip to measure the magnetic field of a small magnet placed on the eyelid.³
- The piglet is increasingly being recognized as a premiere model for preclinical neurodevelopmental research, and as such, there exists a need for a sensitive behavioral assessment to elucidate developmental differences in the piglet model.⁴

Objective
The objective of this study was to determine if piglets were capable of exhibiting conditioned responses using a delay eyeblink conditioning paradigm.

Methods
- N=12, 4-week-old male piglets, artificially reared from 2 d of age until study end. Piglets were further stratified into habituation + acquisition (n=6) and acquisition only (n=6) groups.
- Data was acquired using the P-Blink system (Neurasmus, Rotterdam, Netherlands). This system captures eyelid movement using MDMT, in which the magneto-sensitive chip is placed just above the brow line and a small magnet is placed on the eyelid. A custom airpuff delivery system was attached to the pig’s face using hook-and-loop fastener. Data analysis was performed using custom analysis software.
- P-Blink stimuli:
  - Conditioned Stimulus (CS): blue LED light presented bilaterally approximately 8 inches from each eye
  - Unconditioned Stimulus (US): corneal airpuff, approximately 7-14 psi presented to the left eye

Trial Parameters
- Inter-trial interval- 10x2 s
- Inter-stimulus interval- 500 ms
- Go-No Go criteria- eyelid open and stable threshold for 2 s

Habituation Session: 5 blocks of 8 trials

Acquisition Session: 5 blocks of 8 trials

Data Acquisition of individual piglets

Results

Conclusion & Future Directions
- Four-week-old piglets exhibit nearly 85% conditioned responses within five sessions of EBC.
- MDMT is an effective and sensitive measure of piglet eyelid movement, allowing for head movement without restraint.
- Providing three sessions of CS or US only habituation does not appear to enhance acquisition of conditioned responses (data not shown) and may result in slower learning.
- Future work will focus on ensuring piglets maintain the same level of alertness throughout the duration of the trials, without having to be manually manipulated to remain alert.
- Data shows piglets that exhibit frequent spontaneous blinking when fully alert (~0.5 Hz). Analysis methods more specialized for piglets may be warranted.


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