Can Molecular Imaging Help Move Forward Endocannabinoid-focused Treatments For Psychiatric Illness?
The Endocannabinoid System and FAAH

Anabolic and catabolic pathways of endocannabinoids and their most likely subcellular localization.

- **DAGL-α**: in most instances are located on postsynaptic neurons in the adult nervous system.
- **DAGL-β**: seems to be most abundant on neurons postsynaptic to CB1 receptors.
- **EMT**: seems to facilitate both endocannabinoid release and re-uptake.
- **FAAH** and **MAGL**: for 2-AG inactivation is localized in presynaptic neurons.

**2-AG biosynthesis**
- **sn-1-Acyl-2-arachidonoylglycerol**
- **PLC**
- **NAPE-PLD**

**2-AG inactivation**
- **MAOL**
- **EMT**

**Endocannabinoid enzymes** as drug targets

- **NAPE-PLD** (Box 1) for 2-arachidonoylglycerol (2-AG) biosynthesis, the phospholipases C (PLC) might be localized on both pre- and postsynaptic neurons.

**Endocannabinoid membrane transporter(s)**

- **NArPE**

**Phosphatidylethanolamine (EMT)** seems to be mostly localized on the plasma membrane. The DAGLs, in particular, are located on postsynaptic neurons in the adult nervous system.
Joanne Cameron’s “World without Pain”: the coinheritance of two FAAH mutations

Microdeletion in a FAAH pseudogene identified in a patient with high anandamide concentrations and pain insensitivity

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Photograph by Kamila Lozinska for The New Yorker
ECS Enzyme Inhibitors: Inspired By The Medicinal Properties Of Cannabis

CB₁/CB₂ receptors

Anti-inflammation, analgesia, anti-anxiety and anti-depression

Endocannabinoids

FAAH/MAGL inhibitors

Arachidonic acid

J. Med. Chem. 2017, 60, 1, 4–46
# Development of CNS Disorders Drugs: Can PET help?

## REQUIREMENTS FOR SUCCESSFUL NEW TREATMENTS:

1. **The disease / condition must be well understood**
2. **A molecular target linked with the disease process must be identified**
4. Advance to IND-enabling studies and Phase 1-III

## PET CAN SUPPORT: KEY DECISIONS

1. Providing proof of concept (proof of biology) CNS imaging in humans
2. Detect and measure expression of molecular target
3. Measure and track drug engagement with molecular target (Target occupancy relationship) and
4. Establish evidence of treatment response
[\textsuperscript{11}C]CURB: PET Tracer for Fatty Acid Amide Hydrolase

\begin{itemize}
\item Baseline [\textsuperscript{11}C]CURB
\item PF-04457845 (4 mg)
\end{itemize}
Objective

To use brain PET imaging of FAAH with $^{[11}\text{C}]\text{Curb}$ to gain a better understanding of whether endocannabinoid metabolism in humans relates to:

- Psychiatric illnesses (substance use disorders, PTSD and anxiety disorder)
- Risk phenotypes
FAAH C385A polymorphism: loss-of-function mutation

A functional non-synonymous missense mutation $\rightarrow$ ↑ sensitivity to proteolytic degradation

Cytosine $\rightarrow$ Adenosine

FAAH expression and activity in human peripheral blood T-lymphocytes.

(Sipe et al., 2002; Chiang et al. 2004)
BRIEF COMMUNICATION
The fatty acid amide hydrolase C385A variant affects brain binding of the positron emission tomography tracer $[^{11}C]CURB$

Isabelle Boileau$^{1,2,3,4,5,6}$, Rachel F Tyndale$^{3,5,7}$, Belinda Williams$^{1,2,3,4}$, Esmaeil Mansouri$^{1,2,3,4}$, Duncan J Westwood$^{1,2,3,4,6}$, Bernard Le Foll$^{3,5,6,7}$, Pablo M Rusjan$^{4,5}$, Romina Mizrahi$^{3,4,5,6}$, Vincenzo De Luca$^{3,5,6}$, Qian Zhou$^{7}$, Alan A Wilson$^{3,4,5}$, Sylvain Houle$^{3,4}$, Stephen J Kish$^{2,3,4,5,6,7}$ and Junchao Tong$^{2,3,4,5}$

Evidence that a SNP affecting FAAH (FAAH C385A) is functional in brain.

[Graph showing brain regions with statistical significance for FAAH C385A variant]

- Amygdala
- Hippocampus
- Occipital Cortex
- Parietal Cortex
- Prefrontal Cortex
- Cingulate
- Temporal Cortex
- Ventral Striatum
- Dorsal Striatum
- Thalamus

* indicates statistical significance
§ indicates trend towards significance
FAAH C385A Behavioral phenotype: Human

Avoidance Behavior

- ↓ Trait Anxiety
- ↓ Amygdala response to threat
- ↑ FC in amygdala fear circuits
- ↓ Withdrawal symptoms in CUD
- ↓ Negative effects for heavy drinking

Reward / Approach Behavior

- ↑ Trait Impulsivity
- ↑ Drug and alcohol use
- ↑ Obesity
- ↑ Ventral striatum response to reward
- ↑ Rate of SUD (sedative drugs)
- ↑ Problematic drinking in AUD and in AUD risk
FAAH KO & C385A KI Behavioral phenotype: Mice

- ↓ Fear avoidance
- ↑ Pain threshold
- ↑ FC in amygdala fear circuits
- ↑ Alcohol intake
- ↓ Acute effects of alcohol
Is FAAH elevated in “fear” related conditions (PSTD and Social anxiety) and associated with “fear” circuits?
FAAH is negatively associated with amygdala functional connectivity in HC (N = 31)

• FAAH affects amygdala circuitry involved in fear and emotion processing
• Drug that inhibit FAAH may regulate this circuit know to be abnormally functioning in PTSD, anxiety and mood disorder.

Green et al. 2020, JNP
FAAH is positively associated with amygdala reactivity to threat in HC (N = 28)

- Higher FAAH levels is associated with amygdala hyper reactivity to threat
- FAAH inhibitors may decrease hyper-reactivity to threat
FAAH inhibitor PF-04457845

- ↑ levels of AEA (10 fold)
- ↓ fear learning (startle)
- ↓ autonomic stress reactivity (EDR)
- ↓ stress-induced affective responses (facial EMG)
- FAAH inhibition = ↓ response to fear and anxiogenic effects of stress.
Cross-sectional study design

Screening Visit
- Informed consent
- Urine drug screen
- Urine pregnancy screen
- Socio-demographics
- Medical history
- LSAS
- SCID-5-RV
- SCID-5-PD

MRI Session
- Scanner MR750 3T
- Urine drug screen

PET Session
- Scanner: CPS-HRRT
- Tracer: [C-11]Curb
- Urine drug screen
- Urine pregnancy screen
- Expired CO
FAAH is marginally elevated in Social Anxiety (N = 12 SAD vs 34 HC)
FAAH is NOT elevated in PTSD (N = 16 PTSD vs 29 HC)
Summary

• Multimodal imaging data in HC are in line with models suggesting that up-regulated FAAH may contribute to abnormal fronto-amygdala circuit function.
• These findings support use of FAAH inhibitors in disorders in which abnormalities in these circuits are suspected.
• Our finding in SAD support the view that upregulated FAAH may contribute to the pathology.
• Our findings in PTSD are not in line with “imperfect” animal models of PTSD in which differences in timeline or acute state may explain discrepant findings.
Is FAAH lower in substance use disorders?

Testing the hypothesis that SUD would be associated with lower levels of FAAH
Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial

Deepak Cyril D’Souza, Jose Cortes-Briones, Gina Creatura*, Grai Bluez*, Halle Thurnauer*, Emma Deaso, Kim Bielen, Toral Surti, Rajiv Radhakrishnan, Aarti Gupta, Swapnil Gupta, John Cahill, Mohamed A Sheriff, Alexandros Makriyannis, Peter T Morgan, Mohini Ranganathant†, Patrick D Skosnik†
FAAH is lower in CUD in early abstinence and correlated with THC metabolites

Heavier more recent use of cannabis ↓ FAAH
Alcohol-induced changes in brain FAAH levels in preclinical studies and clinical investigations of AUD

FAAH KO/KI ↑ DRINKING AND AUD SEVERITY

FAAH IN PMB – INCONSISTENT FINDINGS

Involvement of Endocannabinoids in Alcohol “Binge” Drinking: Studies of Mice with Human Fatty Acid Amide Hydrolase Genetic Variation and After CB1 Receptor Antagonists

Yan Zhou, Ted Huang, Frank's Lee, and Mary Jeanne Kreek

Selective alterations of the CB1 receptors and the fatty acid amide hydrolase in the ventral striatum of alcoholics and suicides

K. Yaragudri Vinogd,b,c, Suham A. Kassird, Basalingappa L. Hungundo,d,e, Thomas B. Cooperb,c,e, J. John Mannd,e, and Victoria Arangod,e

Severity of alcohol dependence is associated with the fatty acid amide hydrolase Pro129Thr missense variant

Matthew E. Sloan1, Joshua L. Gowin1, Jia Yan1, Melanie L. Schwandt2, Primavera A. Spagnolo3, Hui Sun2, Colin A. Hodgkinson2, David Goldman2,3 & Vijay A. Ramchandani1

The endocannabinoid system is altered in the post-mortem prefrontal cortex of alcoholic subjects

Amaia M. Erdozan1,2, Marina Rubiob, Elsa M. Valdizant,4, Angel Pazos2,4, J Javier Meana1,2,5, Javier Fernández-Ruiz2,4,7, Stephen P. H. Alexander9 & Luis F. Callado1,2,5
FAAH is lower in AUD in early abstinence and correlated with drinks a week (N = 14 AUD vs 25 HC)

Best et al. 2020 NPP
Low FAAH may be transient
FAAH substrates are elevated in AUD in early abstinence

Best et al. 2020 NPP
Brain FAAH is marginally lower in people that relapse

Best et al. 2020 NPP
Impaired FAAH function: Phenotype for high alcohol intake and potential vulnerability factor

Hansson 2007
Do FAAH levels vary with risk for AUD?

Family history

Self-reported effects of alcohol during alcohol infusion

HRV during alcohol infusion

Best et al. unpublished
FAAH in brain marginally related to AUDIT Scores and drinks/week

Best et al. unpublished
FAAH polymorphism related to higher AUDIT scores in heavy drinking youth

Univariate ANCOVA
Race as covariate

AUDIT Score

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p = 0.049

AUDIT-C Score

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p = 0.02
Low FAAH: Less negative Effects of Heavy Drinking

Max Alcoholic Beverages (Standard Drinks) Tolerated before Experiencing Negative Effects of Heavy Drinking
Alcohol Sensitivity Questionnaire

Subjective Effects of Alcohol Scale
Maximum Low Arousal, Negative Affect

Best et al. unpublished
Low FAAH related to low HRV during alcohol infusion
Summary

- FAAH levels are lower in CUD and related to heavier use / could mask withdrawal
- FAAH levels are transiently lower / peripheral AEA is higher in recently-abstinent subjects with AUD compared to controls and related to heavier recent alcohol use and relapse
- Family history of AUD is not related to brain levels of FAAH
- Low FAAH is associated with greater rates of drinking (AUDIT), decreased self-reported negative effects of alcohol and lower HRV during alcohol infusion.
Lower FAAH (C385A) is associated with D3 receptor in mice and men

Mansouri et al. 2020, NPP
Lower FAAH (C385A) is associated with D3 receptor in mice and men

Mansourī et al. 2020, NPP

[Graphs and images showing data comparisons and mRNA levels]
Conclusion

- Low FAAH and increased endocannabinoid tone may be related to motivation to use drugs or increased tolerance.

- Whether low FAAH is an acute compensatory response or an inherited or acquired biological vulnerability is not known.

- The exact mechanism linking low FAAH with more drinking or greater tolerance is not known but may involve decreased GABA / increased mesolimbic dopamine signaling.

- Longitudinal studies comparing FAAH vs. clinical symptoms should help in understanding whether FAAH might represent a useful therapeutic target.
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