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Acetylcholine signaling in the medial prefrontal cortex increases learned helplessness behavior in mice

Zuhair I. Abdulla[1], Yann S. Mineur[1], Richard B. Crouse[1], Ian Etherington[1,2], Hanna Yousuf[1], 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

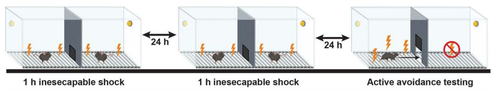
Introduction

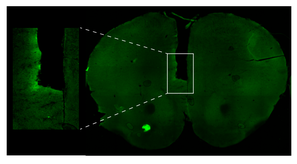
- Several lines of evidence suggest that acetylcholine (ACh) is important in the etiology of depression.
- Blocking the breakdown of ACh produces depressive symptoms in healthy subjects while exacerbating them in depressed patients.
- ACh is also important for learning, memory, and – especially in the 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*).

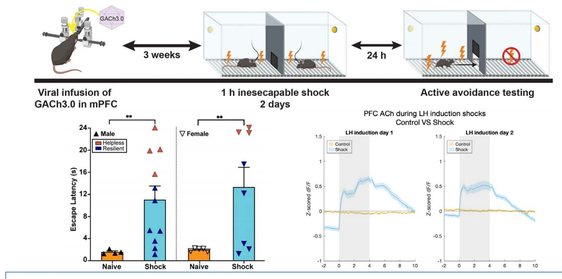
Systemic Pharmacological Increases of ACh

Physostigmine, an ACh esterase inhibitor, or saline was administered i.p. to male (n=8) and female (n=7-8) mice 30 m prior to the second LH induction session. Physostigmine was not administered on either day 1 of induction or prior to the active avoidance test.

Chemogenetic Activation of ACh release

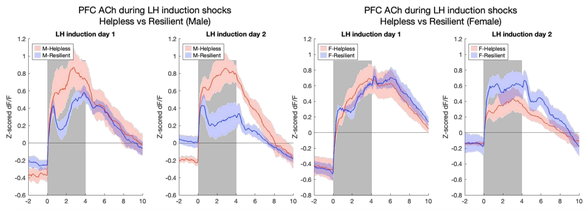
Retrograde, Cre-dependent AAVs expressing HMD3(Gi), HM3D(Gq) or an mCherry control virus were bilaterally injected into the mPFC of male (n=36) and female (n=36) ChAT-Cre mice. LH behavior was carried out a minimum of 4 weeks after surgery to allow for adequate viral expression. Mice were administered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

Inescapable shocks induce escape deficits



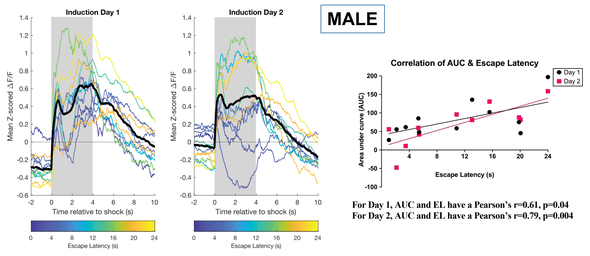
Top. Experimental design. **Left.** Escape latencies were significantly longer in mice following exposure to inescapable shock compared to control mice that received no shock (naïve) during induction ($F(1,24)=11.5$, $p<0.01$) and did not differ between sexes. Furthermore, the ratio of helpless:resilient mice was approximately the same for both male and female mice. **Right.** Inescapable shock produced a robust ACh response in the mPFC. Shaded portion represents the duration of the shock. Lines are average z-scored ΔF/F for each induction day.

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

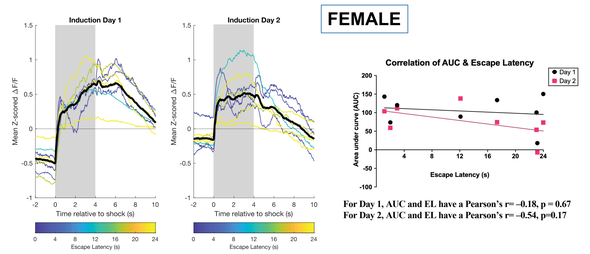


Differences between helpless and resilient mice are more apparent when the sexes are assessed separately. The biggest differences in ACh signaling between helpless and resilient mice are observed in males **(left),** with more separation evident on Day 2 of Induction.

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

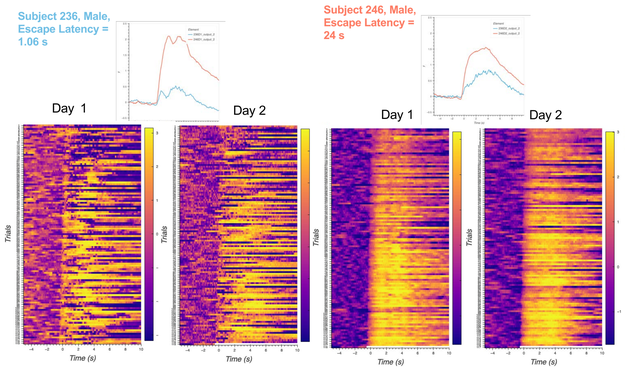


When assessed on an individual basis, the area under the curve (AUC) for the average ACh transients per day positively corelated with escape deficits, here measured as escape latency, in male mice (Day 1: $r=0.61$, $p<0.05$; Day 2: $r=0.79$, $p<0.01$).



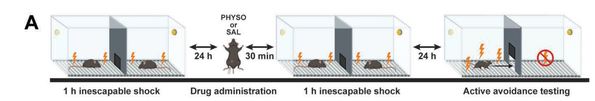
Although females experienced the same rate of helplessness as males, AUCs were not correlated with escape latency in female mice, with those with longer escape latencies (yellower lines) having AUCs both above and below the mean (thick black line).

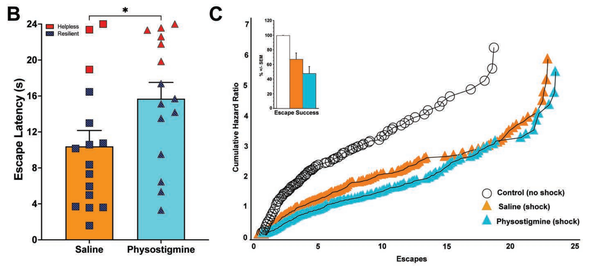
ACh AUCs during induction increase across sessions for helpless mice



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.

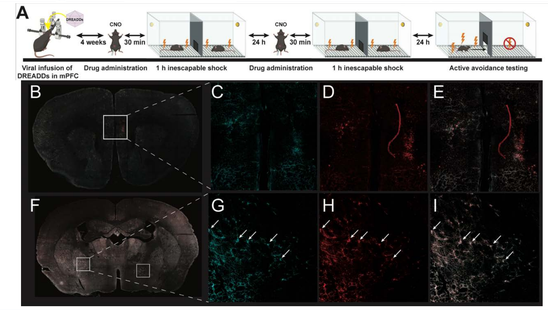
Pharmacologically prolonging ACh signaling during LH induction increases escape deficits in later active avoidance testing



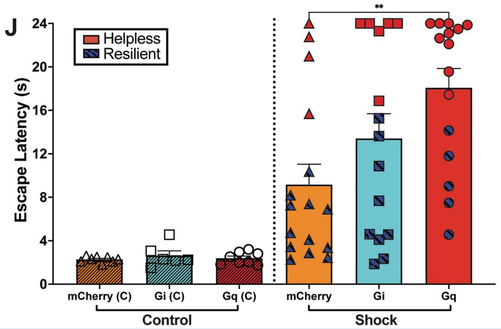


A. Experimental design. **B.** Physostigmine administration 30 min prior to the second induction session increased escape latencies ($t(29)=2.10$, $p<0.05$) and **C.** decreased overall number of escapes (**insert**; $U=-5.16$, $n_1=16$, $n_2=15$). Furthermore, a Trend LogRank for escapes (censored for no escapes) indicated a significant difference in escape performance between mice that received physostigmine and those that received saline, indicating that even when excluding all mice that failed to escape at all, mice that received saline reached full escape efficacy sooner than those treated with physostigmine (x^2:135.9, df=1, P<0.0001).

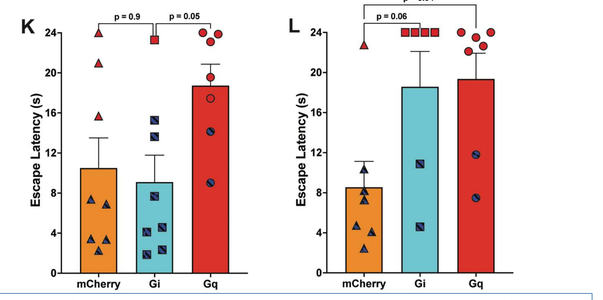
DREADD-mediated manipulation of ACh signaling during LH induction alters later escape behavior



A. Experimental design. **B.** Coronal slice containing bilateral mPFC (box, blowup in **C, D,** & **E** showing ChAT stain, DREADD tagged with mCherry, and merged images, respectively). **F.** Coronal slice containing bilateral basal forebrain (box, blowup in **G, H,** & **I** showing ChAT stain, DREADD tagged with mCherry, and merged images, respectively. Arrows indicate cell bodies.).



Shock exposure during induction increased escape latency in all groups relative to controls. Controls did not differ from each other. Gq-mediated excitation of ChAT-expressing terminals in the mPFC during induction sessions increased escape latencies in later active avoidance testing ($F(5,62)=11.51$, $p<0.001$).



K. In male mice, Gq-mediated excitation of ChAT-expressing terminals in the mPFC increased escape latencies ($F(2,20)=3.59$, $p<0.05$), while Gi-mediated inhibition of the same terminals drove resilience ($x^2(1, N=15)=5.40$, $p<0.05$). **L.** In females, both Gi-mediated inhibition and Gq-mediated excitation of mPFC cholinergic terminals resulted in increased escape latencies ($F(2,17)=4.57$, $p<0.05$).

Conclusions

- ACh signaling in the mPFC is critical in setting the balance between susceptibility and resilience to helplessness following exposure to inescapable stressors.
- ACh dynamics in the mPFC during LH induction predict failure to escape effectively in male, but not female mice.
- Increasing cholinergic activity in the mPFC increases maladaptive coping in the LH paradigm, while decreasing ACh activity in the mPFC has sex specific effects.
- Data suggests that resilience to inescapable stress can be conferred in males by reducing mPFC ACh levels, but that ACh levels are optimal in female mice at baseline, and either increasing or decreasing levels results in greater susceptibility to stress.
- For female mice, this is similar to how cholinergic signaling follows an inverted U-shaped curve, in which both too much or too little ACh is detrimental to cognitive processes.
- 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.