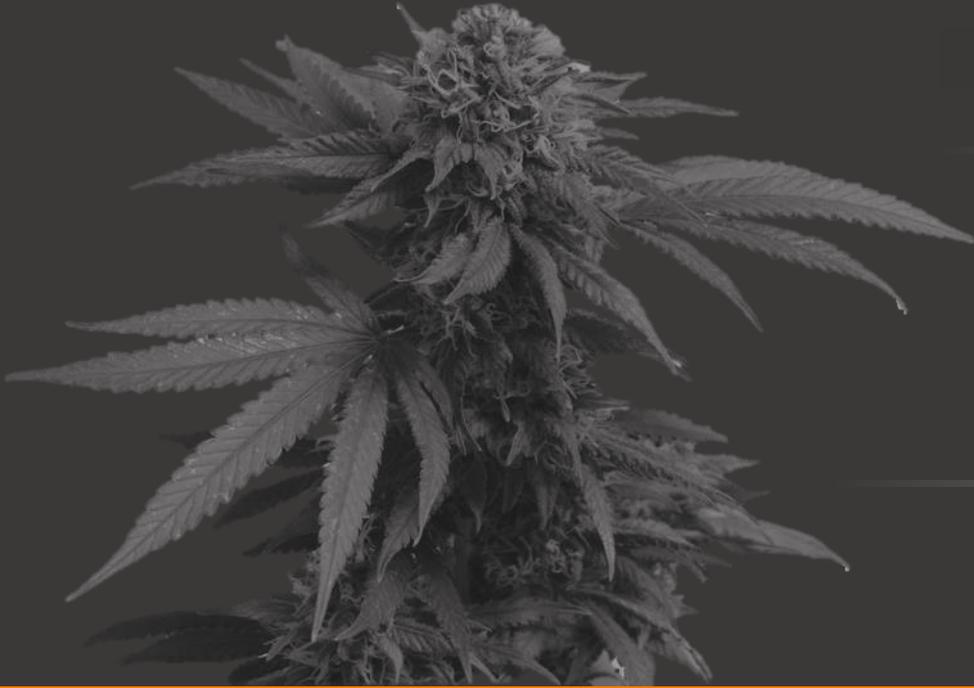


Cannabinoids and the Microbiome:

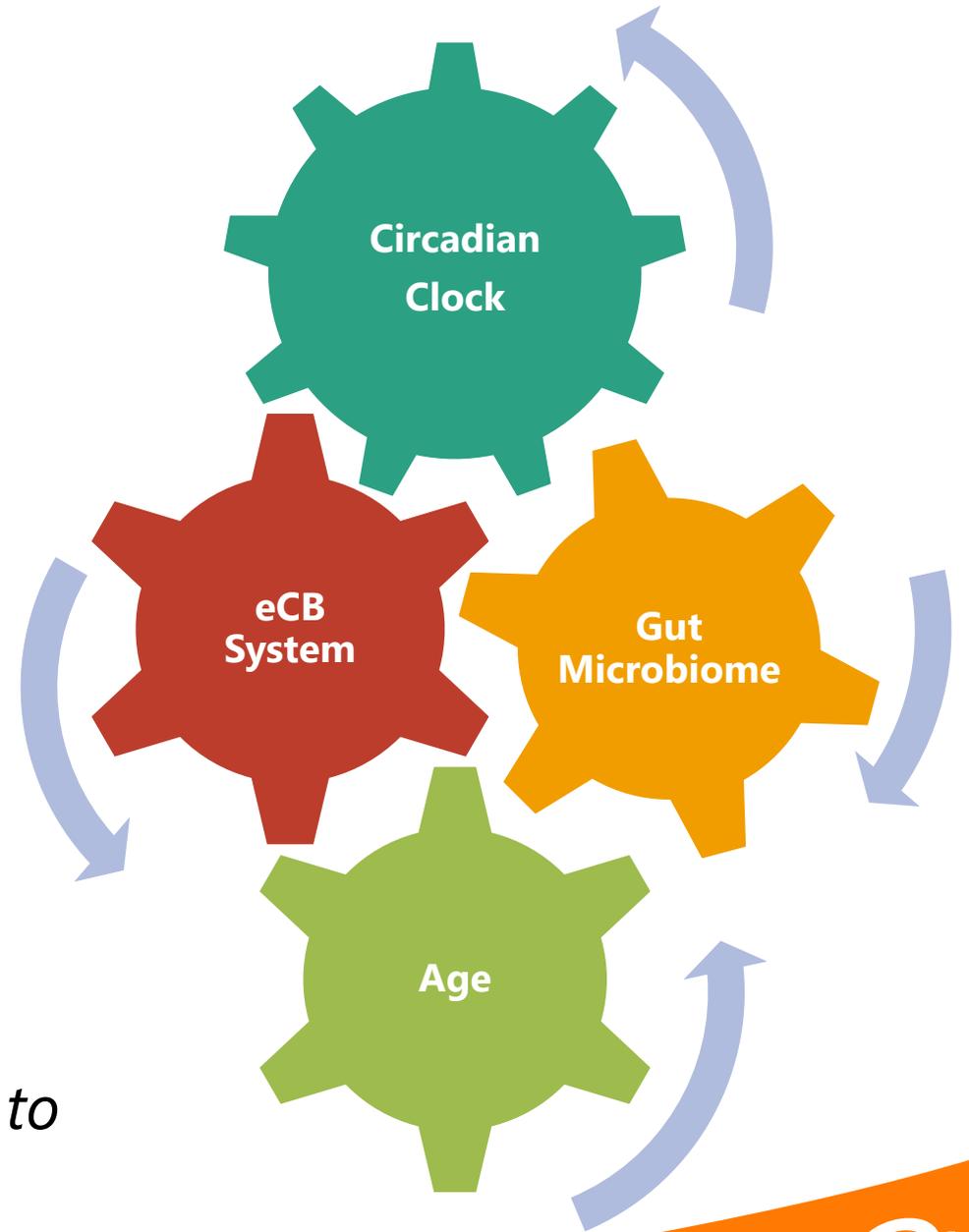


How the Tone Impacts Clinical Trial Strategy



Najla Guthrie
President/CEO
October 2019

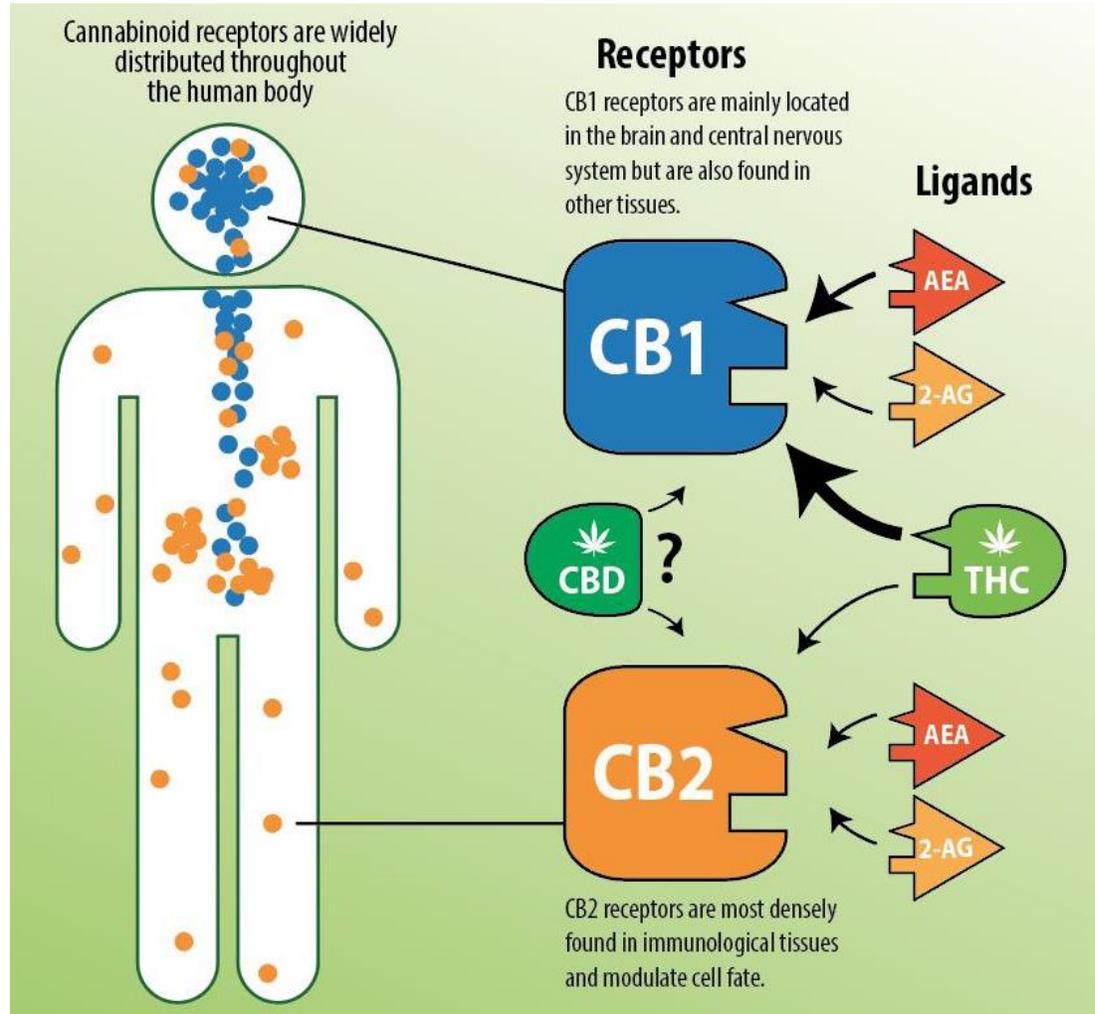
- The circadian clock regulates the eCB system and the gut microbiota
 - Human and animal studies
- Cross-talk between the eCB system and the gut microbiota
 - Most studies are in animal models
- Aging affects circadian rhythm, the eCB system and the gut microbiota
 - Human and animal studies
- Cannabinoids may be beneficial for gut health
 - Emerging area in clinical trials



What factors do we need to consider as we start to conduct cannabis clinical trials in the GI space?

The Endocannabinoid (eCB) System

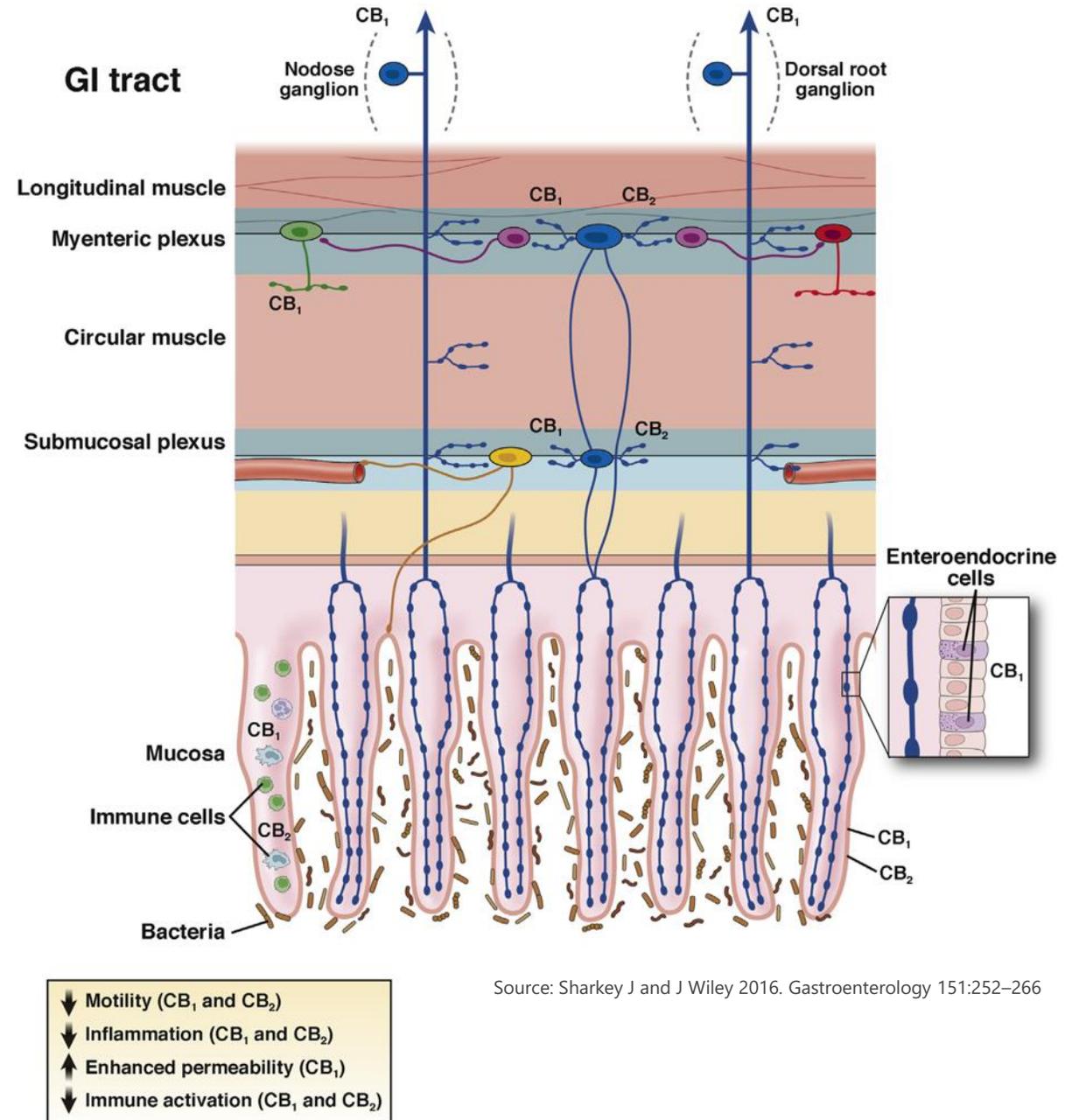
- Identified in 1990s
- Two receptors (CB1 and CB2) and their ligands (2-AG and AEA) have been identified
- Phytocannabinoids (THC and CBD) act on the CB1 and CB2 receptors
- The eCB system regulates many processes, including inflammation, immunity and metabolism
- Endocannabinoid tone: expression and activity of the eCB system components



Source: Nahtigal et al. 2016 J Pain Manage 9(4):481-491

The eCB System in the GI Tract

- Cannabinoid receptors are distributed widely in the GI tract
- CB1 is expressed in neurons, endocrine and epithelial cells
- CB2 is expressed in neurons and immune cells
- CB receptor activation is associated with reduced gut motility, reduced inflammation and increased permeability
- Polymorphisms in eCB system genes are associated with inflammatory bowel syndrome (Ambrose and Simmons 2019 *Journal of Crohn's and Colitis*, 2019, 525–535)

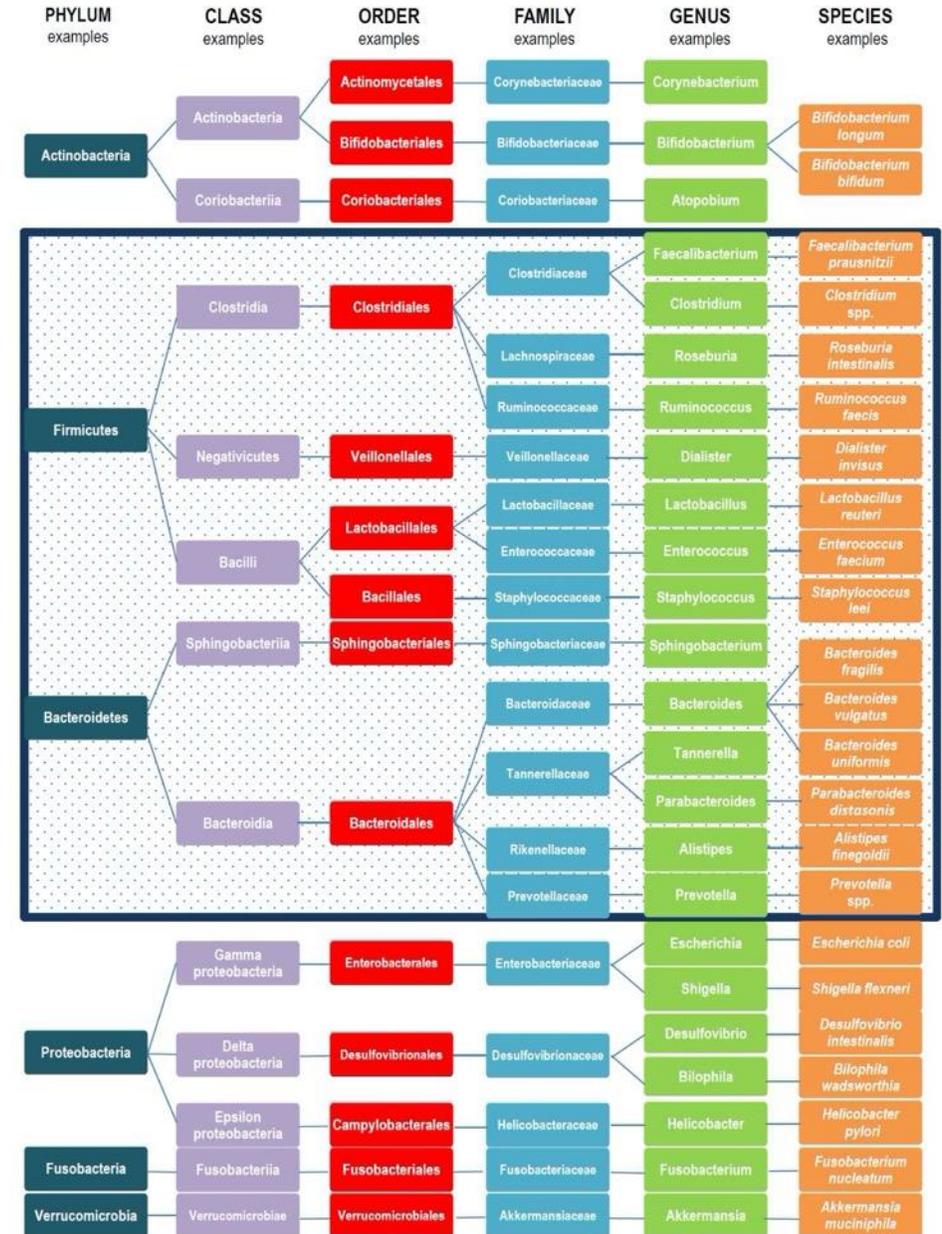


Gut Microbiota

- More than 1 trillion organisms
- The host and the inhabiting organisms = “superorganism”
- Immune and metabolic functions
- Maintenance of intestinal epithelium integrity
- Composition varies in individuals and can change depending on diet, age and health status
- A high F/B ratio is associated with obesity

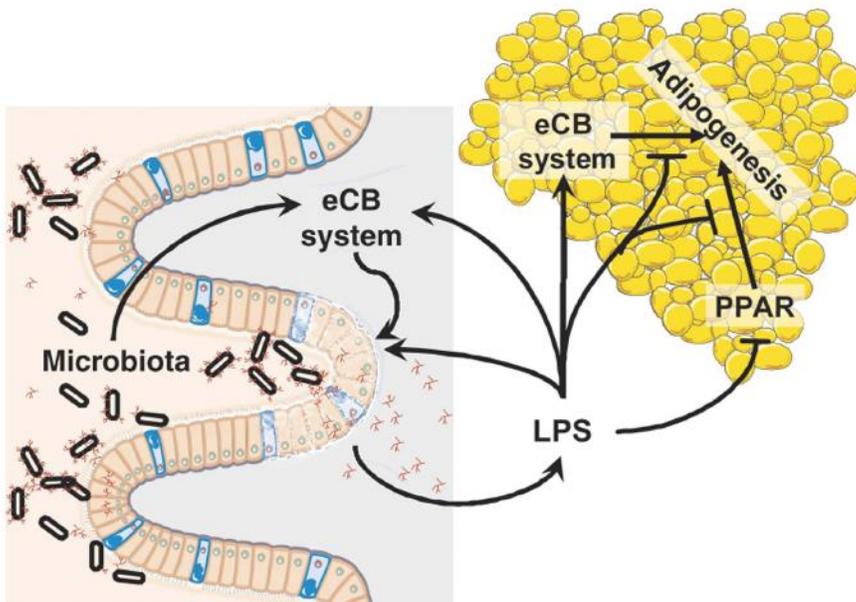
Firmicutes

Bacteroidetes



90%

eCB System/Gut Microbiome Cross-Talk

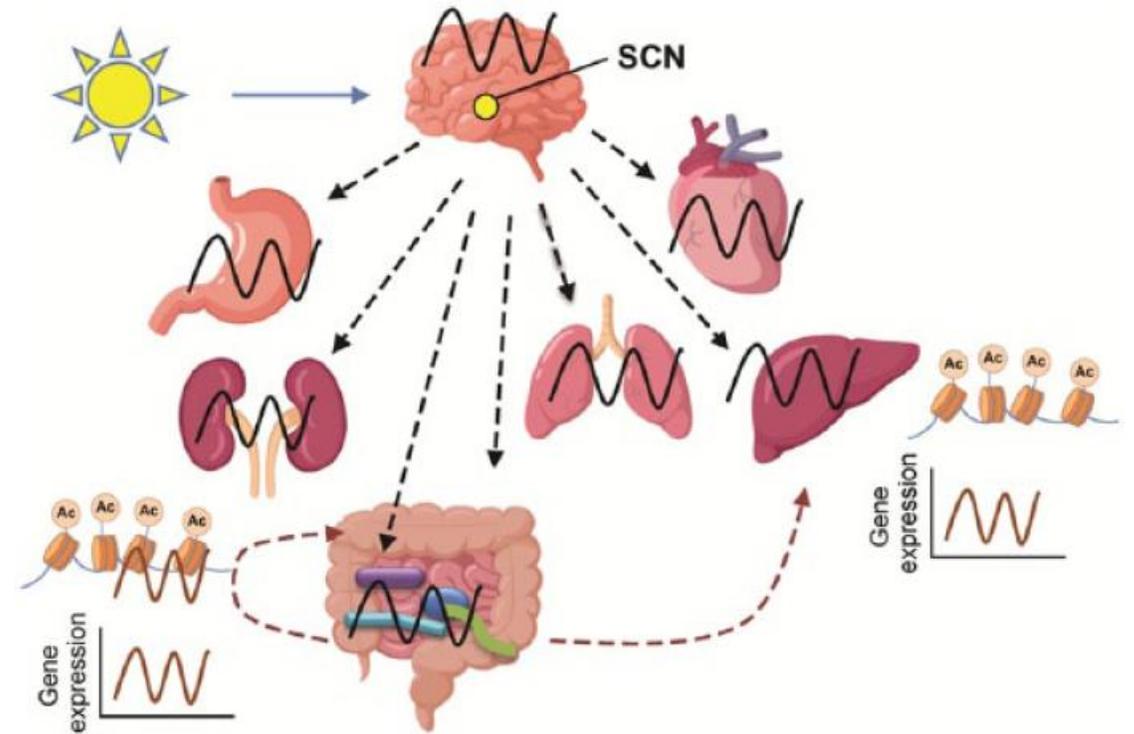


Source: Muccioli et al. 2010 Mol Syst Biol 6: 392

- eCB system is overactivated in conditions of microbiome dysbiosis
 - Increased gut permeability and release of bacterially-derived LPS into the circulation
 - Increased eCB tone in adipose tissue leading to increased fat mass
 - Feedback to the gut further increasing permeability and leading to a vicious cycle
- THC administration prevented the high-fat diet-induced increase in the Firmicutes:Bacteroidetes ratio (Cluny et al. 2015. PLoS ONE 10(12): e0144270)
- THC+CBD treatment attenuates gut microbiome dysbiosis and reduces neuroinflammation in a mouse model of multiple sclerosis (Al-Ghezi et al. 2019. Brain, Behavior and Immunity, *in press*)

The Circadian Clock

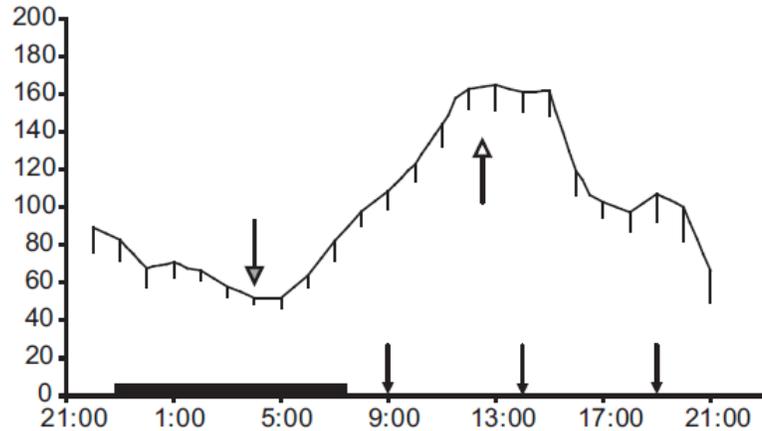
- Adaptation to the 24-h light/dark cycle
- Controls feeding behavior, body temperature, sleep-wakefulness, hormone secretion and metabolism
- The master clock is in the suprachiasmatic nucleus (SCN) in the hypothalamus
- There are peripheral clocks in most organs, which control their function and feedback to the master clock
- The gut clock regulates multiple functions, including nutrient absorption and colonic motility



Source: Liang X and G FitzGerald 2017 J Biol Rhythms 32(6) 505–515

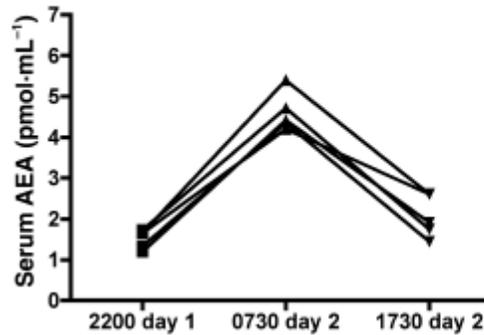
Circadian Rhythm: *The Endocannabinoid System*

Serum 2-AG level (% of 24-hr mean)

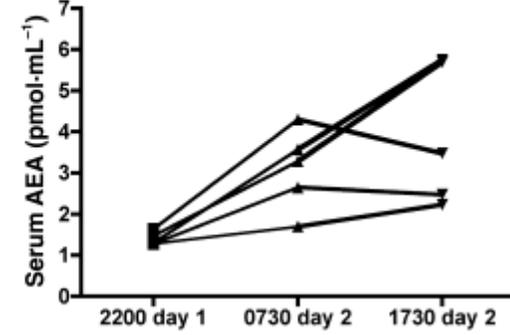


Source: Hanlon et al 2015 Clin Endocrinol Metab 100(1):220–226

A



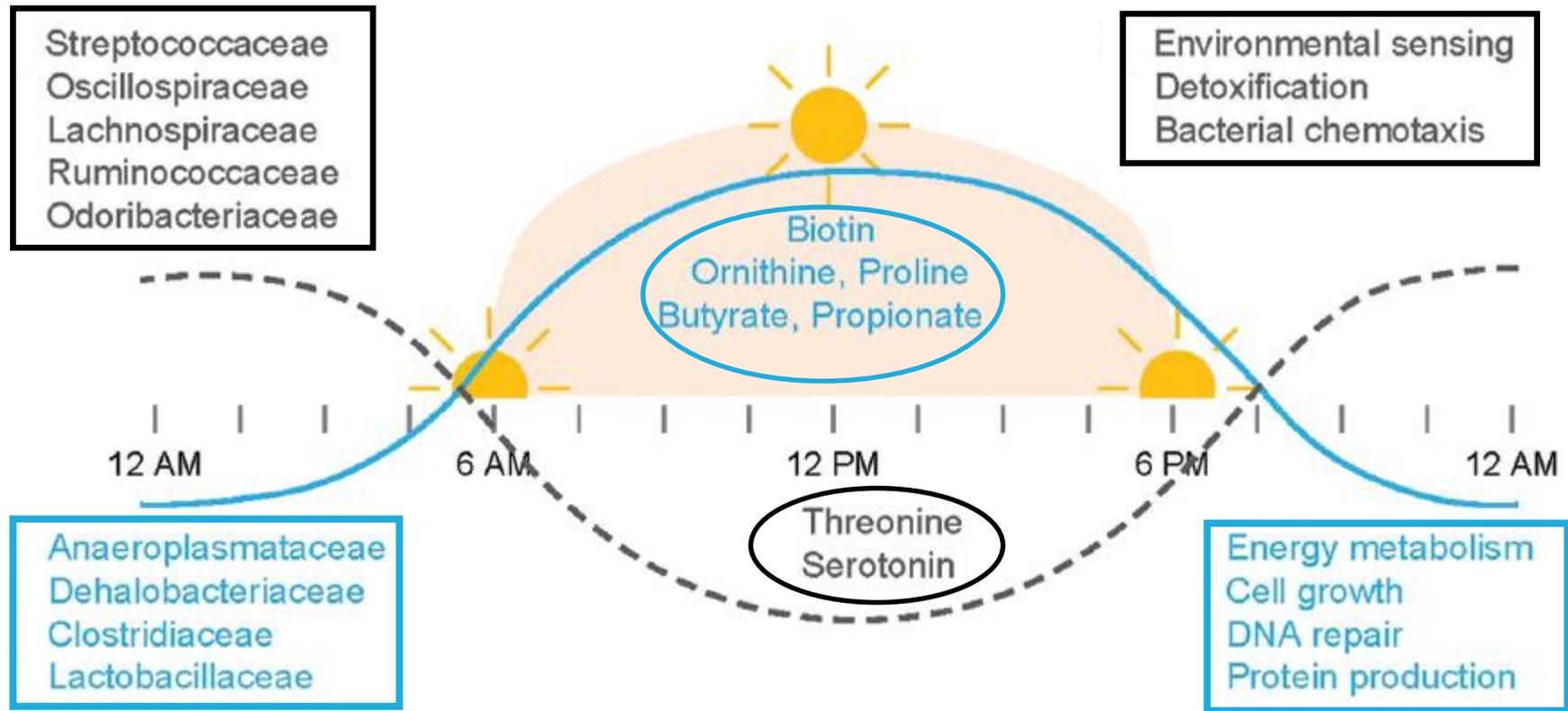
B



Source: Vaughn et al. 2010 Br J Pharmacol 160 530–543.

- 2-AG levels are low at night and increase during the day
- AEA levels are low in the evening, peak in the morning and decrease during the day
- Sleep deprivation affects AEA levels

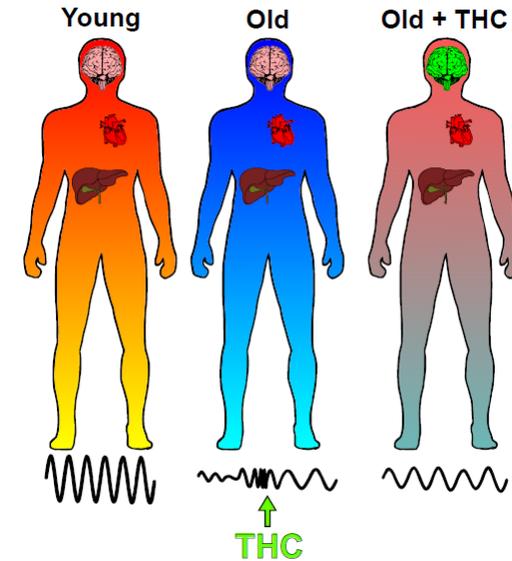
Circadian Rhythm: *The Gut Microbiome*



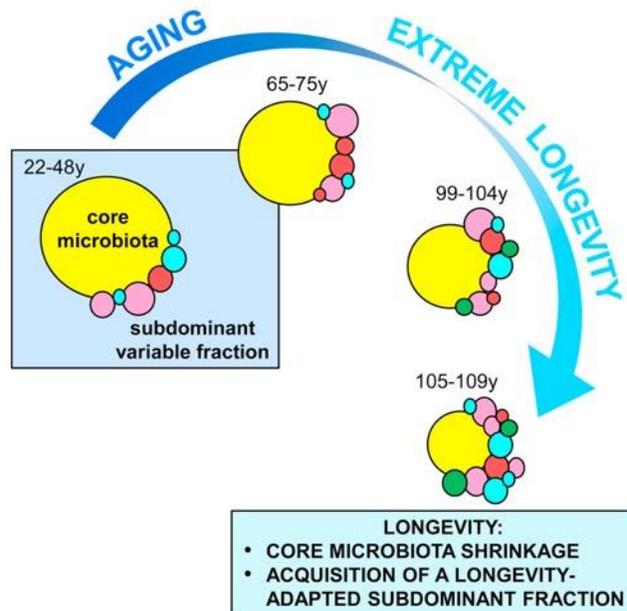
Source: Liang X and G FitzGerald 2017 J Biol Rhythms 32(6) 505–515

Aging: Circadian Rhythm, eCB System and the Gut Microbiome

- eCB tone decreases as we age
- Aging is associated with disrupted sleep patterns and reduced ability to maintain core body temperature
 - Low dose cannabinoids may be effective in restoring circadian rhythm and thermoregulation



Source: Hodges E & N Ashpole. 2019 Neurobiology of Aging 79:110e118

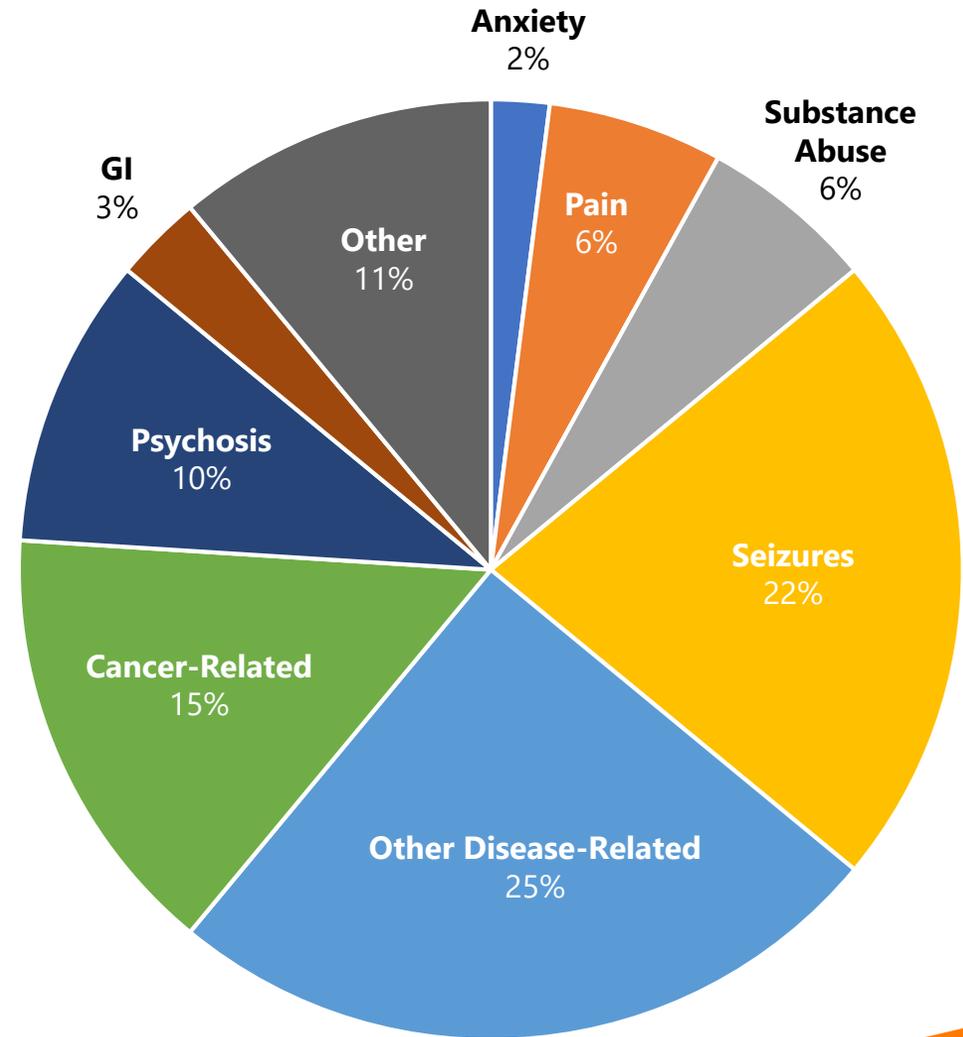


Source: Biagi et al. 2016 Current Biology 26, 1480–1485

- The composition of the gut microbiota changes with age
 - There is an enrichment of health-associated bacteria in very old individuals

Clinical Trial Strategy: *Opportunities and Challenges*

- Healthy population recruitment
- Study design
- Outcomes



Source: www.clinicaltrials.gov

Clinical Trial Strategy: *Recruitment*

- Participant health status and age
- Participant lifestyle
 - Sleep habits
 - Diet
- Participant genetic profiles
 - SNPs in eCB system genes are associated with IBD (Ambrose and Simmons 2019 *Journal of Crohn's and Colitis*, 2019, 525–535)

What is a Healthy Population? *KGK Survey (48 Respondents)*

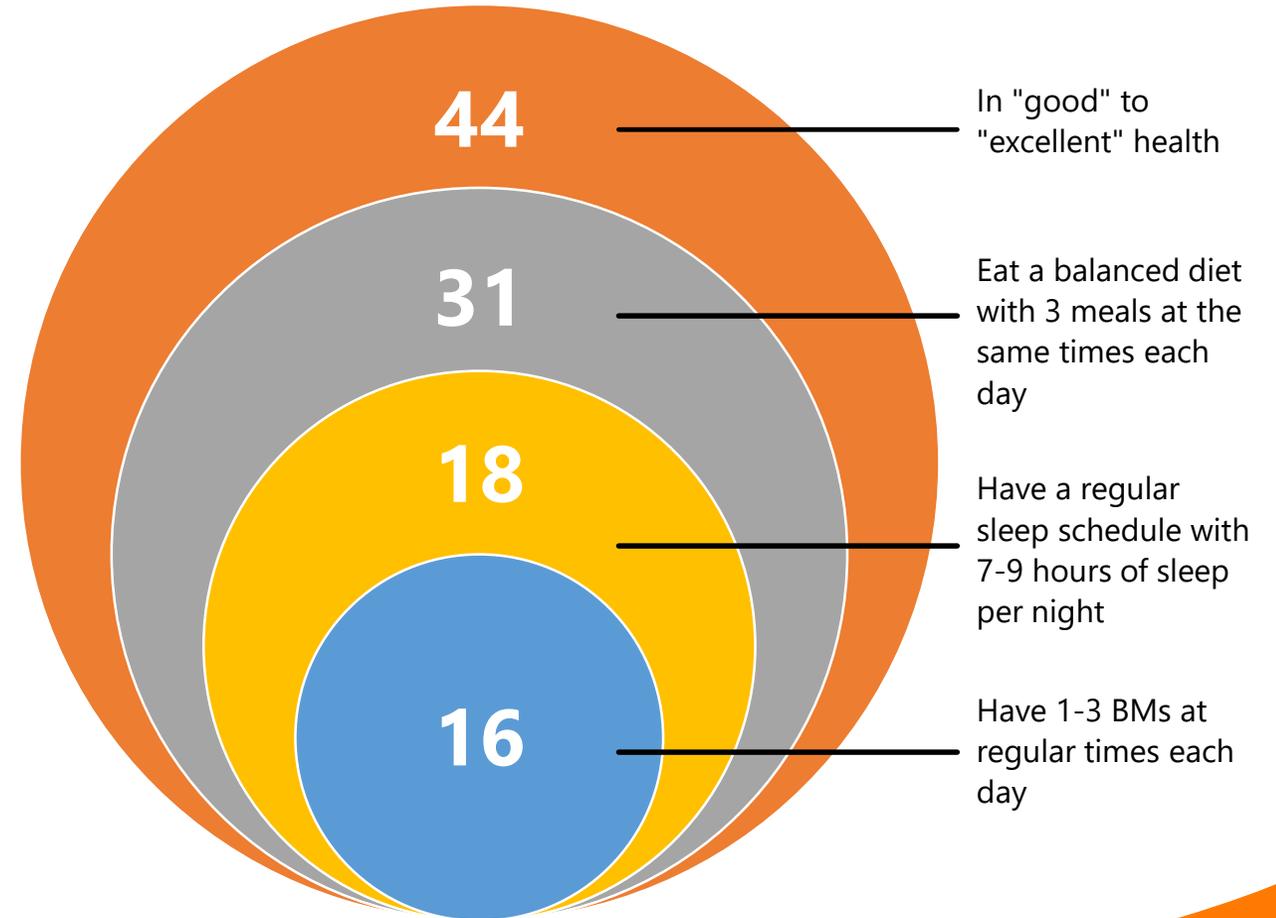
1. In general, how would you rate your own health?
2. Do you eat a balanced diet?
3. How many scheduled meals do you eat in a day?
4. Do you eat these meals at the same times each day?
5. Do you have a regular sleep schedule?
6. How much sleep do you get per night?
7. Do you wake up feeling well-rested?
8. How many bowel movements do you have per day?
9. When do you usually have your bowel movement(s)?

Self-Reported Health Status



Clinical Trial Strategy: *Recruitment*

- Only **36%** of our respondents who reported that they were in good, very good, or excellent health had a balanced diet, a regular sleep pattern, and regular bowel movements



Clinical Trial Strategy:

Study Design

- Run-in period
 - Ensure all participants have similar dietary and sleeping habits prior to the study
 - Account for large placebo effect, which is often observed with GI studies
- Time and length of study visits
- Time of investigational product administration
- eCB tone of participants (circulating levels of 2-AG and AEA)

Outcomes for GI Health Studies

- Microbiome screening of individuals via fecal collection and subsequent extraction of RNA/DNA sequences
- Gastric emptying (via radiopaque markers), gas concentration (intestinal gas capsules) and/or GI inflammation (fecal calprotectin or lactoferrine)
- Validated gut-health questionnaires (bowel habits, GI symptoms, and quality of life)

Conclusions

- The cross-talk between the eCB system and the gut microbiota is an emerging field
- eCB tone should be considered in clinical trial design
- Cannabinoids may be beneficial for gut health

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