Neural mechanisms associated with context-dependent pain tolerance.

Michael Kuczajda, Kathleen Reyna, Rachel Riley, Sydney Thrasher, Katelyn E. Sadler & Sydney Trask
Department of Psychological Sciences, Purdue University

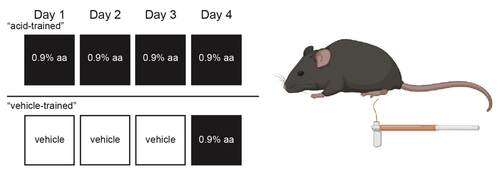
Introduction

Previous work has demonstrated that mice can develop a context-dependent conditional pain tolerance when repeatedly given presentations of a noxious stimulus (i.e., acetic acid) in that environment (Trask et al., 2022). These findings suggested that female mice were better equipped to form these associations, in line with human research demonstrating that women are not only more susceptible to chronic pain, but they are also able to endure higher severity of pain in comparison to men due to their greater nociceptive processing (Traub & Ji, 2013).

The present study aimed to examine neural activity in several brain regions implicated in contextual learning, pain processing, or both, including the medial orbital cortex (MO), the pre- and infralimbic cortices (PL, IL), the anterior cingulate cortex (ACC), the basolateral amygdala (BLA), the dorsal and ventral hippocampi (DH, VH), the anterior and posterior retrosplenial cortices (aRSC, pRSC) and the dorsolateral periaqueductal gray (dlPAG). We measured the immediate early gene zif268 as a proxy for neural activity following behavioral testing following development of a context-dependent pain tolerance or a control procedure. We predicted that the developmental of pain tolerance would be associated with changes throughout this circuit.

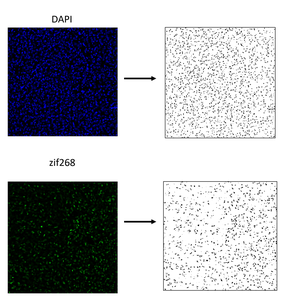
Methods

Behavior. This between-subject experiment used both male and female C57 mice from ages 8-12 weeks. Two distinct 10cm x 10cm x 15cm plexiglass chambers served as the context. On days 1, 2, and 3, animals received an IP injection of either 0.9% acetic acid or vehicle before being placed in the one of the two conditioning chambers. On day 4, all animals received an IP injection of 0.9% acetic acid before being placed in their training context. Withdrawal threshold using a Von Frey procedure was measured as the behavioral variable of interest (see below).



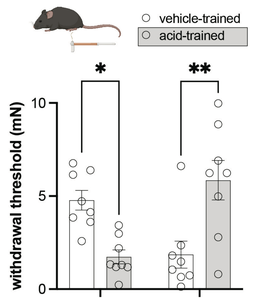
Immunofluorescence. Brains were extracted, sectioned, and placed on slides. Slides were then incubated briefly in a solution containing the zif268/EGR1 antibody, an Alexa 488 secondary, and then stained with the nuclear stain DAPI (as in Trask et al., 2021).

Microscopy and analysis. Images were captured on the Leica Thunder Imager microscope using a 20x objective lens and quantified with ImageJ software by converting them to 32-bit, applying Gaussian filtering, and then counting particles greater than 4 pixels in diameter within the ROI. This results in a binary image with minimal background. By quantifying the amount of black relative to white and comparing it to the total amount of DAPI present on the same section, we were able to determine an estimate of neural activity in each brain region.



Results

Figure 1. Direct comparison between contexts is not needed to demonstrate a contextually-induced tolerance.
We found that the vehicle-trained subjects had a higher withdrawal threshold (less pain) on day one, while the acid-trained subjects had a higher withdrawal threshold on day four when both groups were given acid injections. *$p < 0.05$, **$p < 0.01$.



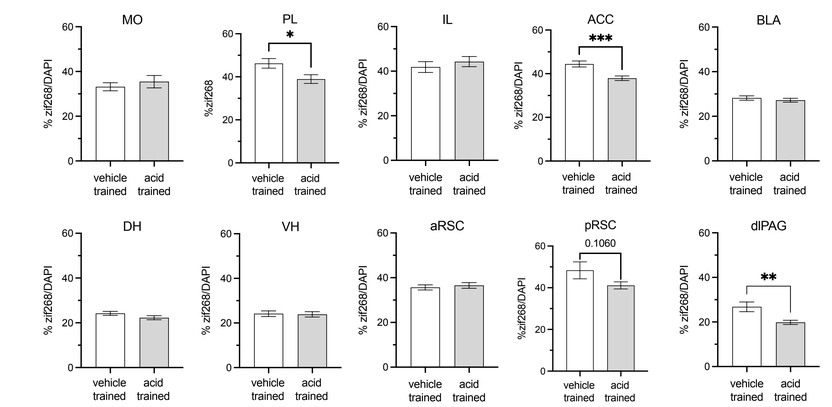


Figure 2. Mean zif268/DAPI percentages per brain region for vehicle- and acid-trained groups.
We found less expression of zif268 in acid-trained animals in the PL, ACC, and dlPAG regions, with a slight trend towards a decrease in the pRSC. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.

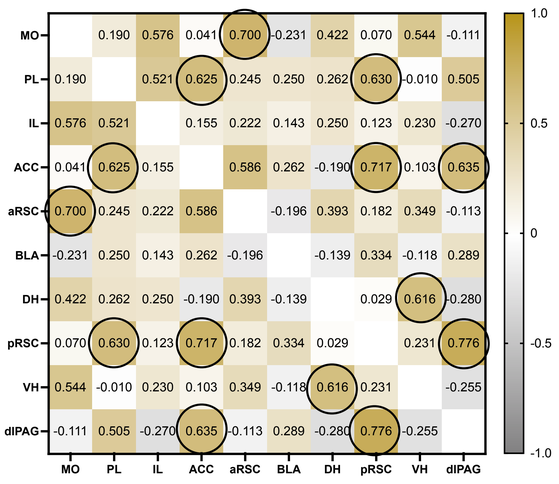


Figure 3. Heat map depicting correlational significance between brain regions. .
We found correlations in brain activity between all the regions in the circled comparisons. These results, as well as those from Figure 2, suggest that circuit-level activity between the PL, ACC, pRSC, and dlPAG might be responsible for encoding of pain memory during initial CS-UCS pairings.

Conclusions

Here, we found that activity was increased in the PL, ACC, pRSC and dlPAG in mice who were given context-acid acid pairings for the first time. Further analyses demonstrated that correlated activity between these regions is needed for memory encoding of this association.

These results are in line with others that demonstrate repeated administration of a painful unconditional stimulus results in less neural activity than the initial exposure (Williams et al., 2019). Together with those results, our data suggest that rather than seeing reduced activity in the acid-trained group, we are observing increased activity in the vehicle-trained group associated with the first context-UCS pairing.

These findings suggest that the PL may be involved in the top-down processing of contextually-induced pain tolerance through connections with the dlPAG (Floyd et al., 2000), which controls behavioral output in species-specific defense behaviors, including pain. In line with our results, previous work has demonstrated that the ACC is important for conditional, but not unconditional pain in a conditioned responding (Johansen et al., 2001). Our results for the anterior and posterior RSC are in line with what other research has found: Only the posterior, and not anterior, retrosplenial cortex was involved in context-dependent memory (Trask et al., 2021).

Future work will manipulate this circuit to examine its effects on contextually-gated pain tolerance.

References

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Contact: smtrask@purdue.edu trasklab.com