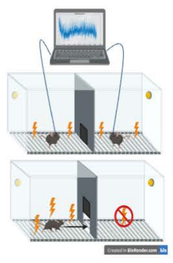
Cholinergic Signaling in the Medial Prefrontal Cortex Potentiates Learned Helplessness

Zuhair I. Abdulla[1], Richard B. Crouse[1], Ian Etherington[1,2], Jessica J. Na[3], and Marina R. Picciotto[1,2]
[1]Department of Psychiatry, Yale University School of Medicine, New Haven, CT
[2]Interdepartmental Neuroscience Program, New Haven, CT; [3]Yale University, New Haven, CT

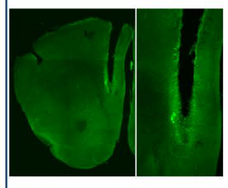
Introduction

- Several lines of evidence suggest that acetylcholine (ACh) is important in the etiology of depression.
- Blocking the breakdown of ACh produces depressive symptoms in healthy subjects while exacerbating them in depressed patients.
- ACh is also important for learning, memory, and – especially in the prefrontal cortex – attention, suggesting that optimal levels are beneficial, while excessive increases are detrimental to affective health.
- Prolonged ACh signaling during highly stressful events could therefore lead to a ***negative encoding bias***, in which stressful experiences are both attended to, and encoded, more potently, leading to increased depressive symptoms.
- We therefore recorded ACh transients from medial prefrontal cortex (mPFC) during learned helplessness (LH) to evaluate parameters under which cholinergic signaling might alter information processing during stressful events.

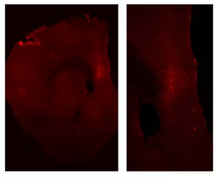
Methods



Learned Helplessness
Mice received 120, 4-s inescapable shocks (0.3 mA) delivered semi-randomly (~26 s ITI) over the course of 1 h during each of 2 induction trials, ~24 h apart. Control mice are placed in shock chambers but receive no shocks. ~24 h following induction trial 2, mice undergo active avoidance testing consisting of 30 escapable shocks that terminate either upon escape or after 24 s, with an ITI of 10 s. A *k*-means clustering algorithm is used to categorize mice as helpless or resilient based on escape latency and escape failures.

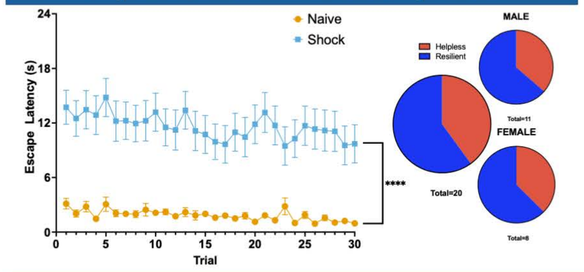


Fiber Photometry to Measure ACh
$GRAB_{ACh}$ 3.0 was injected into the mPFC of male (n=15) and female (n=13) mice, and a fiber was implanted above the injection site. 4 weeks following surgery (to allow for viral expression) mice were run in LH and ACh levels were evaluated during both induction trials. Data were analyzed with Matlab code and GuPPy. (*Representative image of injection and fiber site*).



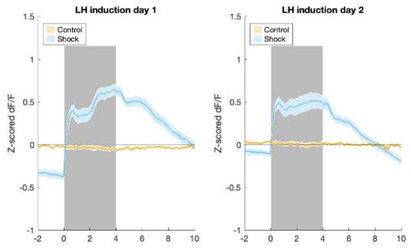
Chemogenetic Activation of ACh release
Retrograde, Cre-dependent AAVs expressing HMD3(Gi), HM3D(Gq) or an mCherry control virus were bilaterally injected into the mPFC of male (n=36) and female (n=36) ChAT-Cre mice. LH behavior was carried out a minimum of 4 weeks after surgery to allow for adequate viral expression. Mice were administered 7 mg/kg clozapine N-oxide dihydrochloride (Hello Bio. Princeton, NJ) 30 m prior to both induction sessions. (*Representative image of injection site*).

Inescapable shocks induce escape deficits



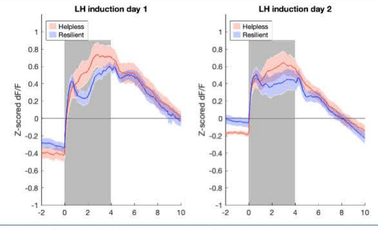
Escape latencies were significantly longer in mice following exposure to inescapable shock compared to control mice that received no shock during induction ($F(26,754)=76.51$, $p<0.0001$). The ratio of helpless:resilient mice was approximately the same for both male and female mice.

ACh is released in mPFC in response to shock



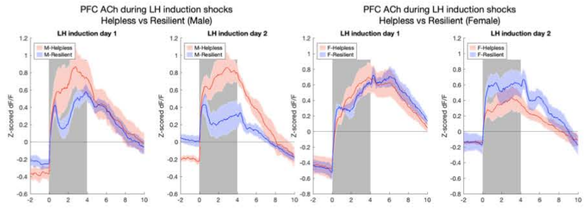
A robust ACh signal is elicited by inescapable shocks. The shock occurs from 0 – 4 s but mPFC ACh levels are elevated for several seconds after termination.

ACh levels in mPFC during LH induction differ in resilient and helpless mice



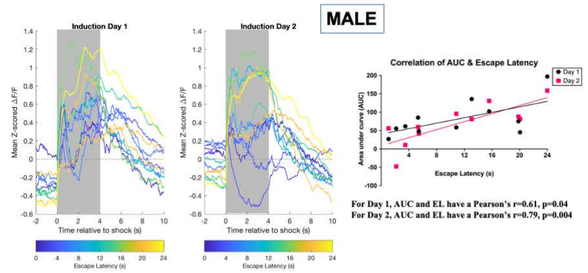
ACh levels in the mPFC of helpless and resilient mice as recorded during two 1-h sessions of inescapable shock. Sexes combined.

ACh differences during LH induction are prominent in male, but not female, mPFC

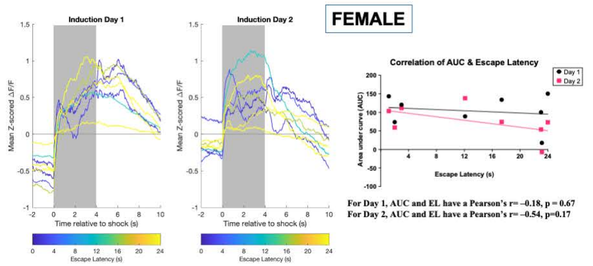


Differences between helpless and resilient mice are more apparent when the sexes are assessed separately. The biggest differences in ACh signaling between helpless and resilient mice are observed in males, with more separation evident on Day 2 of Induction.

ACh levels in mPFC during LH induction are correlated with escape deficits in male mice

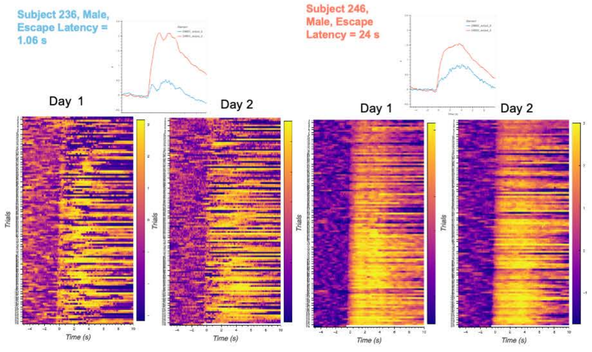


When assessed on an individual basis, the area under the curve (AUC) for the average ACh transients per day positively corelated with escape deficits, here measured as escape latency, in male mice.



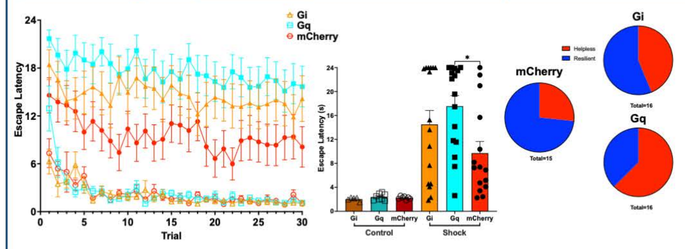
Although females experienced the same rate of helplessness as males, AUCs were not correlated with escape latency in female mice.

ACh AUCs during induction increase across sessions for helpless mice

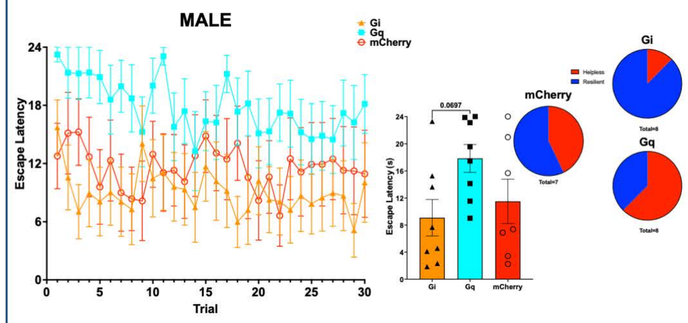


Heatmaps displaying ACh signal per trial for two individual male mice: the ones with the fastest (left, 'resilient') and slowest (right, 'helpless') escape times. In mice with increased escape latencies, the signal appears to increase across trials.

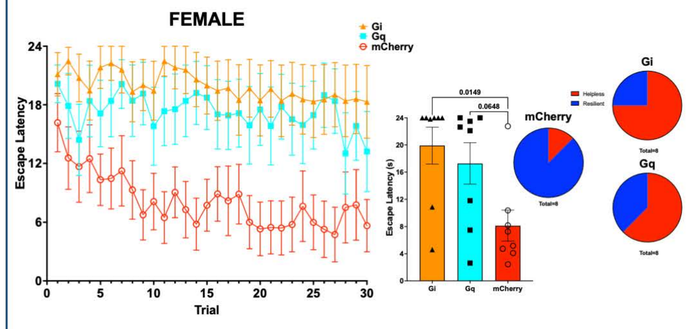
DREADD-mediated manipulation of ACh signaling during LH induction alters later escape behavior



Gq-DREADD-mediated excitation of cholinergic neurons in the mPFC was sufficient to increase escape latencies in an active avoidance test. No differences were observed between control groups.



Gq-DREADD-mediated excitation of cholinergic neurons in the mPFC increased escape latencies in male mice compared to Gi-DREADD-mediated inhibition of the same population, which induced an increase in resiliency to inescapable shocks.



Both Gi-DREADD-mediated inhibition and Gq-DREADD-mediated excitation of cholinergic cells in the mPFC was sufficient to increase escape latency and drive helplessness in response to inescapable shocks.

Conclusions

- Helpless male mice during testing show larger ACh transients in the mPFC in response to inescapable shock during training.
- mPFC ACh transients during LH induction trials are positively correlated with escape latency in active avoidance testing of male mice.
- Although proportions of helplessness did not differ between sexes, ACh dynamics in the mPFC only correlated with escape behavior in males. However, shifting ACh activity either up or down in females using Gq- or Gi-DREADDs increased escape deficits during active avoidance testing and shifted the ratio of helpless:resilient mice, suggesting that unlike males, changes from optimal release in either direction resulted in increased helplessness for female mice.
- Male mice only displayed increased escape deficits in active avoidance testing when ChAT-positive cells were stimulated with a Gq-DREADD. Unlike female mice, decreasing ACh levels via Gi-DREADD-mediated inhibition of ChAT-positive cells increased the proportion of resilient mice, with escape latencies in active avoidance testing similar to control animals.
- The current study provides evidence that increased mPFC ACh results in greater behavioral responses to inescapable stressors, supporting the possibility of a ***negative encoding bias***, in which stressful experiences are more potently attended to and encoded.