**Thesis Goals and Experiments:**

* + **Aim 1: Viral Tracing**
    - Identify putative convergence site – Agranular Insular cortex (AIc).
  + **Aim 2: Functional Calcium Imaging**
    - Characterize AIc cell activity in response to tastants, odorants, and mixtures.
    - Examine how representations change in animals with mixture experience.
  + **Aim 3: Behavioral Paradigm**
    - Investigate whether silencing AIc impairs animal's ability to discriminate flavor mixtures.
    - Shows selective impairment in task performance related to flavor.

**Summary of "Odor Taste Convergence in Agranular Insular Cortex" Section:**

* **Identification of Integration Site:**
  + Used AAV(Synapsin-tdTomato) in posterior piriform cortex and AAV (Synapsin-GFP) in anterior gustatory cortex.
  + Identified Agranular Insular cortex (AIc) as the primary convergence site for taste and odor cortical outputs.
* **AIc Characteristics:**
  + AIc is a five-layer cortical region adjacent to primary gustatory and olfactory cortices.
  + Previous rat studies suggested AIc as a site of odor-taste integration based on tracing studies.
* **Tracing Studies:**
  + Studies using biotinylated dextran amine and biocytin injection demonstrated AIc as a multimodal sensory convergence zone.
  + Brain-wide tracing studies continued to support AIc as a distinct target of convergence.
* **Single Cell Convergence Testing:**
  + Employed a modified conditional rabies viral tracing strategy to test for single-cell convergence.
  + Injected AAV-WGA-cre into piriform cortex and AAV with cre-dependent glycoprotein coat into AIc.
* **Results of Single Cell Convergence:**
  + Demonstrated convergent input from piriform and gustatory cortex onto individual cells in AIc.
  + Utilized conditional rabies tracing to show individual cells in AIc receiving input from both piriform and gustatory cortices.
* **Discussion on Anatomical Convergence:**
  + Argued that AIc is the primary site of anatomical taste-odor convergence.
  + Emphasized the importance of understanding multisensory integration for a comprehensive understanding of brain functions.
* **Challenges and Future Considerations:**
  + Acknowledged limitations of the modified rabies strategy.
  + Discussed alternative approaches, such as optogenetics, for more thorough quantification of convergence probability.
* **Overall Implications:** The study provides evidence supporting AIc as a key site for the convergence of taste and odor signals, shedding light on the anatomical basis of multisensory integration in the brain. The findings contribute to the understanding of how the brain processes and integrates information from different sensory modalities.

Calcium-Imaging:

* **Objective:**
  + Building on anatomical evidence of integration in Agranular Insular cortex (AIc), the study aimed to characterize taste-odor integration at the single-cell level.
* **Findings:**
  + Taste and odor representations found in AIc.
  + Flavor mixtures altered populations by:
    - Modulating responses of odor and taste responsive cells.
    - Recruiting neurons that don't respond to component stimuli but respond to the mixture.
  + Integration of taste and odor influenced by experience, leading to an increased population of cells responding only to the mixture.
* **Limitations of Previous Approaches:**
  + Previous studies focusing on taste-odor integration typically looked for cells responding to taste and odor presented separately.
  + Suggests that this approach may not capture the fundamental transformation that occurs when stimuli are presented together.
* **Considerations for Limited Cells Responding to Both:**
  + Vast olfactory chemical space (estimated at 400,000 detectable molecules).
  + Sparse activation of piriform cells by any one odorant.
  + Constrained number of odors tested in each animal.
* **Mode of Delivery and Future Considerations:**
  + Odorants delivered retronasally to mimic processes creating flavor.
  + Future work could evaluate AIc odor responses with a broader array of odors and concentrations.
* **Additivity Index and Interpretation:**
  + Used an additivity index to characterize how mixture responses differ from responses to component stimuli.
  + Originated from superior colliculus where super-additive responses are often seen.
  + Psychophysical studies in humans suggest a suppressive mixture effect for unfamiliar odor-taste pairings, while familiar pairings can be perceived as more intense.
  + Suggests future work could explore if shifts in population activity are accompanied by increased sensitivity to the mixture.

**Summary of "Silencing Agranular Insular Cortex During a Flavor Discrimination Task" Chapter:**

* **Introduction:**
  + Perception and decision-making understanding derived from experiments measuring motor outputs in response to controlled stimuli.
  + Psychophysical tasks applied to rodents offer insights into somatosensory, auditory, and visual perception and decision-making.
  + Two-alternative forced choice design used to test AIc's role in odor-taste integration in water-deprived animals.
* **Experimental Design:**
  + Animals presented with odor, taste, or a mixture at a center sampling port.
  + After sampling, animals indicate perception by licking at a reward port to the left or right.
  + One side rewarded after sampling the mixture; the other rewarded after sampling either mixture component.
* **Results:**
  + Animals demonstrate high accuracy (>90%) in discriminating between stimuli.
* **Silencing AIc:**
  + AIc targeted for silencing using several measures:
    - Lesioning cortical area encompassing AIc with unilateral injection of ibotenic acid.
    - Relying on anatomical outputs; injecting retrograde AAV-cre into infralimbic cortex and cre-dependent inhibitory DREADD into AIc.
* **Preliminary Findings:**
  + Selective activation of AIc disrupts perception of flavor mixture.
  + Animals treat the mixture as one of its components.
  + Preliminary results indicate a significant impact (n=3, p=0.008, paired t-test, baseline vs. silenced mixture).
* **Distinct Responses in Agranular Insula Cortex (AIc):**
  + Imaging of AIc reveals that responses to flavor mixtures are distinct from responses to individual component stimuli.
* **Behavioral Discrimination Task:**
  + Performed perceptual discrimination task coupled with transient inactivation of AIc.
  + Demonstrates that flavor responses observed in AIc are utilized to drive behavioral discrimination.
* **Potential Distal Effects and System Perturbation:**
  + Acknowledges the possibility of distal effects in a dynamic brain system due to perturbation in a single region.
  + Silencing experiments include component stimuli as a control to show that perturbation doesn't affect systems essential for task performance.
  + Earlier experiments with misplaced DREADDs injections occasionally affected unisensory responses without impacting mixture responses, suggesting AIc's ability to detect weakened unisensory input through experience-dependent enhancement.
* **Sensory Selection in Multimodal Stimuli:**
  + Discusses the challenge of understanding what an animal perceives when simultaneously experiencing taste and odor but not the mixture.
  + Refers to sensory selection studies and anticipatory biasing of sensory input through prefrontal modulation of thalamic input.
  + Suggests that disambiguating perceived stimuli could involve separating odor and taste responses with an additional reward port, but this would increase task complexity.
  + Proposes the possibility of recording from downstream neurons accumulating evidence and analyzing unique activity patterns for each stimulus category, comparing them to silenced trial mixture responses.