The role of microRNA in PFC development and neuroplasticity mechanisms

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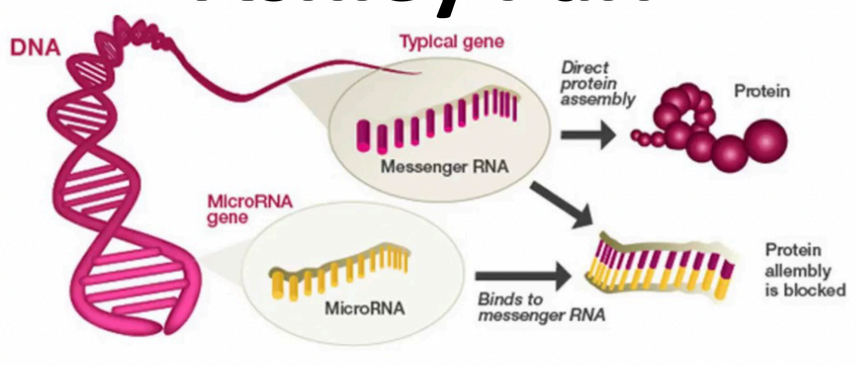


Takehome(S)

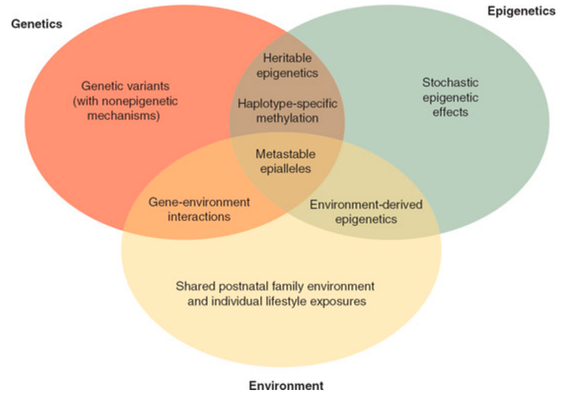
- PFC development throughout adolescence is sensitive to experience, and microRNAs can shed light on the epigenetic processes facilitating critical period plasticity and experience-dependent plasticity mechanisms in the PFC

- microRNA expression changes throughout the lifespan, including in adolescence, controlling gene networks that guide PFC and cognitive development

- microRNA as a tool to understand individual differences in vulnerability to neuropsychiatric disorders with adolescent onset (and resilience)

We proposed to study several microRNAs in the new 7T grant that will advance our understanding of pruning, critical period plasticity, and PFC development more broadly.

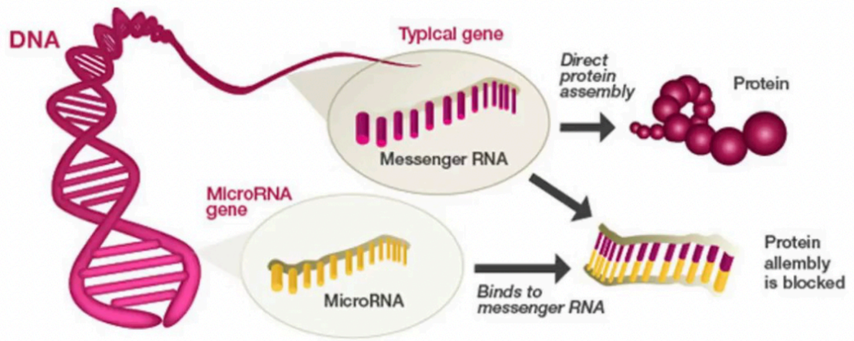
Gene-Environment Interactions throughout Adolescence



- Individual differences in vulnerability (and resilience) during adolescence result from the complex interaction between genetic and environmental factors
    - Protracted maturation of PFC during adolescence makes it sensitive to the surge in novel experiences (experience-dependent plasticity)
- Studies on epigenetic processes implicated in PFC function and development highlight **microRNAs** as promising predictive markers that could inform impact of environment on PFC maturation (I.e., stress)
- **Adolescence coincides with drastic changes in local miRNA expression, including miRNA that control gene networks involved in PFC and cognitive development (Morgunova & Flores, 2021; Somel et al., 2010)**
    - Linked to brain development and critical period plasticity (Beveridge et al., 2014; Ziats & Rennert, 2014), and to puberty-related neuronal changes (Sangiao-Alverellos et al., 2013)
    - Altered levels of miRNA in the PFC are associated with adolescent onset psychopathology, including depression & schizophrenia (Morgunova & Flores, 2021)
    - Can inform preventative and therapeutic programs

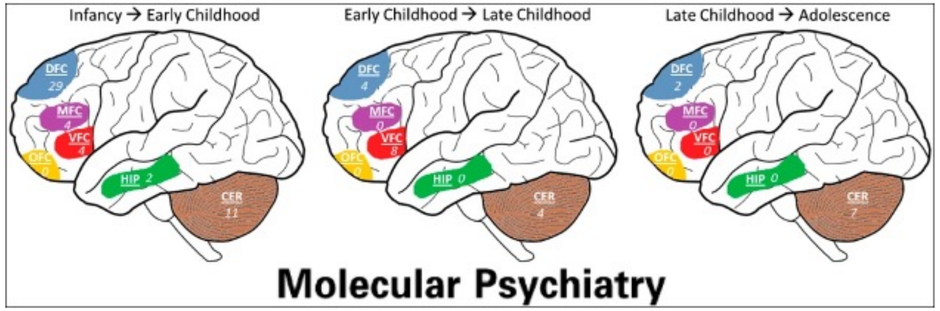
Function of microRNAs

- microRNAs are small, single stranded noncoding RNAs that **regulate gene expression of several messenger RNAs** (mRNA; Morgunova & Flores, 2021)
    - Excreted from cells into peripheral fluids, regulating gene expression at far away targets (Rajman et al., 2017)
- Essential coordinators of developmental programming, and mediators between environment and changes in gene expression
    - Key regulators of large-scale gene expression - **allows a single microRNA to control and coordinate activity of entire gene networks and cellular pathways** (Rajman et al., 2017)
- Binds to mRNA sequences of target genes usually induces transcript degradation and/or prevention of mRNA translation (Ha & Kim, 2014)
- Mature microRNA can be found in the cell's cytoplasm and in the nucleus, and their distribution can change in response to environmental factors (Li et al., 2013; Turunen et al., 2019)



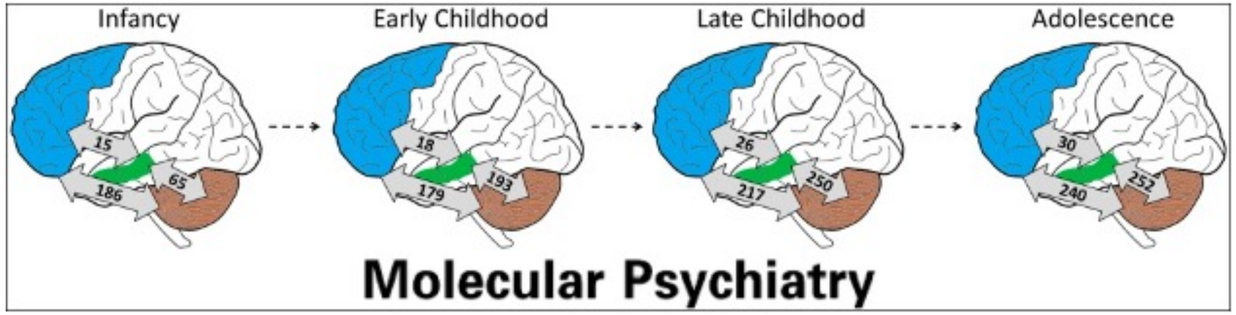
microRNA & Cortical Development

- microRNA are essential regulators of cortical development (Volvert et al., 2012)
  - Production of progenitor cells (Desai et al., 2000; Bian et al., 2013; Abdullah et al., 2016)
  - Survival, differentiation, and spatiotemporal organization of cortical neurons (Tonelli et al., 2008; Schwambom et al., 2009; Cremisi et al., 2013)
  - Shaping development of corticospinal and callosal projection neurons (Diaz et al., 2020)
    - Disruption of microRNA function induces dramatic disruptions to cortical development (Barca-Mayo et al., 2014; Marinaro et al., 2017; Wang et al., 2007)
  - **This control over cortical development extends to adolescence (Torres-Berrio et al., 2020), when the PFC continues to undergo maturation**



microRNA & PFC Development

- Modulate synaptogenesis and axon extension (Kos et al., 2016, Zhang et al., 2013; Li et al., 2014; Kos et al., 2017) (cultured cortical neurons)
- Regulate cortical pyramidal neuron dendritic structure (Christensen et al., 2010), spine morphology (Siegel et al., 2009) - key in intercellular communication & circuit organization
- Establishing synaptic connections, dendritic spine morphology and plasticity (Siegel et al., 2009)
- Mediates neuronal size (Rajkowska et al., 2001), dendritic outgrowth (Black et al., 2004), spine density (Broadbelt et al., 2002)
- **Synaptic pruning and the balance of excitatory and inhibitory signaling (Roshan et al., 2014, Lewis et al., 2005)**
- Together with the adolescent switch in global microRNA expression occurring in the human PFC (Morgunova & Flores, 2021), microRNA provide info about PFC developmental trajectories



Developmental shifts in patterns of microRNA

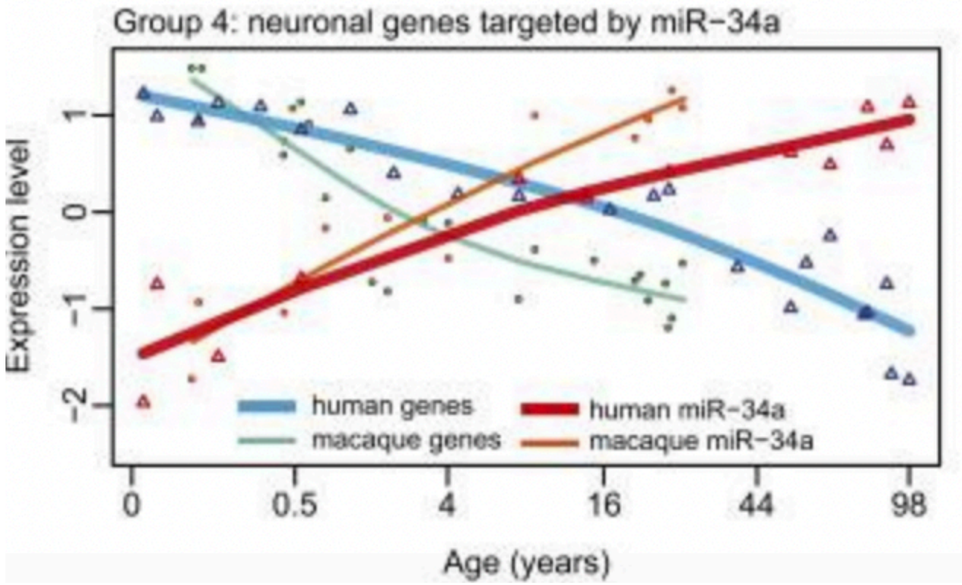
- Postmortem studies show that miRNA expression in the PFC is highly influenced by age, with microRNAs being differentially expressed between infancy, early and late childhood, and adolescence

- **Adolescence coincides with a shift in the pattern of global miRNA expression. Specifically, miRNAs in the PFC show that the pattern of miRNA expression in the PFC splits into two diverging directions:**

  - Some miRNAs begin to be upregulated, some are downregulated (age-associated miRNA expression "**split**") with this new pattern of expression maintained for the rest of life (Beveridge et al., 2014)

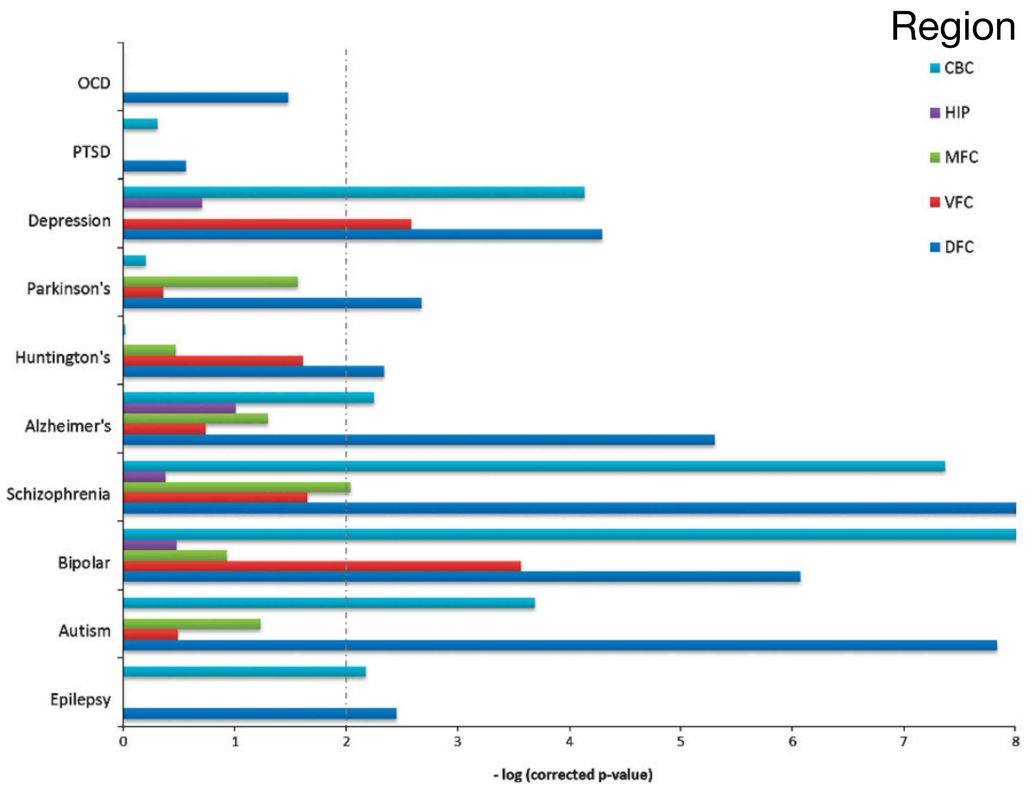
  - Gene targets of the "split" miRNAs are involved in system development and function, cell to cell signaling and interactions (e.g., synaptic transmission and axon and neuritis morphology)

  - **adolescent pattern of miRNA expression trajectories in the PFC could lead to predisposition to mental illness**

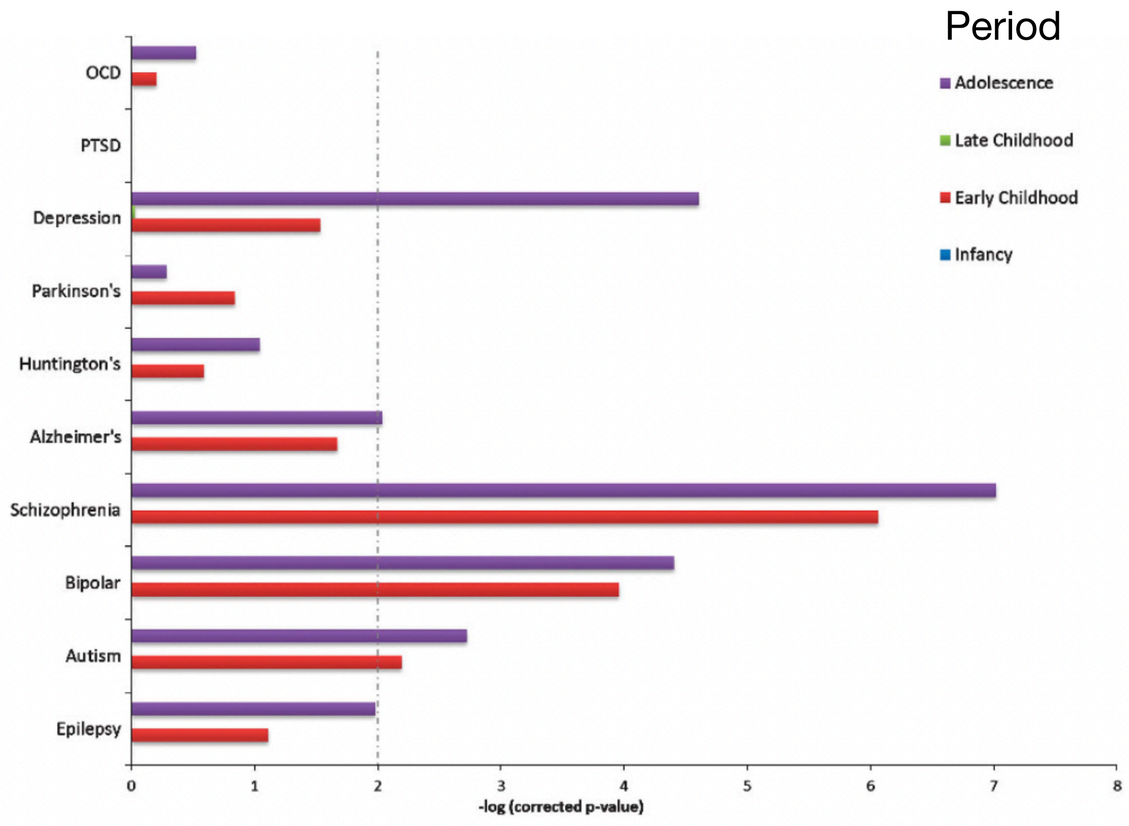


Different patterns of miRNA expression

Disease

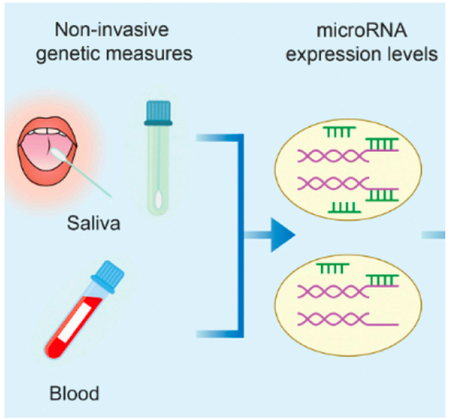


Sex



Ziats & Rennert, 2014

How do we measure it?

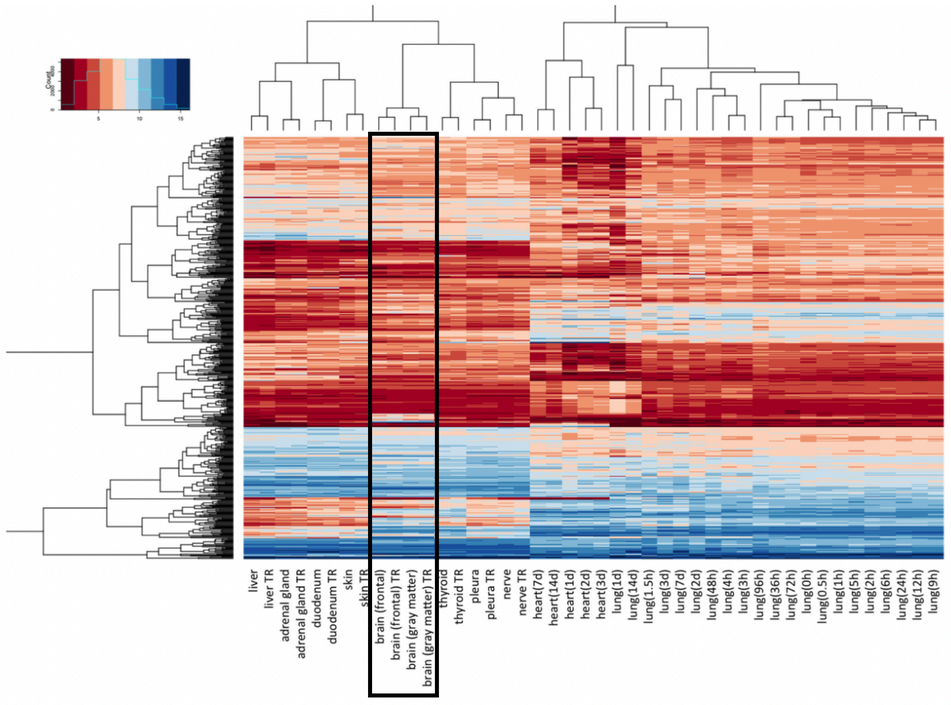


- microRNA are expressed organs all over the body - some are preferentially expressed in the brain, and highly prevalent in the PFC (Ludwig et al., 2016)

- Stable, do not degrade due to heat, or after prolonged storage (Nelson et al., 2006, Mitchell et al., 2008), abundant and quantified in a variety of peripheral fluids including **saliva** (Layne et al., 2019; Fujimoto et al., 2019; Year et al., 2017)

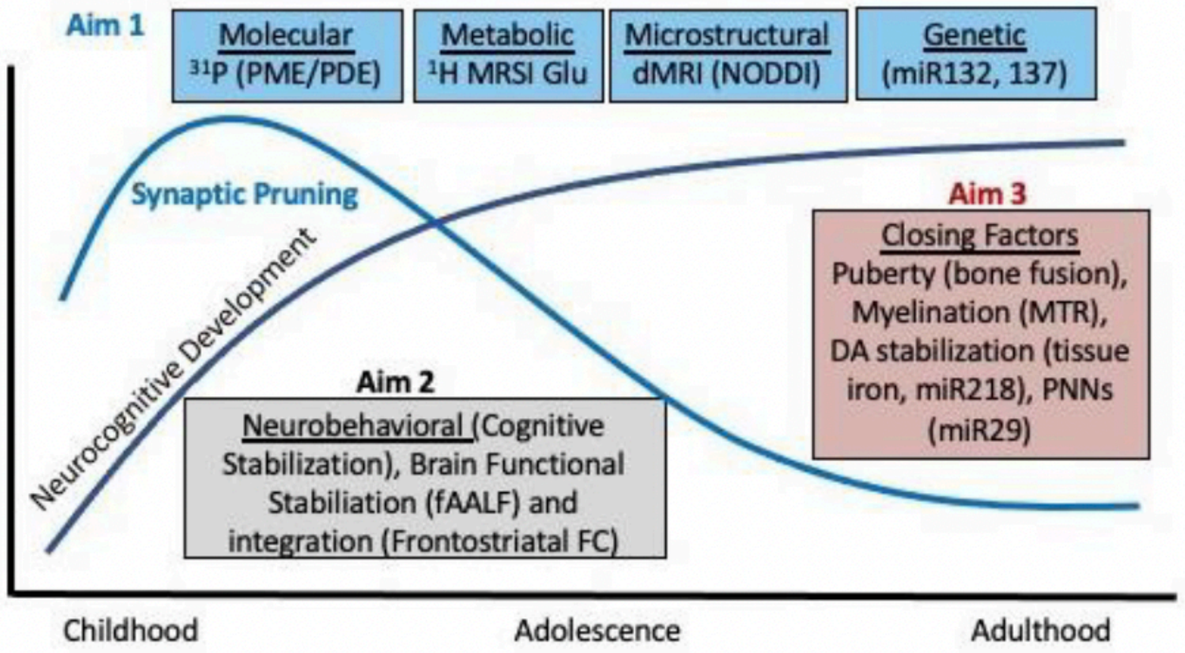
- Changes in microRNAs measured from peripheral fluids show close correspondence with changes occurring in the brain, notably the PFC (Torres-Berrio et al., 2021, Issler et al., 2014, Torres-Berrio et al., 2017)

- Therefore, microRNAs obtained from peripheral fluids can serve as an adequate readout of their expression in the brain (Roy et al., 2020), and longitudinally, can provide indices of mechanisms underlying PFC maturation

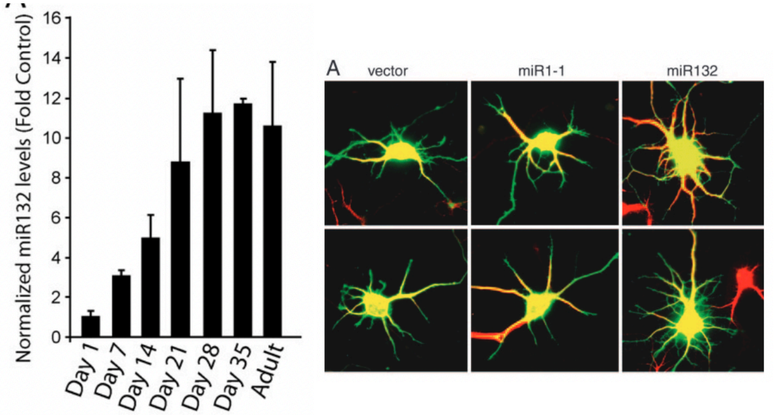


Ones we care most about (Grant Aims)

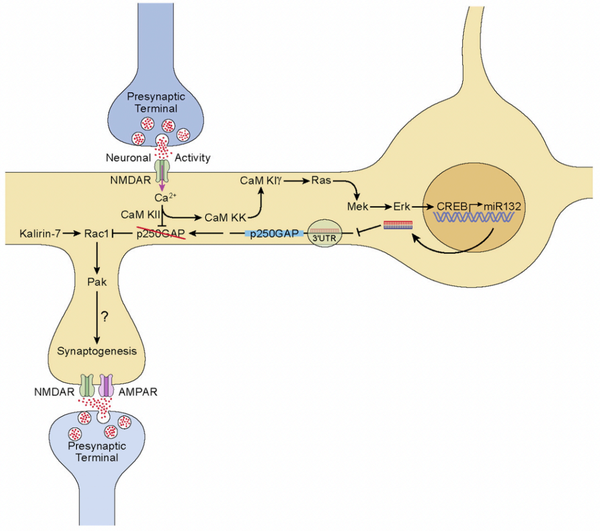
- **Aim 1: Characterize changes in indirect indices of synaptic pruning through adolescence**
    - Assess whether microRNAs involved in synaptic pruning will show associated changes throughout adolescence, and will be associated with indirect MR indices reflecting synaptic pruning (i.e., NODDI, 31^{P}-MRSI, glutamate)
- **Aim 3: Characterize the role of critical period closing factors into adulthood**
    - Assess whether microRNAs involved in critical period closing factors (i.e., perineuronal nets) will show associated changes throughout adolescence, and will be associated with other closing markers including growth plate fusion evidence of completion of puberty, stabilization of tissue iron indices of DA availability, and increased myelination
- **Aim 2: Characterize associations between synaptic pruning, CPP closing factors, and neurocognitive development**
    - Link microRNA (and other imaging metrics of pruning and CPP closing factors) to neuroimaging and behavioral indices reflecting stabilization of neurocognitive processes into adulthood



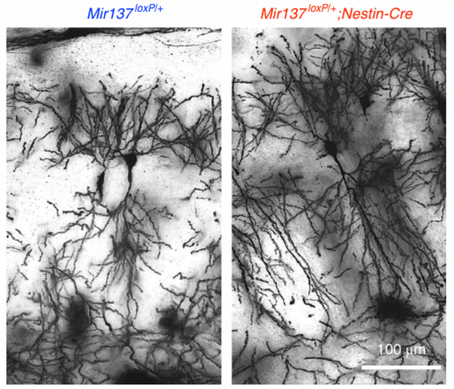
Synaptic Pruning: microRNA-132



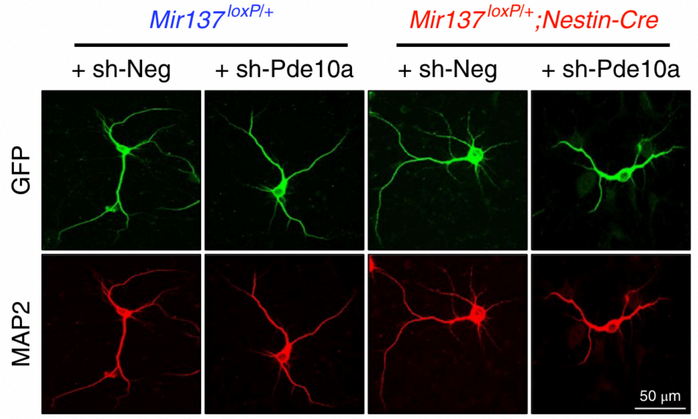
- **microRNA-132:** involved in neurite outgrowth (Vo et al., 2005, 2010) and dendritic spine formation & plasticity (Impey et al., 2010)
- Experience-dependent plasticity
  - Regulates ocular dominance plasticity in visual cortex of juvenile mice (Tognini et al., 2011) - acts through modulation of dendritic spine plasticity
  - Increases its expression in the visual cortex, hippocampus, olfactory bulb, and striatum in response to visual (Tognini et al., 2011), olfaction, fear, and cocaine stimuli (Nudelman et al., 2010), respectively
- Involved in simulating glutamate release (Tognini et al., 2010), increases in ado
- *Correlate with our MR metrics of neurite density (NODDI), indicating a role in the development of grey matter microstructure - providing a link to ongoing neural plasticity processes*



Synaptic Pruning: miR-137

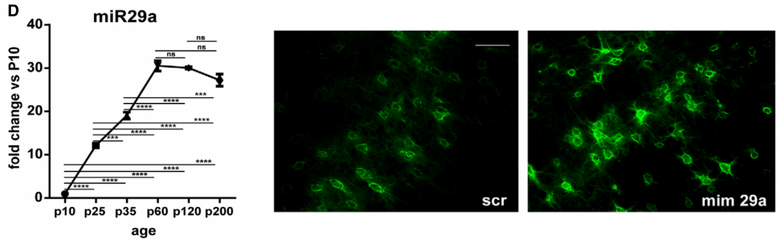


- **microRNA-137**: involved in synaptic pruning in the PFC, maintenance of synaptic plasticity, neurogenesis, neuronal maturation, and dendritic morphogenesis during development (Cheng et al., 2018; Rombaut et al., 2021)
  - Overexpression impairs synaptic plasticity and hippocampal-dependent learning and memory (Nudleman et al., 2010)
  - Dysregulation linked to schizophrenia & autism (Cheng et al., 2018)
  - *Correlate with our MR metrics of synaptic plasticity (PDE10a expression in the PFC, which is one of the phsophodiesterases contributing to the 31^{P} MRS signal)*



Critical Period Closing Factors: miR-29

- **microRNA-29**: involved in cortical maturation (Kole et al., 2011), neuronal differentiation (Zhang et al., 2017), supports cell survival and suppression of apoptosis (Roshan et al., 2014), and synapse formation (Lippi et al., 2011)

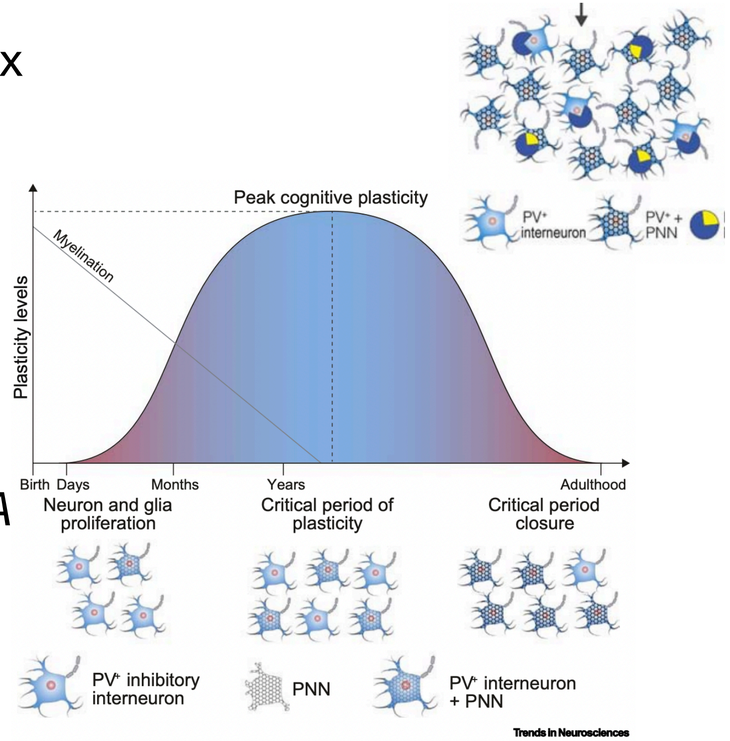


- **Most interestingly, it regulates plasticity brakes promoting age-dependent stabilization of cortical connections - involved in the generation of perineuronal nets (PNNs), a critical period braking factor (Napoli et al., 2020; Tognini et al., 2010; Nudelman et al., 2010; Mellios et al., 2011)**

  - Associations between microRNA-29 and PNN formation found in the visual cortex (Napoli et al., 2020; Tognini et al, 2010)

  - **Though never tested in PNN formation in the PFC, microRNA-29 expression increases during adolescence and we hypothesize that similar mechanisms of PNN formation in the PFC will be mediated by miR-29 (braking factor)**

  - *Correlate with other closing factors including puberty progress, stabilization of DA of cognition (i.e., decreased variability and stabilization of BOLD and connectivity)*



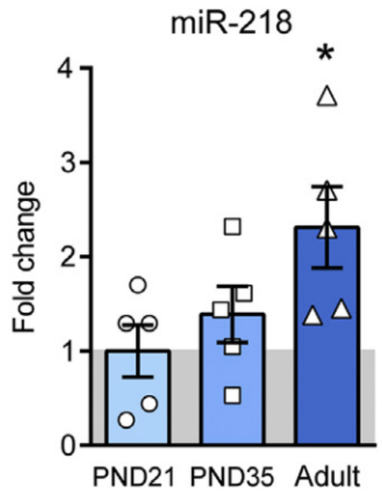
Organization of DAergic PFC synaptic circuits: miR-218

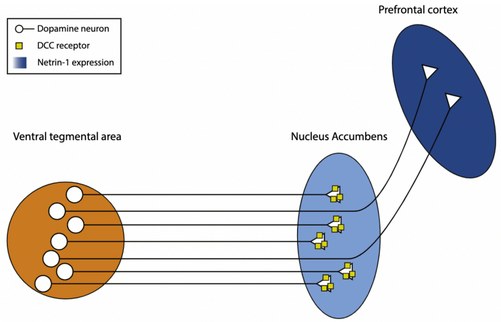
- **microRNA-218**: regulator of genes that are known to control adolescent PFC development and guidance cues for DAergic PFC projections
(also involved in adolescent-onset disorders; Torres-Berrio et al., 2020, 2021)

  - Expression of microRNA-218 in mouse PFC increases from early adolescence to adulthood, with the same pattern observed in blood

  - Represses the DCC gene, a receptor for the guidance cue Netrin-1.
Involved in the formation of PFC networks in development - specifically, DCC mediated Netrin-1 signaling controls maturation of PFC circuitry in adolescence (Morgunova & Flores, 2021)

  - **Netrin-1/DCC pathway involved in axonal targeting and growth in adolescence, while controls refinement of already established circuitries in adulthood by modifying neuronal structure, including dendritic spine morphology (Vosberg et al., 2019; Torres-Berrio et al., 2020)**





Novelty/impact

- Link to neuroimaging and behavioral measures of PFC maturation throughout adolescence. **Will provide mechanistic insight into synaptic pruning, stabilization of cognitive functions, and timing of critical period plasticity (and its closure)**

- Longitudinal characterizing *in vivo* of miRNA in humans throughout development is lacking

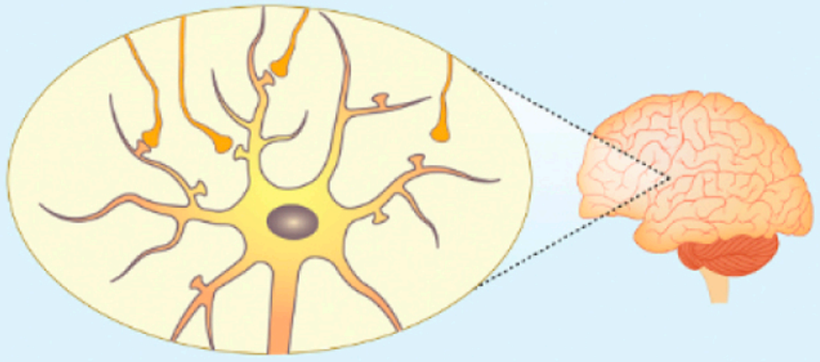
    - Lots of animal

    - Lots of post mortem & culture studies

- Candidate (ala SNP) versus high-throughput analysis: Longitudinal noninvasive high-throughput analysis of microRNA profiles from peripheral fluids in adolescents with and without early adversity and/or psychiatric condition

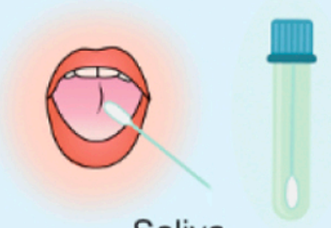
    - We will collect microRNA at each visit allowing us to characterize changes in expression levels across the adolescent period, likely contributing to dynamic changes in PFC signaling, pruning, and CPP

- Objective longitudinal markers of psychiatric risk could help guide early diagnosis, treatment, and prognosis



Adolescents

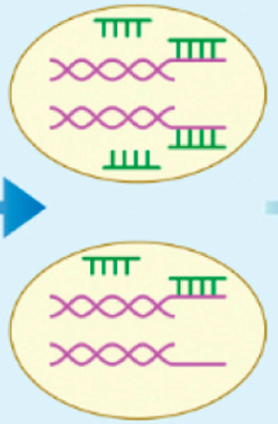
Non-invasive genetic measures



Saliva

Blood

microRNA expression levels



Identification/prediction

Adverse event

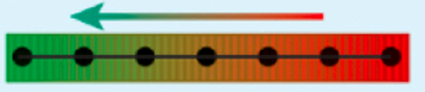
risk or resilience

Teen

Adult

Prevention / Intervention

Track



Resilience

Susceptibility