**Metabolomics of dietary switch and serotonin involvement**

**Section I: Background**

In previous experiments, we identified an acute change in mortality rate, neurometabolomes and individual neurometabolites in flies that are transferred from a fixed diet (SY10) to a choice diet (S10 vs Y10) within 2 days (Fig. 1). Such response requires the involvement of a specific serotonin receptor *5-HT2a*. These results raised a series of follow-up questions including 1) what is the critical macronutrient component(s) that elicits such changes? Is the effect of dietary switch due to the exposure to pure sugar (S10), the exposure to pure yeast (Y10), or the decision-making process that evaluates these two macronutrients? And 2) what is the role of *5-HT2a* in individual macronutrient sensing and decision-making process, respectively? To answer these questions, we designed the following metabolomics experiment to determine the critical macronutrient that modulate mortality rate and metabolomes in a serotonin dependent manner.

Figure

**Section II: Experimental design**

**160 samples in total:** (16 treatments and 10 replicates for each treatment)

**Factorial design**

* Genotypes (w1118 vs *5-HT2a*)
* Diets (fixed to sugar, fixed to yeast, fixed to choice and fixed to fixed)
* Tissues (head vs body)

**Treatment**

|  |  |  |
| --- | --- | --- |
| Group | Treatment Description | Procedure |
| Group 1 (n=10) | W1118; fixed to sugar; heads | Collect newly emerged w1118 flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to S10 diet. After 2 days collect heads (n=50 for each replicate). |
| Group 2 (n=10) | W1118; fixed to sugar; bodies | Collect ~30 bodies from the same flies in Group 1. |
| Group 3 (n=10) | W1118; fixed to yeast; heads | Collect newly emerged w1118 flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to Y10 diet. After 2 days collect heads (n=50 for each replicate). |
| Group 4 (n=10) | W1118; fixed to yeast; bodies | Collect ~30 bodies from the same flies in Group 3. |
| Group 5 (n=10) | W1118; fixed to choice; heads | Collect newly emerged w1118 flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to choice diet (S10 | Y10). After 2 days collect heads (n=50 for each replicate). |
| Group 6 (n=10) | W1118; fixed to choice; bodies | Collect ~30 bodies from the same flies in Group 5. |
| Group 7 (n=10) | W1118; fixed to fixed; heads | Collect newly emerged w1118 flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to fixed diet (SY10 | SY10). After 2 days collect heads (n=50 for each replicate). |
| Group 8 (n=10) | W1118; fixed to fixed; bodies | Collect ~30 bodies from the same flies in Group 7. |
| Group 9 (n=10) | 5HT2a; fixed to sugar; heads | Collect newly emerged 5HT2a flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to S10 diet. After 2 days collect heads (n=50 for each replicate). |
| Group 10 (n=10) | 5HT2a; fixed to sugar; bodies | Collect ~30 bodies from the same flies in Group 1. |
| Group 11 (n=10) | 5HT2a; fixed to yeast; heads | Collect newly emerged 5HT2a flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to Y10 diet. After 2 days collect heads (n=50 for each replicate). |
| Group 12 (n=10) | 5HT2a; fixed to yeast; bodies | Collect ~30 bodies from the same flies in Group 3. |
| Group 13 (n=10) | 5HT2a; fixed to choice; heads | Collect newly emerged 5HT2a flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to choice diet (S10 | Y10). After 2 days collect heads (n=50 for each replicate). |
| Group 14 (n=10) | 5HT2a; fixed to choice; bodies | Collect ~30 bodies from the same flies in Group 5. |
| Group 15 (n=10) | 5HT2a; fixed to fixed; heads | Collect newly emerged 5HT2a flies using SY10 bottles. After 2 days, sort males into 12-well chamber, 30 flies per vial, preconditioned use SY10 plates. Flip flies for 20 days w/SY10 plates. Transfer flies to fixed diet (SY10 | SY10). After 2 days collect heads (n=50 for each replicate). |
| Group 16 (n=10) | 5HT2a; fixed to fixed; bodies | Collect ~30 bodies from the same flies in Group 7. |