Endocannabinoids, the Amygdala and Anxiety

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Cannabis and the Endocannabinoid System
Cannabinoid CB₁ Receptor
– Presynaptic, suppresses neurotransmitter release

Endogenous ligands
• Similar to endorphins
• Two ligands:
  – Anandamide (AEA)
    • Metabolized by fatty acid amide hydrolase (FAAH)
  – 2-Arachidonoylglycerol (2-AG)
    • Metabolized by monoacylglycerol lipase (MAGL)
The Endocannabinoid System

Morena, Patel, Bains and Hill, *Neuropsychopharm* 2016
Tonic eCB Signaling as a Gatekeeper of Stress and Anxiety
Tonic eCB Signaling Constrains Stress and Anxiety

Patel et al., 2004

Patel and Hillard, 2006
Tonic eCB Signaling Constrains Stress and Anxiety

- Local blockade of CB1 receptors within the BLA:
  - Increases activation of the HPA axis (Hill/Hillard, Akirav)
  - Increases anxiety (Gilpin/Tasker, Currie)
  - Impairs fear extinction (Lutz/Pape)

Hill et al., 2009 NPP
Acute stress is known to increase excitatory drive to BLA pyramidal neurons.

Unpublished data, in collaboration with Shona Chattarji.
Tonic eCB Signaling Constrains Stress and Anxiety

- Blockade of CB1 receptors increases glutamate release onto pyramidal neurons in the BLA similar to acute stress

Unpublished data, in collaboration with Shona Chattarji
Tonic eCB Signaling Constrains Stress and Anxiety

- **Interim Summary**

- Disruption of tonic eCB signaling produces a stress-like state characterized by alterations in behavior, neuronal activation and activation of stress-responsive hormonal cascades.
- These effects can largely be recapitulated by local disruption of eCB signaling only within the BLA.
- Blockade of CB1 receptors in the BLA increases glutamatergic drive to pyramidal neurons known to be involved in the assembly of a stress response.
- This suggests tonic eCB signaling gates excitatory inputs to the BLA, and that at rest there is constitutive eCB signaling at these synapses restricting afferent drive to the BLA.

- If disrupting eCB signaling can produce a stress-like response, is a disruption of eCB signaling part of the normative cascade involved in mounting a stress response?
Exposure to acute stress rapidly reduces AEA levels within the amygdala (Hill, Patel, Hillard, Finn).

This is mediated, in part, by a rapid induction of AEA hydrolysis by the enzyme FAAH.

Gray et al., 2015 J Neurosci
Tonic eCB Signaling in the BLA Constrains Stress and Anxiety

- This stress-induced reduction in AEA is not sexually dimorphic as similar effects are seen in female rats

Vecchiarelli et al., in preparation
Tonic eCB Signaling in the BLA Constrains Stress and Anxiety

- Genetic or pharmacological disruption of FAAH, systemically or in the BLA, can attenuate:
  - Stress-induced activation of the HPA axis (Hillard, Hill, Gaetani)
  - Stress-induced anxiety (Hill, Centonze, Lutz, Patel)
  - Stress-induced impairments in fear extinction (Akirav)

![Graph showing open arm time (s) for VEH and FAAH-I conditions with CON and STRESS groups.](image)

Hill et al., 2013 *Mol Psychiatry*
In preparation, collaboration with Shona Chattarji

Tonic eCB Signaling in the BLA Constrains Stress and Anxiety

- Inhibition of FAAH suppresses stress-induced increased in glutamate release in the BLA.
AEA as a Gatekeeper of the Amygdala

- Our working model of stress-induced loss of AEA signaling in the amygdala and generation of a stress response

Hill and Tasker, 2012 Neuroscience
Mechanism of Dynamic AEA Changes from Stress
The effect of stress on FAAH and AEA in the amygdala can be recapitulated by administration of CRH.

Gray et al., J Neurosci 2015
Co-expression of CRHR1 and FAAH in BLA

- In the BLA, FAAH is co-expressed in glutamatergic pyramidal neurons with CRHR1

Gray et al., *J Neurosci* 2015
CRHR1 on Glutamatergic Neurons Mediates the Effects of Stress on FAAH/AEA

Gray et al., J Neurosci 2015
FAAH Activation Mediates the Anxiogenic Effects of Stress

Gray et al., *J Neurosci* 2015
**AEA as a Gatekeeper of the Amygdala**

- Our working model of acute stress-induced loss of AEA signaling in the amygdala and generation of a stress response

Gray et al., *J Neurosci* 2015
Translational Relevance
In humans, there is a functional polymorphism in the FAAH gene (C385A; P129T) where a C (proline) is substituted for an A (threonine).

A carriers of this polymorphism exhibit reduced levels of FAAH expression and elevated levels of AEA.
• There is reduced binding of a FAAH PET tracer probe in humans carrying an A allele
To explore these mechanisms we developed a mouse possessing the C385A polymorphism in FAAH.

These mice have reduced FAAH activity and elevated AEA levels in the forebrain.

Dincheva et al., 2015 Nat Commun

AEA Signaling Regulates Anxiety and Activation of the Amygdala in Humans
• Both human and mouse A carriers exhibit reduced levels of anxiety

Dincheva et al., 2015 Nat Commun
Consistent with our model of AEA gating the amygdala, humans who carry the A allele of FAAH gene exhibit reduced activation (Hariri et al., 2009) and accelerated habituation of the amygdala in response to stress (Gunduz-Cinar et al., 2013).
AEA Signaling Regulates Anxiety and Activation of the Amygdala in Humans

- Similar to the humans, A carriers also exhibit reduced activation of the amygdala in response to stress

Dincheva et al., 2015 *Nat Commun*
• Similar to our data indicating CRH drives FAAH activity, a SNP in CRHR1 cancels out the FAAH SNP effect on amygdala habituation.
- The FAAH C385A SNP A/A allele prevents stress-induced declines in AEA signaling the circulation.

Mayo et al., submitted
The FAAH C385A SNP A/A allele prevents stress-induced declines in AEA signaling the amygdala.

Mayo et al., submitted
The FAAH C385A SNP A/A allele prevents stress-induced increases in negative affect

Mayo et al., submitted
Summary
Endocannabinoid Signaling and the Stress Response

- **Summary**

- Anandamide signaling tonically constrains glutamatergic inputs to the BLA
- Acute stress, through CRH activation of CRHR1, reduces AEA by increasing FAAH activity.
- Inhibition of FAAH can reduce stress induced glutamatergic drive to the BLA, anxiety, activation of the HPA axis and impairments in fear extinction
- These indicate AEA signaling is a critical regulator of excitability of the amygdala and the generation of stress and anxiety
Endocannabinoid Signaling and the Stress Response

**Summary**

- Genetic variance in FAAH/AEA signaling, resulting in elevated AEA/CB1 receptor signaling, may confer a resilience to the neurobehavioral effects of stress and anxiety.
- Impairments in AEA/CB1 receptor signaling may promote states of perpetual arousal and stress and increase vulnerability to stress-related psychiatric illnesses, such as PTSD

![Graph showing AEA content in control, trauma-exposed, and PTSD groups]

Neumeister et al., 2013 *Mol Psychiatry*
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