T.C.

DOKUZ EYLUL UNIVERSITY

IZMIR INTERNATIONAL BIOMEDICINE AND GENOME INSTITUTE

INSULIN DEPENDENT REGULATION of the SEROTONIN TRANSPORTER (SERT or 5-HTT) FUNCTION in FOOD INTAKE using *Drosophila melanogaster as a* MODEL SYSTEM

MERVE TORUN

MOLECULAR BIOLOGY AND GENETICS

MASTER OF SCIENCES THESIS

IZMIR-2020

Thesis ID: DEU.IBG.MSc.2017850031

T.C.

DOKUZ EYLUL UNIVERSITY

IZMIR INTERNATIONAL BIOMEDICINE AND GENOME INSTITUTE

INSULIN DEPENDENT REGULATION of the SEROTONIN TRANSPORTER (SERT or 5-HTT) FUNCTION in FOOD INTAKE using *Drosophila melanogaster as a* MODEL SYSTEM

MOLECULAR BIOLOGY AND GENETICS

MASTER OF SCIENCES THESIS

MERVE TORUN

Supervisor: Assoc. Prof. Duygu SAĞ
Prof. Henrike Scholz

Thesis ID: DEU.IBG.MSc.2017850031

T.C.
DOKUZ EYLÜL ÜNİVERSİTESİ

İZMİR ULUSLARARASI BİYOTIP VE GENOM ENSTİTÜSÜ

Dokuz Eylul University Izmir International Biomedicine and Genome Institute Department of Genomics and Molecular Biotechnology, Molecular Biology and Genetics graduate program Master of Science student Merve TORUN has successfully completed her Master of Science thesis titled **'INSULIN DEPENDENT REGULATION of the SEROTONIN TRANSPORTER (SERT or 5-HTT) FUNCTION in FOOD INTAKE using *Drosophila melanogaster as a* MODEL SYSTEM'** on the date of 23.03.2020.

Assoc. Prof. Duygu SAĞ
CHAIR

MEMBER
Asst. Prof. Yavuz OKTAY
Dokuz Eylül University

MEMBER
Asst. Prof. Çiğdem TOSUN
Izmir Institute of Technology

SUBTITUTE MEMBER
Prof. Şermin Genç
Dokuz Eylül University

SUBTITUTE MEMBER
Asst. Prof. Anne FRARY
Izmir Institute of Technology

T.C.
DOKUZ EYLÜL ÜNİVERSİTESİ

İZMİR ULUSLARARASI BİYOTIP VE GENOM ENSTİTÜSÜ

Dokuz Eylül Üniversitesi İzmir Uluslararası Biyotıp ve Genom Enstitüsü Genom Bilimleri ve Moleküler Biyoteknoloji Anabilim Dalı,

İngilizce Moleküler Biyoloji ve Genetik Yüksek Lisans program öğrencisi Merve TORUN '***Drosophila melanogaster*** **MODEL ORGANİZMASI KULLANILARAK GIDA ALIMINDA SEROTONİN TAŞIYICISININ FONKSİYONUNUN İNSÜLİNE BAĞLI REGÜLE EDİLMESİ' konulu Yüksek Lisans tezini** 23.03.2020 tarihinde başarılı olaraktamamlamıştır.

Doç. Dr. Duygu SAĞ
BAŞKAN

ÜYE
Dr. Öğr. Üyesi Yavuz OKTAY
Dokuz Eylül Üniversitesi

ÜYE
Dr. Öğr. Üyesi Çiğdem TOSUN
İzmir Yüksek Teknoloji Enstitüsü

YEDEK ÜYE
Prof. Dr. Şermin GENÇ
Dokuz Eylül Üniversitesi

YEDEK ÜYE.
Prof. Dr. Anne FRARY
İzmir Yüksek Teknoloji Enstitüsü

TABLE of CONTENTS

ABBREVIATIONS

AL	Antennal lobe
AP	Anterior within protocerebrum
ADP	Adenosine diphosphate
AKH	Adipokinetic hormone
AMPK	AMP-activated protein kinase
ASM	Anterior superior medial
CaFe	Capillary Feeder
CSD	Contra-laterally projecting deutocerebral
DILP	Drosophila insulin like-peptide
DL	Dorso-lateral
DM	Dorso-medial
DP	Dorsal protocerebrum
GR	Gustatory receptor
GRN	Gustatory rceptor neurons
IGF	Insulin-like growth factor
InR	Insulin-like receptor
IP	Inferior medial protocerebrum
IPC	Insulin producing cells
LP	Lateral protocerebrum
PAM	Protocerebral anterior medial
PB	Protocerebral bridge
PBS	Phosphate buffered saline
PI	Pars intercerebralis
PPL	Protocerebral posterior lateral
PPM	Protocerebral posterior medial
RTK	Receptor tyrosine kinase
SEZ	Subesophagal zone
Sert	Serotonin Ttansporter
SOG	Suboesophagal ganglion
SP	Superior protocerebrum

TAG	Triglycerides
TBS	Tris buffered saline
ERp44	Endoplasmic Reticulum Resident Protein 44

LIST of TABLES

LIST of FIGURES

ACKNOWLEDGEMENTS

During my master education, I am grateful to my advisor Assoc. Prof. Duygu SAĞ. She encouraged me, admired me, followed me, supported me, influenced me, treated me right, and most importantly never lost her faith in me through my research. I would like to thank to my co-advisor Prof. Henrike Scholz for giving me the opportunity to be involved in her project at the University of Cologne.

I am also grateful to Assoc. Prof. Hatice Güneş ÖZHAN BAYKAN and Prof Dr Bünyamin Akgül for supporting me to do my external master thesis externally.

Of course my gratitude should be extended to my friends Tuğçe ÇAKIR, , Gizem Tuğçe ULU, İrem ALEMDAR, Miray KURTOĞLU, Eda Deniz ERDEM, Sezgin BAL, the previous labmates from Meuwissen Lab Ayşe Tuğşen AYDEMİR and İrem Şimşek for their encouragement, support, faith and their endless friendships.

I would like to thank Jasper RUMMENY for his support, encourage, friendship, faith, his great family (especially his mother Sussan STEİNMANN), and endless love. He was there for me as always!

Lastly, first and foremost I am so grateful to my family for their endless support, faith, love, behaviour and encourage. No words can describe how I am so lucky to be a family member of this family. Without them, none of these would have happened!

INSULIN DEPENDENT REGULATION of the SEROTONIN TRANSPORTER (SERT or 5-HTT) FUNCTION in FOOD INTAKE using *Drosophila melanogaster as a* MODEL SYSTEM

Merve TORUN

Izmir International Biomedicine and Genome Institute, Dokuz Eylül University

merve.torun@msfr.ibg.edu.tr

ABSTRACT

Eating disorders occurring by food intake such as obesity, and type II diabetes, are one of the most prevalent diseases in the world. They have become a topic of various research to understand its psychological and molecular mechanisms for a long time. However, the exact mechanisms are still not known. Thus, more research is needed to understand detailed mechanisms that are triggered by food intake.

How serotonin, insulin and their signaling pathways have been shown separately to regulate food intake. This project addressed how serotonin transporter is regulated by insulin signaling by food intake in *Drosophila melanogaster*. ERp44 is responsible for glycosylation of the Sert in Asp208 and Asp 217 via insulin signaling. So, the ERp44 protein should be researched and targeted for its function in food intake.

In this thesis, the metabolic and taste-dependent regulation was discovered by developmentally decreased insulin signaling on Sert3-GAL4 targeted neurons. The taste-dependent regulation was observed by developmentally enhanced serotonin signaling on Sert3-GAL4 targeted neurons. The carbohydrate specific uptake was decreased by developmentally diminished ERp44 on Sert3-GAL4 targeted neurons. The possible interaction between serotonin transporter and insulin signaling via ERp44 protein was tried to understand in food intake.

Key Words: Eating Disorders, Drosophila melanogaster, insulin, insulin signaling, serotonin, serotonin transporter, serotonin signaling, endoplasmic reticulum, endoplasmic reticulum stress, ERp44

Drosophila melanogaster MODEL ORGANİZMASI KULLANILARAK GIDA ALIMINDA SEROTONİN TAŞIYICISININ FONKSİYONUNUN İNSÜLİNE BAĞLI REGÜLE EDİLMESİ

Merve TORUN

İzmir Uluslararası Biyotıp ve Genom Enstitüsü, Dokuz Eylül Üniversitesi

merve.torun@msfr.ibg.edu.tr

ÖZET

Obezite, tip II diyabet gibi gıda alımından kaynaklanan yeme bozuklukları, dünyadaki en yaygın hastalıklardan biridir. Yeme bozukluklarının psikolojik ve moleküler mekanızmalarını anlamak, uzun zamandır çeşitli araştırmalara konu olmuştur. Ancak, bütün mekanizmalar hala bulunamamıştır. Dolayısıyla, gıda almını tetikleyen ayrıntılı mekanizmaları araştırmaya ihtiyaç duyulmaktadır.

Serotonin, insülin ve bunların sinyal yolaklarının gıda alımını nasıl regüle ettiği ayrı ayrı gösterilmiştir. Bu projede, *Drosophila melanogaster'da* insülin sinyal yolağının serotonin taşıyıcısını gıda alımı sırasında nasıl regüle ettiğini ele almak amaçlanmaktadır. ERp44, Sert'ü insulin sinyali aracılığıyla Asp208 ve Asp217 konumlarından glikosilat etmektedir. Bu nedenle, ERp44 araştırılmalı ve gıda alımındaki fonksiyonu anlaşılmaya çalışılmalıdır.

Bu tezde, Sert3-Gal4 hedefli nöronlar üzerinde gelişimsel olarak azaltılmış insulin sinyali ile metabolic ve tada bağımlı düzenleme bulunmuştur. Tada bağımlı düzenleme Sert3-GAL4 hedefli nöronlar üzerinde gelişimsel olarak arttırılmış serotonin sinyali ile gözlenmiştir. Karbonhidrat spesifik alımının azalımı Sert3-GAL4 hedefli nöronlar üzerinde gelişimsel süreçte azaltılmış ERp44 proteini ile bulundu. ERp44 proteini aracılığıyla gıda salımı sırasında serotonin taşıyıcısı ve insulin yolağı arasındaki olası etkileşim anlaşılmaya çalışıldı.

Anahtar Kelimeler: Yeme bozuklukları, Obezite, Tip II Diyabet, Drosophila melanogaster, insulin, insülin sinyal yolağı, serotonin, serotonin taşıyıcısı, serotonin sinyal yolağo, endoplazmik reticulum, endoplazmik reticulum stresi, ERp44

1. INTRODUCTION and OBJECTIVES

1.1 Problem Definition and Importance

To understand how neurotransmitters regulate proper food intake which is of key importance to resolve the mechanism underlying eating disorders. Advanced genetic manipulation technologies allow us to have target gene analysis of before, during and after food intake. Since multiple signaling pathways are involved whether to activate or to regulate during food intake, the exact mechanism is still unknown.

Hence, there is a clear need to identify further regulatory mechanisms of food intake.. Insulin regulates carbohydrate intake and is important to maintain body homeostasis. Insulin signaling is highly conserved between mammals and insects. The neurotransmitter serotonin is one of the neurotransmitters that regulates food intake and insulin production. Also, insulin regulates the serotonin transporter as post translational manner inside the Endoplasmic reticulum An ideal start to address the relationship between neuropeptide signaling and neurotransmitter signaling in the regulation of food intake lies the identification of insulin dependent regulation of Serotonin transporter (Sert) function. Our preliminary experiments of the Scholz group at the University of Cologne shows that serotonergic neurons expressing the insulin receptor and serotonin transporter are important for the regulation of carbohydrate specific food intake.

In our project, we focused on determining whether insulin receptor function regulates Sert function on a cellular level. Towards this end, we used behavioral experiments to dissect the relationship between insulin receptor signaling and serotonin transporter function at different time points of food intake using *Drosophila melanogaster* as a model system. The analysis were complemented by neuroanatomical experiments out. With this project, the gene responsible from the regulation of Sert via insulin signaling might be found out to address in eating disorders.

1.2. Research Objective

In this project, The UAS/GAL4 system was utilized to alter the gene function. Sert3-GAL4 driver line was used to target subset specific serotonergic neurons including SE1, LP1, and IP neurons. The taste specific regulation of carbohydrate intake was pointed out with developmentally enhanced 5-HT signaling due to dominant negative serotonin transporter function. The taste and metabolic specific modulation were figured out via dominant negative insulin receptor. The possible gene that plays central role in insulin dependent regulation of serotonin transporter was trying to address. If my aim is achieved, the gene might responsible for eating disorders like obesity, diabetes could be targeted further.

1.3 Research Hypothesis

We hypothesize that *homo sapiens* ERp44 ortholog of CG9911 protein in *Drosophila melanogaster* positively regulates Serotonin transporter in carbohydrate intake on SE1, LP1, and IP serotonergic neurons via insulin signalling.

To test our hypothesis, we aim;

-Firstly to investigate whether the inactivation of insulin receptor (InR) changes food intake in serotonergic neurons via CaFe assay.

-Secondly to determine how inactivation of the serotonin transporter (Sert) whether influences the food intake on SE1, LP1, and IP neurons via CaFe assay.

-Thirdly to understand possible sereptor that interact with taste neuron IR60b on SE1 neurons via CaFe assay.

-Finally to address whether the limited carbohydrate intake would be based on ERp44 signaling with CaFe assay and qPCR.

2. LITERATURE REVIEW

2.1. Food Intake

Behavior is defined as the change in the activity and mannerism of individual organism. It is allied to stimuli whether external including other organisms, systems and environment or internal such as hormones, genes and neurotransmitters (Minton and Khale, 2014). One of the fundamental activity of the animalis food consumption. Inclusive fitness is the ultimate purpose of the organism. Inclusive fitness is achieved by optimized food intake based on the organism's internal state to survive, grow and reproduce. (Illius et al., 2002). To understand how neurotransmitters regulate proper food intake which is of key importance to resolve the mechanism underlying eating disorders. Previous studies revealed a tendency to eat more protein-loaded or overconsumption of low-protein loaded food because of deprived protein state in people (Gosby et al., 2011; Griffioen-Roose et al., 2014). Several studies revealed, people who are on low-carb diets and having appetency for carbohydrate intake to compensate diet and it is also common among the organisms as primates (Felton et al., 2009), rodents (Deutsch et al., 1989; Sorensen et al., 2008), and *Drosophila melanogaster* (Itskov and Riberio, 2013) to maintain internal homeostasis. Foraging behavior of organism evolved as functional and nutritional outcome depending on physiological and molecular mechanisms. But the mechanisms of food intake are still unknown (Illius et. al., 2002). To understand the complex mechanism of food intake regulation, we performed our experiments using *Drosophila melanogaster*.

2.2 Drosophila Melanogaster

Drosophila melanogaster, the fruit fly, is found around rotten and unripe fruits (Itskov, 2013). It is one of the model organism for research in a variety disciplines among fundamental genetics and the development of structures as tissues and organs. Almost 60 percent of *Drosophila* genome is homologous to humans. 75% ortholog human genes involving in diseases, are found in *Drosophila* (Deutsch, 1989). It also has a

short generation time, small size, low maintenance costs, easy manipulation and sexually dimorphic features (Deutsch, 1989).

2.3 Life Cycle of *Drosophila Melanogaster*

- ➔ Day 0: Mating and eggs
- ➔ Day 1: Hatched eggs
- ➔ Day 2: First instar (one day length)
- ➔ Day 3: Second instar (one day length)
- ➔ Day 5: Third instar (two days in length)
- ➔ Day 7: Roaming of pupa/Pupariation
- ➔ Day 11-12: Eclosion from pupa case

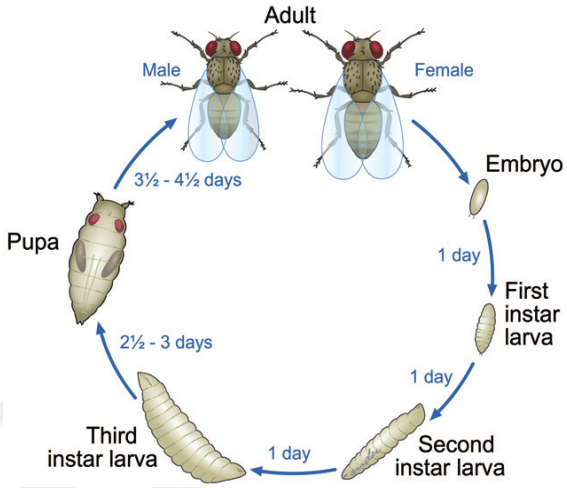


Figure 2.1. The life cycle of *Drosophila melanogaster.* Drosophila has four dioristic stage; embryo, larva, pupa, adult. Modified after Ong C., et al. (2014)

The life cycle of *Drosophila* includes metamorphosis with four distinguishable stages: eggs, larva, pupa and mature.

- **Mating and Eggs:** Mated females, which store sperm to make sure desired cross are occurring before laying their fertilized eggs. Oviposition of female flies are initiated after emerging from their pupal cases. And oviposition is increased through the week till 50-75 eggs are laid per day (≈ 400-500 eggs in 10 days). Ovoid eggs are protected by chorion, which is a strong and thin cover, but at the anterior end of it, there is a micropyle to pass down the spermatozoa. Other spermatozoa, are placed in the developing embryonic tissues.
- **Larva:** A segmented, white worm shaped, with black mount parts in the head part is called as larva. It develops spiracles for breathing, located in the anterior and posterior ends. Until larva become adult, the cuticle is periodically shed by along with the mouth. The periodic times are called as first instar, among hatching and first molt, and determined by the number of teeth on jaw hooks and size the larva has reached. Third instar is fed till pupation is occurred and crawls out from the culture to a dry place.

- **Pupa:** Prepupa will be formed after anterior spiracles everting, body of larva shortens and cuticle is getting hardened and pigmented. The stage is followed by formation of head, pads, wings, and legs, formation of preadult tissues known as imaginal discs. While using breakdown substances of larval tissues for pupal metabolism.
- **Mature:** Adult stage, which includes compound eyes, head, thorax and abdomen, wings and six jointed legs covered with bristles and hairs to have a typical insect anatomy. Virgin female flies cannot mate during several hours (around 8 hours at 25°C)* so they could be easily collected separately from male flies for genetic crosses to vials.

*The generation time of the *Drosophila melanogaster* depends on the temperature. The above cycle description is based on 22°C (77°F) for 12-15 days.
Flies which are raised at low temperatures as 20°C, need more time to develop as 19 days. Besides, at high temperatures like 29°C, the generation time period takes around 7 days. The optimum condition for it is 25°C with taking about 9-10 days long.

2.4 Feeding in *Drosophila melanogaster*

Drosophila, intermittent feeder, usually eats up sugary fruits and plants. Also, the smell of yeast inside the plant materials attracts it (Becher et al., 2012). Although main carbohydrate sources for *Drosophila* is sucrose, glucose or fructose (Ashburner, 2005), different caloric and/or taste content of sugar also attract the Drosophila as arabinose, mannose (Huetteroth W., 2015). Since ethanol is produced by rotting fruit, low ethanol concentrations, 5-10%, attracts Drosophila (McKenzie and Parsons 1972; Zhu et al., 2003; Ogueta et al., 2012). Optimize lifespan is provided by 1:2 protein-carbohydrate ratio (Itskov et al., 2013) . The enhancement in protein level increases the egg production (Lee et al., 2015).

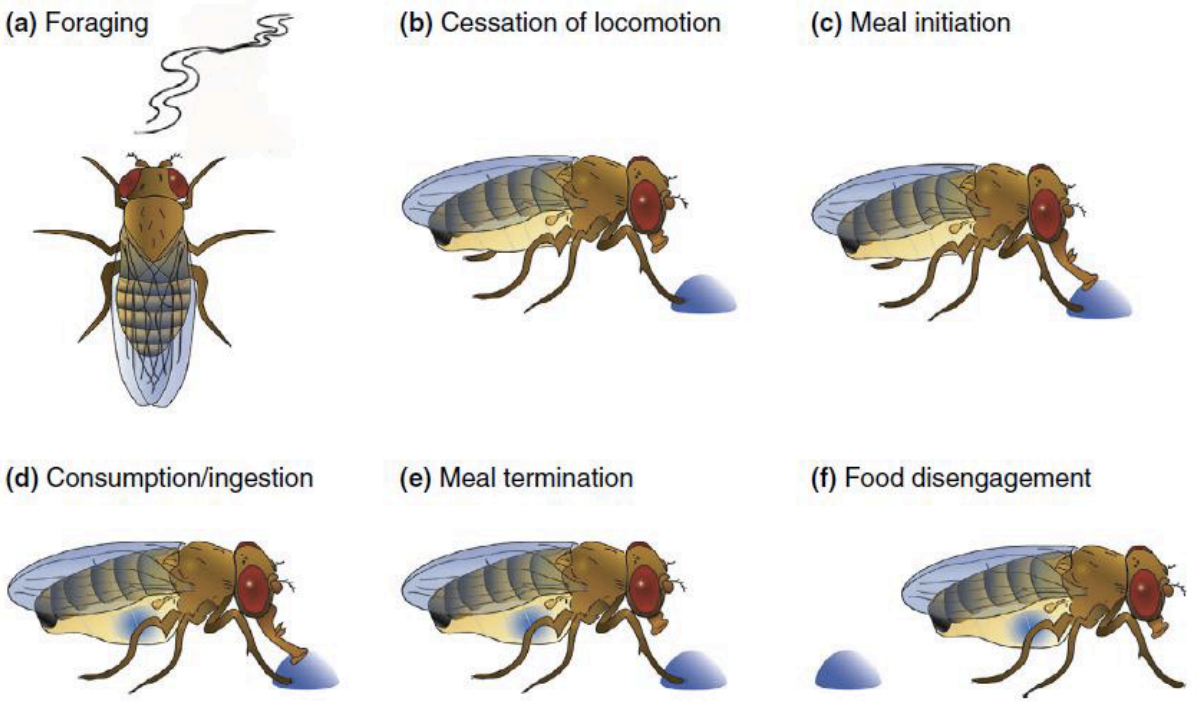


Figure 2.2. Different behavioral states of feeding in *Drosophila*. The food source is searched by odor attraction (a). Locomotion is inhibited when a food source is found (b). The proboscis extension response occurs by gustatory receptors (GR) in gustatory receptor neurons (GRN) with the palatability of food and nutrient content food(c). Food ingestion started with consumption (d). Internal state feedbacks to terminate the consumption (e). Food disengagement and reactivation of locomotion (F) (Taken from Pool and Scott, 2014).

The feeding behavior in *Drosophila* includes food source detection, physical contact with food, sense of nutrient content information, ingestion initiation, and discrete meal consumption (Figure 1.2) (Bernays, 1985; Gelperin, 1972) based on internal state and environmental factors (Root et al., 2011). When the information of food source is identified as caloric, taste, liquid or solid, the feeding is started with proboscis extension ressponse. Although liquid food directly is taken, the solid food needs to be predigested with extracted saliva, including digestive enzymes (Bay, 1978; Boer and Hickey 1986). The enhanced starvation state increases feeding time, but not the feeding speed (Qi et al., 2015). Around 260 taste sensilla is distributed primarily in the tarsi, wings and labellum of *Drosophila* (Amrein and Thorne, 2005). Each of sensilla includes multiple gustatory receptor (GR) neurons (GRNs). These neurons provides detection of taste, of salts, water, bitter compounds, caffeine, fatty acids, amino acids, carbohydrates (Amrein and Thorne 2005; Cameron et al., 2010; Scott, 2005; Lee et al., 2009; Masek and Keene, 2013; Montell, 2009) in taste pegs of the inner labellum and the internal taste organs lining inside pharynx, lateral sensory organ (LSO), ventral cibarial organ (VCSO), and dorsal cibarial sensory organ (DCSO). Gr64f is one of the

gustatory receptors to respond variety of sugars as glucose, fructose, maltose, trehalose to promote consumption (Dahanukar et al., 2007; Jiao et al., 2007; Slone et al., 2007; Fujii et al., 2015). Several receptors could sense the same sugars just as trehalose sensing receptor Gr5a (Chyb et al., 2003). Many clade of ionotropic receptors (IR), IR20a, was found in the labellum, legs, and pharynx (Koh et al., 2014; Stewart et al., 2015). Although IR functions are still unknown, two of the IR20a receptors are responsible in male mating behavior. There is one sucrose sensing receptor, IR60b to limit sucrose consumption (Joseph et al., 2017). IR25a and IR76b also provide amino acid sensation in larvae (Steck et al., 2018; Croset et al., 2016). *Drosophila* percepts post-ingestive cues like gut stretch, nutrient value so these cues are feedback to stop ingestion and it disengage from food source via reactivated locomotion (Olds and Xu et al., 2014; Fujita and Tanimura; 2011).

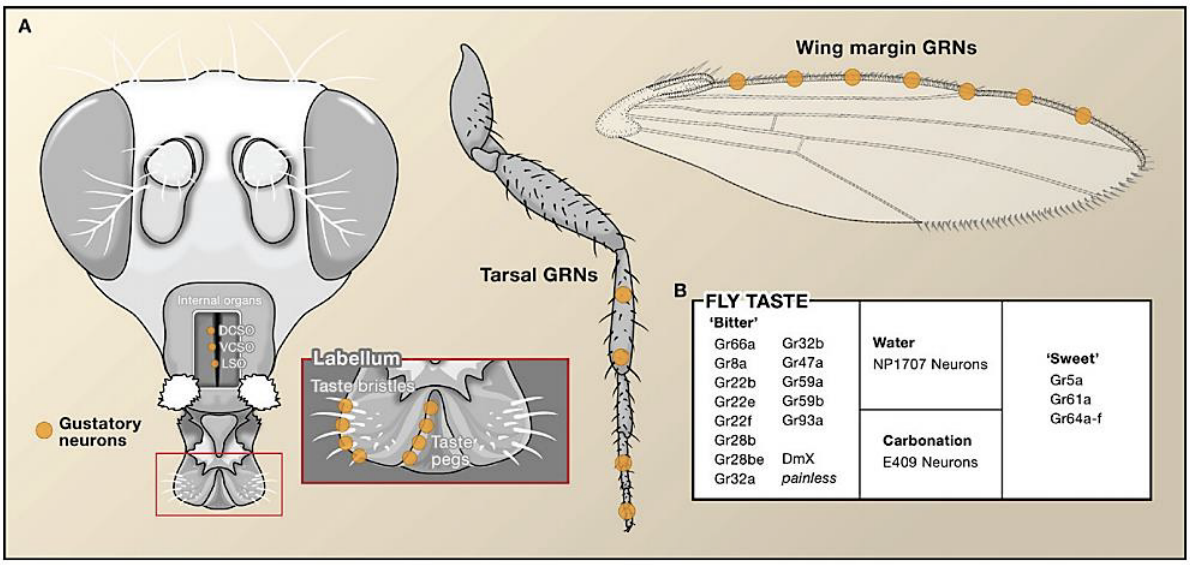


Figure 2.3. Fly taste sensing. Gustatory receptor neurons (GRN) of sensilla are stimulated with taste in labella, wings and legs. Several gustatory receptors are responsible for attractive and aversive taste sensing as in mammals. (Modified after Yarmonlisky et al., 2009)

2.5 Energy Homeostasis

Heterotopic organisms including *Drosophila*, and mammals, obtain energy from the ingested and catabolized carbon sources as fats, carbohydrates, and proteins. Energy is released during catabolism as heat using to convert the ADP (adenosine diphosphate) with inorganic phosphate (Pi) into ATP (Adenosine triphosphate) (Hardie, 2011). The intracellular high energy level of ATP to ADP is used in anabolic reactions

via AMP-activated protein kinase (AMPK) for cellular maintenance and growth (Hardie, 2011). The energy homeostasis depends on availability of food (Ohtsu et al., 1992). In the case of starvation, the storage compounds as fatty acids, triglycerides (TAG) (Ohtsu et al., 1992; Gade et al., 2003; Zhang and Xi et al., 2015) glycogen are used to maintain homeostasis in specialized cells like fat body, liver, adipose tissues (Hardie, 2014).

In *Drosophila*, when potential food source is detected, a fly could either start to ingest the food or search another source based on its internal state. The internal state modulation includes different internal sensors and regulatory pathways (Buchon et al., 2013). Gut which provides absorption of consumed nutrient, is a part of the segmented digestive system homologous to the stomach and intestine in vertebrates. Through digestive tract, almost 349 digestive enzymes are found (Lemaitre and Miguel-Aliaga, 2013). Enterocytes provide absorption of nutrients to transcellularly transport them into the hemolymph to trigger receptors with different expression patter in different tissues.

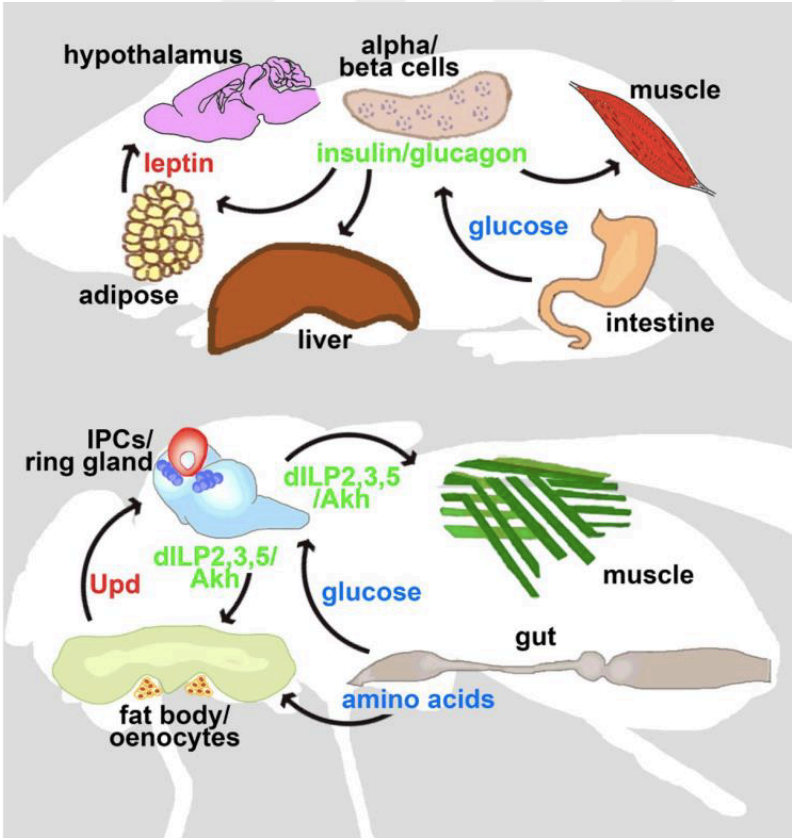


Figure 2.4 Energy homeostasis regulation in *Drosophila* and vertebrates.
Homologous tissue interactions to maintain similar pathways for energy homeostasis was shown in mice (top) and *Drosophila* (bottom) and the regulation is conserved between invertebrates and vertebrates (modified after Rahul and Dobens, 2015)

2.6 Insulin Like Signaling in *Drosophila melanogaster*

Insulin which is peptide hormone, triggers the metabolic process such as glycogen and fatty acid synthesis and cellular glucose uptake to maintain blood/hemolymph glucose level (Bantig and Best, 1922). It also acts as mitogen about fibroblasts. Regulatory system to maintain energy homeostasis from circulating sugars is highly conserved between mammals and *Drosophila.* In mammals, there is one insulin secretion interacting with insulin receptor (InR) with high affinity while expressing two insulin-like growth factors (IGF); relaxin and insulin-like peptides (INSL 3-7) to interact with InR with low affinity (Nasel and Broeck, 2016). The insulin gene codes for preproinsulin, insulin precursor composed of A-chain, C-peptides and B-chain. Cleavage of preproinsulin to polypeptide is occurred to transport into the endoplasmic reticulum (ER) to form proinsulin. The proper folding is applied in ER for transportation into trans-Golgi network where immature granules made upon by proinsulin. The maturation will occur when disulfide bond formation occurs after separation of C peptide of proinsulin from A and B-chains by cleavage. And, the insulin receptor has two spliced variants; InR-A and InR-B with receptor tyrosine kinase activity (RTKs) (Belfiore et al., 2009).

In *Drosophila*, sugars in food are in taken and transport to fat body to convert into theralose. Theralose is stored as glycogen in fat body to release into hemolymph when energy homeostasis is imbalanced. The sugar level in *Drosophila* is controlled antagonistically by Adipokinetic hormone (AKH), *Drosophila* insulin-like peptides (DILPs), and glucagon-like peptides (Fernandez et al., 1995). Together with DILPs, they are released into the hemolymph circulation. The InR proreceptor is proteolytically processed to generate binding site, alpha subunit (InR 120 kDa) and beta subunit (InR 170 kDa or 90 kDa) with protein kinase domain for DILP. The InR 170 is composed of carboxyterminal side that produce alpha 120, beta 170, beta 90, and free 60 kDa C terminus after proteolytic cleavage to mature InR (alpha2(Beta170)$_2$) and InR (alpha$_2$(Beta90)$_2$ (Fernandez et al., 1995). There are eight DILPs (1-8) in *Drosophila.* They are structurally similar to insulin, IGFs and relaxin in mammals due to their conserved Cys position and disulfide bonds. Except the DILP8, all of them bind to InR. DILP8 stimulates the relaxin-type membrane receptor, Lgr3. Each of DILP has its

unique expression pattern tied to developmental stage. Although DILP 1, 4 are restricted developmentally, DILP 6 is found in fat body while showing homology with IGF. DILP 2, 3, and 5 is found in adult to regulate energy homeostasis and they are procured by insulin producing cells (IPCs) in pars intracerebralis in the brain. DILP 2, 3, 5 projects into aorta, corpora cardiaca and crop for hemolymph circulation (Nasel and Broeck, 2016). DILP 6, unpaired 2 (Upd 2) , neuropeptide F (NPF), serotonin, GABA, octopamine, alllatostatin A, corazonin, limostatin and AKH modulate DILP 2, 3 and 5 expression. 20 cells in ventral nerve cord (VNC) generate DILP7 which projects into the hindgut and in female reproductive organ in adult fly. Larval imaginal discs and adult ovaries express DILP 8. DILP7-8 shows homology with human relaxin (Nassel and Vanden Broeck, 2016).

DILP and InR triggers receptor tyrosine kinase activity to activate phosphoinositide 3-Kinase (PI3K) to create phosphatidylinositol (3, 4, 5) triphosphate (PIP3) with ATP by phosphatidylinositol 4,5-biphosphate (PIP2). PDK is recruited to membrane by PIP3 to activate AKT. AKT repress FOXO which controls the life cycle of an organism as development and growth, aging and life-span, stress response, neural activity and behavior modulation, fecundity and ovarian development, carbohydrate and lipid metabolism (Baker and Thummel, 2007).

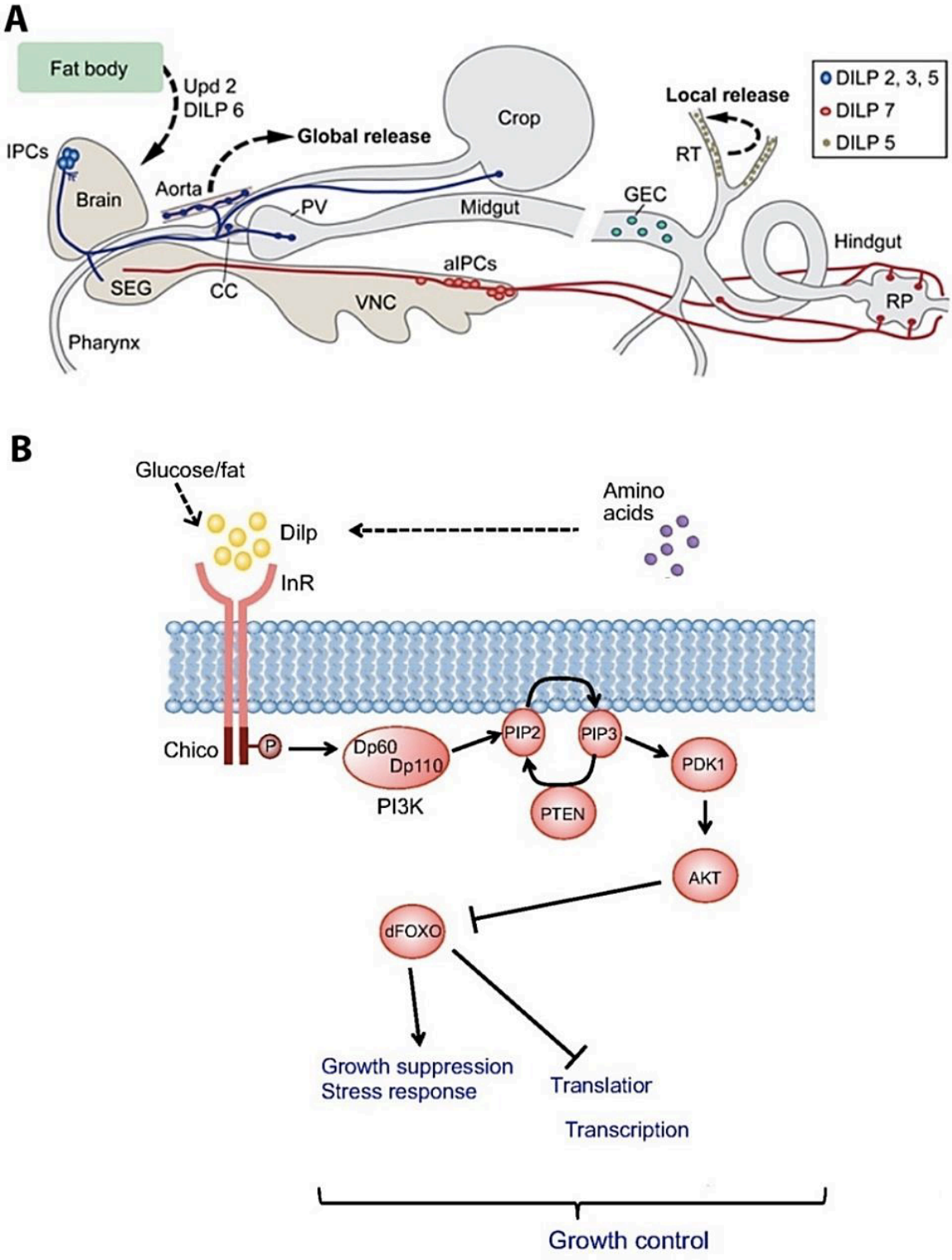


Figure 2.5 Insulin signaling in *Drosophila.* Fat body releases Upd2 and DILP6 in the head and abdomen. DILP 2, 3, and 5 is produced by 14 IPC cells in pars-intercerebralis. 20 cells in the VNC stimulate DILP7 production. Principal cells inside renal tubes generates DILP5 (A). DILPs activated by glucose/fat stimulates InR to activate PI3K with Chico sites of InR. PDK1 is activated by conversion of PIP2 to PIP3 to trigger AKT. Activated AKT results in repressed FOXO to prevent its intercellular transport to nucleus to control growth (Modified after A- Nassel et al., 2013 and B- Shim et al., 2013)

2.7 DILP Signaling Modulates Food intake

Energy homeostasis is maintained by insulin signaling both in the periphery and the brain (Root et al., 2011). As in people with type 1 diabetes, DILP 2, 3, 5 ablations cause reduction of DILP expression and hyperglycemia in the brain (Rulifson et al., 2002). Activated insulin in Neuropepide Y neurons maintains energy homeostasis in flies and mice (Loh K. et al., 2017). Anti-porcine insulin guinea pig serum (AI) causes insulin immune-neutralization in chicken, which limits food intake without affecting hypothalamic transcript (Weglarz M. P., et al., 2017). The food intake, especially non-preferred food intake, is suppressed with the increased DILP levels in hemolymph of both adult and larva (Wu et al., 2005) as enhanced insulin level in vertebrates (Woods et al., 1979; Brown et al., 2006; Jauch-Chara et al., 2012). Insulin signaling in hypothalamic neurons of rodents regulates feeding with activated AgRP neurons and diminish feeding with triggered POMC neurons (Belgardt et al., 2009; Williams et al., 2010; Qui et al., 2014). It has been showed in many researches that insulin signaling controls neuron size, synaptic connectivity, neuronal survival, dendritic arborizations, neurotransmitter reuptake with enhanced dopamine, and altered norepinephrine levels (Boyd et al., 1985; Wan et al., 1997; Govind et al., 2001; Figlewichz et al., 1994; Cheng et al, 2003; Apostolatos et al., 2012).

2.8 Reinforcement Learning and Food Intake Regulation

Decision making is explained as chooses of organisms (Neumann and Morgerstern, 1944). It has been tried to explain how the human preferences are acquired based on different actions and outcomes (Sutton and Barto, 1998). Reinforcement is explained as acquired new knowledge about metabolic state and organism's environment non-dependent on reward and penalty and it changes its value functions that might impress further behaviors to maximize future rewards (Tolman, 1948) as also seen in one of behavior: food intake and its regulatory neurons (Sohn, 2015). Insulin ablated regulatory neurons POMC and AgRP resulted in diminishment in food intake (Belgardt et al., 2009; Williams et al., 2010; Qui et al., 2014). Serotonin has been shown in the appetitive learning formation (Sitaraman et al., 2012) and modulates food intake (Albin et al., 2015; Pooryasin and Fiala, 2015; Ro et al., 2016).

2.9 Serotonin (5-Hydroxytryptamine, 5-HT)

The monoamine serotonin act as neuromodulator and a neurotransmitter in central and peripheral functions in vertebrates and invertebrates. It is observed through the gastrointestinal tract, central nervous system, platelets and the pineal gland. It has been synthesized from basic amino acid, tryptophan, in two steps (Figure 2.6). Firstly, it is hydroxylated at 5' indole ring via TPH and TRH. Then, it is decarboxylated by DDC to produce serotonin.

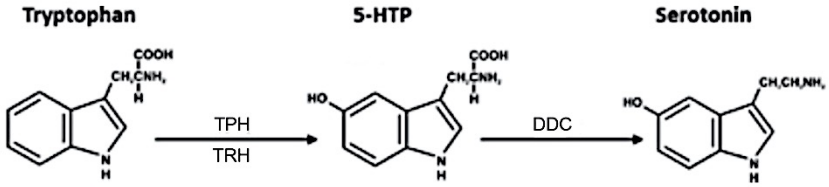
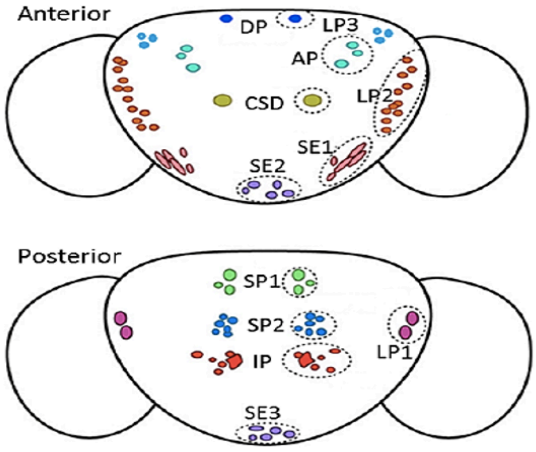


Figure 2.6 Synthesis of Serotonin. Tryptophan is converted into 5-hydroxy-tryptamine (5-HTP) and after, decarboxylation of 5-HTP by 3,4-dihydroxyphenylalanine decarboxylase to produce serotonin. (Modified by J. He, doctoral thesis 2017)

It regulates behavioral approaches as olfaction, memory formation, learning, aggression, feeding (Johnson et al., 2011; Becnel et al., 2011; Vleugels et al., 2014; Lee et al., 2011; Albin et al., 2015; Alekseyeneko et al., 2014). In CNS of adult Drosophila brain, about 92 serotonergic neurons have been identified (Valles and White, 1988; Giang et al., 2011; Pooryasin and Fiala, 2015).

A



B

Clusters	Location	N
DP	Dorsal protocerebrum	2
LP3	Lateral protocerebrum	6
SP1	Posterior medial protocerebrum, dorsal	6
SP2	Posterior medial protocerebrum, medial	12
IP	Inferior medial protocerebrum	14
AP	Anterior within protocerebrum	6
LP1	Posterior lateral protocerebrum	4
LP2	Lateral protocerebrum	22
CSD	Contralaterally projecting deutoreactive deutocerebral	2
SE1	Lateral to the oesophagus foramen	10
SE2	Lateral subesophagal ganglion	4
SE3	Medial subesophagal ganglion	6

Figure 2.7 Serotonergic neurons in CNS of adult brain. In. anterior (A top) and posterior (A bottom), the serotonergic neuron clusters were shown. Table of abbrevations for location and neuron numbers were illustrated (B) (Modified after Xu et al., 2016)

In *Drosophila*, vesicular monoamine transporter (VMAT) is formed to transmit serotonin (Saudou et al., 1992). Calcium influx and neuronal depolarization cause releasing of serotonin into the presynaptic cleft to activate its receptors. Five receptors, 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B and 5-HT7, have been identified in *Drosophila*. They are all G-protein coupled receptors (Saudou et al., 1992; Gasque et al., 2013; Nichols et al., 2008). 5-HT1A and 5-HT1B negatively regulates the adenyl clyclate that repress cAMP activation. 5-HT2A, 5-HT2B and 5-HT7 positively couple to phospholipase C and adenyl cyclase (Nichols et al., 2008; Saudau et al., 1992)

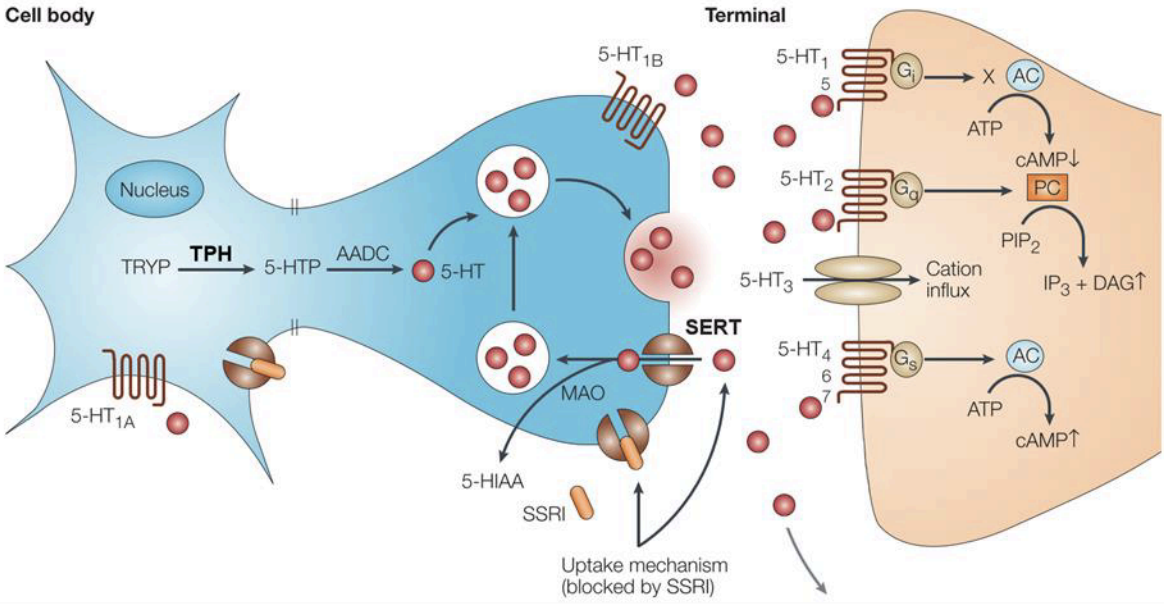


Figure 2.8 Serotonin production and serotonin neurotransmission in vertebrates. The synthesis of serotonin is completed from the essential amino acid tryptophan (TRYP) with TPH, TRH and AADC. The VMAT is occurred with the formation of serotonin to release it in the presynaptic cleft with calcium influx and neuronal depolarization. The serotonin whether is reuptaken or activates the serotonin receptors in post-synaptic cleavage. The cAMP signaling is modulated by Serotonin. AC: adenyl cyclase; cAMP: cyclic adenosine monophosphate; DAG: diacylglycerol; IP3: inositol-1, 4, 5-triphosphate; PIP2: phosphadityinositol-4, 5-biphosphate (Modified from Wong et al., 2005).

2.10 Serotonin Transporter (Sert/5-HTT)

The serotonin transporter is found in the presynaptic membrane to reuptake serotonin from the synaptic cleavage back into the pre-synapse (Rudnick et al., 2006). So that, it determines the amount and the duration of the serotonin in the synaptic cleavage. The outward-open conformation of Sert which contains the central binding site for

serotonin with Na^+ and Cl^-. In the intercellular surface of Sert, K^+ is placed (Rudnick et al., 2013). The outward-open conformation is changed to inward -open state with serotonin binding to central site. The Na^+, Cl^- and serotonin into the cells K^+ are released to the outside of the cell to balance ion concentration (Rudnick et al., 2013). Human 12-transmembrane Sert structure was observed with X-ray crystallography (Coleman et al., 2016). The structure and function is conserved among vertebrates and invertebrates (Zahniser and Doolen; 2001)

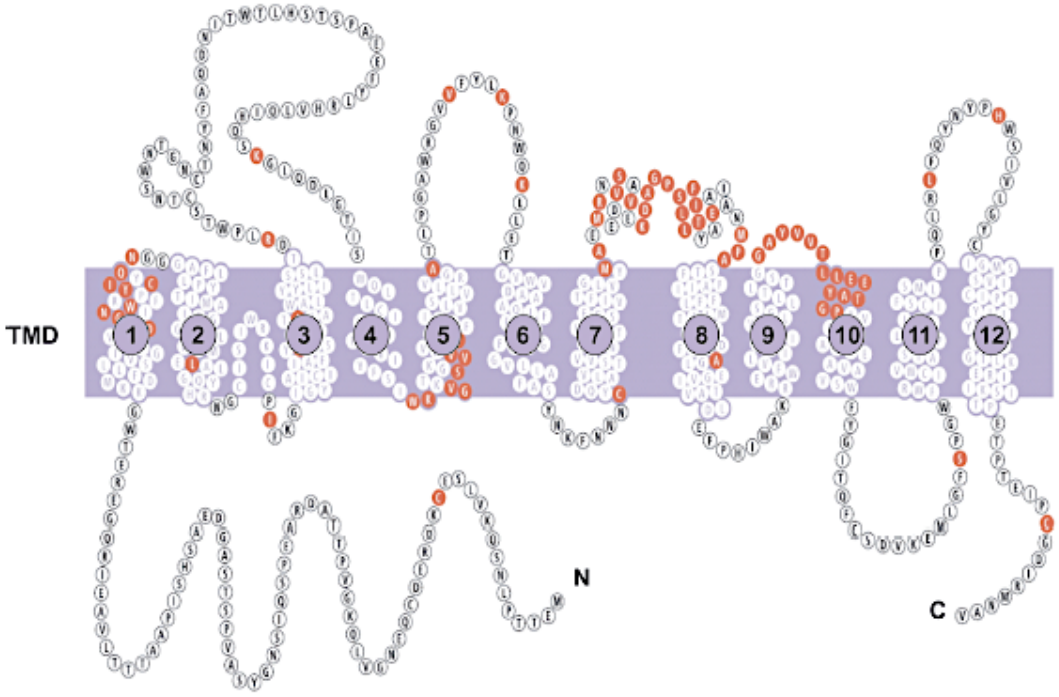


Figure 2.9 The structure of Sert. The figure shows the amino acid sequence composition (a.a) and transmembrane domains (TMD) of Sert. Each letter stands for an amino acid and the number indicate the transmembrane domains located in the cytoplasm (Modified after Rudnick et al., 2006).

The Sert forms either oligomers or dimers to form serotonin channe (Whitworth et al., 2002). Post-translational modification recruits Sert translocation from ER to pre-synaptic membrane. The post-translational modification determines the function of Sert (Ramamoorthy et al., 2007; Ramamoorthy et al., 2010; Zhang et al., 2007; Oz et al., 2010; Whitworth et al., 2002).

2.11 Food Intake Regulation by Serotonin Signaling.

The function of the serotonin transporter has been studied in *Drosophila*. The genomic deletions ranging from 1121 and 1192 position of dSert^{10} and dSert^{16} mutations results deletion of Sert expression. So, increased 5-HT signaling was occurred at the synaptic cleft (Kaiser, diploma thesis 2009) while decreased the carbohydrate intake (El Khadrawe, bachelor thesis 2017; Götz bachelor thesis 2018). The protein is enhanced by serotonin altering (Ro et al., 2016; Albin et al., 2015). In this thesis, the driver line Sert3-GAL4 driver is used to target three serotonergic neurons per hemisphere including IP, LP1, and SE1 neurons. The activation of neurons targeted by Sert3-GAL4 driver line caused negatively food approach (Xu et al., 2016) and carbohydrate intake (Kastenholz, PhD thesis 2019). Non-functional 5-HT2A receptor in flies and knockdown of Trh enzyme in flies resulted in diminish to protein intake (Ro et al., 2016). In this thesis, the Sert3-GAL4 driver line was focused. This line targets 3 serotonergic neuron per hemisphere; SE1, LP1, IP.

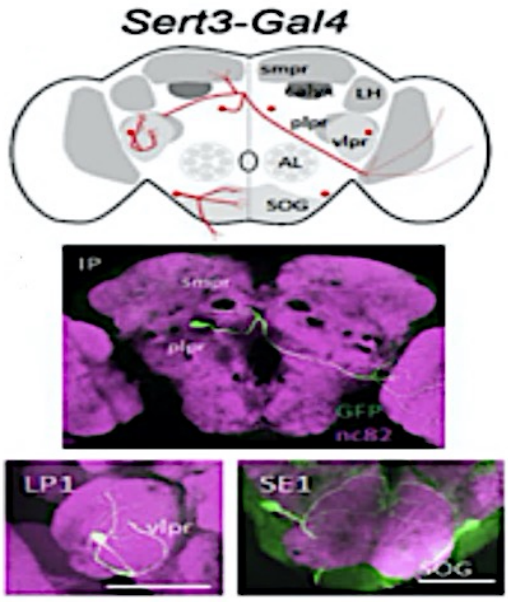


Figure 2.10 Neurons targeted by the Sert3-GAL4 driver line. Neurons targeted by the Sert3-GAL4 driver are visualized using UAS-mcD8::GFP transgene (in green) including the SE1, LP1, and IP neurons. The neutrophil was stained with nc82 (magenta). (Taking from Xu et al., 2016)

2.12 Endoplasmic Reticulum Stress and Endoplasmic Reticulum Resident Protein 44 (ERp44)

The endoplasmic reticulum (ER) is important in folding assembling and post-translational modification of polypeptides translocated to ER lumen in the optimum ionic and redox environment (Chaudhari et al., 2014). Chaperons inside the lumen, limits the aggregation of polypeptides while folding is occurred (Mori, 2000). Insufficient folding capacity or uncompromised folding results in ER stress. Unresolved ER stress gives rise to many metabolic disorders like obesity, type 2 diabetes (Chaudhari et al., 2014). ER stress stimulates unfolded protein response (UPR) to solve misfolded proteins or might triggers programmed cell death to maintain ER homeostasis (Mori, 2000). UPR proteins trigger protein disulfide isomerases (PDIs). PDI catalyze reduction, isomerization and oxidation of protein disulfide bonds to control protein quality (Wanatabe S., et al., 2017).

The ERp44 is a member of the PDI enzymes. Expression of ERp44 is observed in various cell types and tissues.

It is made upon by three thioredoxin (Trx) domains; a, b, c followed with C terminal extension (Pang X et al., 2018). The a domain includes unique CRFS (Cytokine receptor factor stimulators) motifs that provide interaction with cytokines. CRFS also involves Cys29 to make disulfide bonds with the proteins that it regulates as SERT to promote their thiol-dependent retention acting as chaperon proteins (Pang et al., 2018). In the ER lumen, endogenous ERp44 is found as with KDEL (Lys-Asp-Glu-Leu) motifs and insoluble portion for interaction with peripheral and integral membrane proteins. ERp44 also modulates intracellular [Ca^{++}] homeostasis (Nyirimigabo E., et al., 2019). ERp44 adjust blood pressure during cardiac development. ER and ERGIC (Endoplasmic reticulum-Golgi intermediate compartments) where Ero1La, Ero1b, Prx4, Sumf1 and ERAP1 interact with ERp44 to supply their complete function. ERp44 also folds pro-insulin to its mature structure via 3-disulfide bond (Nyirimigabo E. et al. 2019).

2.13 ERp44 Controls Energy Homeostasis

People with diabetic neuropathy, depleted ERp44 lead to impaired transcription factor spliced X-box binding protein 1 (XBP-1). In nuclear level, activation of transcription factor 6 (ATF-6) and C/EBP homology protein (CHOP) are stimulated with impaired XBP-1 to trigger ER stress. Knock-outing entire ERp44 is found as lethal in mice experiments (Pang X. et al., 2018).

In fat mice, ERp44 level is diminished. Overflowed triglycerides and plasma free fatty acids cause clearance of blood glucose, accumulation of lipids ectopically and eventually change in glucose metabolism. Thus, non-adipose tissues shows insulin resistance associated with obesity (Nyirimigabo E., et al., 2019).

SERT has two sites to undergo proper N-linked glycosylation; Asp208 and Asp217. ERp44 binds to Sert to maintain disulfide bond formation at Cys200 and Cys209 where Asp208 is included. In people with Gestational diabetes mellitus disease, ERp44 cannot bind to Asp208 and, Sert post-translational modification is blocked. Thus, GDM associated insulin deficiency shows downregulated uptake of placental serotonin (Li Y., et al., 2014).

3. METHODS

3.1 Type of Research

This study is an in vitro experimental study.

3.2 Time and Place of Research

All experiments were conducted in between July, 2019- February, 2020 at the University of Cologne.

3.3 Universe and Sample of Research

In this thesis, human samples were not used.

3.4 Materials of Research

In this thesis, UAS/GAL4 was used to obtain desired genes with different fly types by me on various times. Table 3.1 contained detail informatin about the fly type including transgene, which chromosome it carries the transgene, the origin of stock, and the number of stock.

Table 3.1 The fly stocks

Transgene	Chromosome	Origin	Stock Number
w^{1118};Sert3-GAL4	2	Andrea Herb	#175
UAS-CG9911 RNAi (RNAi for ERP44)	3	Bloomington Stock Center #29606	#177
w^{1118};;IR60b-GAL4	3	Bloomington Stock Center #60710	#457
w^{1118};UAS-5HT1B RNAi	2	Silva et al. (2014)	#541

		Bloomington Stock Center #25634	
w1118;;UAS-SERT:GFP	3	Sophia Balmert	#574
w^{1118};UAS-$SERT^{DN}$:GFP		Xu et al. (2016)	#160
Y[1]w^{1118};P{w[+mC]=UAS-InR.K1409A}3 (UAS-InRDN)	3	Exelixis, Inc. Bloomington Stock Center #8253	#473
Y[1]w^{1118};P{w[+mC]=UAS-InR.A1325D}2 (UAS-InRCA)	2	Exelixis, Inc. Bloomington Stock Center #8263	#474
w^{1118};Sert3-GAL4, tubGAL80^{ts}	2,2	Henrike Scholz	
W1118;;RN2-E	3	Li	#336
UAS-5HT2B RNAi	2	Qian et al. (2017) VDRC	
UAS-mCD8:GFP;UAS-mCD8:GFP;UAS-mCD8:GFP	X, II, III	Lee and Lou (2001)	#113
Tph-GAL4	III	Henna	#53
UAS-denMark	I, III	Nicole et al. (2010) Bloomington Stock Center #33061	#458
Trh-LexA::p65	II	Bloomington Stock Center #52248	#391
UAS-Trh RNAi	III	S. Birman	#179
UAS-BRP	II	Owald et al. (2010)	#288
w^{1118};sp/cyo;TM2/TM6	II, III	Bloomington Stock Center	#371
cyo/sp; RN2-E/RN2-E	III	Li	#328

3.5 Variables of Research

Agar, dry yeast, polenta, sugar beet molasses, propionic acid, nipagin acid, 10X PBS, PBS to Triton-X ratio (0.5), Glycerol ratio (50% Glycerol/50% PBS), dNTPs, Taq DNA-Polymerase, 10x Thermo Polymerase Buffer, Chloroform, Phenol, Potassium Acetate, Homogenization Buffer, dNTPs, 1 kb DNA Ladder, Loading Dye, Agarose, Sucrose, Fructose, Glucose, Arabinose, Mannose, Yeast are independent variables.
Flies, density of flies, antibody amounts and ratios, Genomic DNA concentration, cDNA concentration are dependent variables.

3.6 Buffer, Solutions and Enzymes of Research

Table 3.2 Brain dissection and immunohistochemistry solutions

Solution	Composition
Phosphate buffered saline (PBS, pH 7.4)	137 mM NaCl, 2.7 mM KCl, 2 mM KH2PO4, 10mM Na2HPO4
PBST (0.5, 0.3 or 0.1%)	1x PBS and 0.5, 0.3 or 0.1% Triton X-100
50% Glycerol	50% Glycerol in 50% 1X PBS

Table 3.3 Genomic DNA and cDNA Isolation, Polymerase Chain Reaction and Agarose Gel Reagents

Solution	Composition
Homogenization buffer stock solutions	5 M NaCl, 10 % SDS, 1 M EDTA, 1 M Tris
Potassium Acetate (KOAc)	8 MKOAc; 78.5 g KOAc in 80 ml ddH_2O
Phenol equilibrated stabilized	A1153,0100 – AppliChem
Chloroform dehydrated	NORMAPUR AR for synthesis, VWR
Trizol Reagent	Invitrogen
Ethanol (≥99.8%) Ethanol (70%)	VWR Chemicals 70 ml Ethanol (≥99.8) bring to 100 ml with ddH_2O

Taq Polymerase	New England Biolabs M0273S
Thermo Pol Buffer	New England Biolabs B9004S
1kb Plus DNA Ladder	New England BioLabs N3200S
Agarose	Sigma Aldrich
50x TAE buffer (pH 8.5)	40 mM Tris base 20 mM acetic acid 1mM EDTA
10x DNA Loading dye	40 g Sucrose 0.2 g Orange G 60 g Glycerol bring to 100 ml with ddH_2O

Table 3.4 Food chemicals using in CaFe Assay

Reagent		**Company**
yeast extract	Lot.: BCBQ1176V	Sigma-Aldrich
D-(+) sucrose	Lot.: 6F014540	Sigma-Aldrich
D-glucose	Lot.: SLBR5156V	Sigma-Aldrich
D-(+) fructose	Lot.: SLBQ0969V	Sigma-Aldrich
D-arabinose	Lot.: MKBW2915V	Sigma-Aldrich
D-mannose	Lot.: BCCB4432	Sigma-Aldrich
red food dye cochineal [E124]		Ruth

3.6.1 Fly food preparation

- 160g agar
- 300g dry yeast
- 1200 g polenta
- 1600 ml sugar beat molasses

Were utilized to prepare 20 ml food. To preserve food 0.28% propionic acid and 0.24% nipagin acid were added.

3.6.2 Fly Maintanance

Fly stocks were taken from stock collection and were put to the 25°C room to lay eggs. Flies were flipped to the new big size vial containing food each 20-21 days to maintain the flies at 25°C with 12h/12h day-night rhythm and other rest of flies were discarded. In addition to that, the two samples from each fly stocks were kept in 18°C room as stock.

3.6.3 Fly mating

To prepare a crossing, each fly type should be amplified. Each fly types were transfered to new big vials every second days. The flies were grown at 25°C with 12h/12h day-night cycle. After 9-10 days, flies were hatched and anesthesized with CO_2. They were placed to flat surface affected by CO_2. The virgin female and male flies were selected based on phenotypical signs. Males are smaller, and have a darker and rounded abdomen. Virgin females are longer, have a pointed abdomen. The sex of flies which carry the interested genes for crossing, were selected based on the chromosome carrying transgene as X, II, III to perform crossing. To obtain desired gene, UAS-GAL4 system was used. The flies were crossed as follows:

-w1118*UAS transgenic flies

-w1118*GAL4 transgenic flies

-UAS transgenic flies*GAL4 transgenic flies

to do behavioral analysis, immunochemistry experiments and cDNA and gDNA isolation..

3.6.4 The *UAS*-GAL4

One of the method to create targeted gene expression in Drosophila is performed with the GAL4/upstream activation sequence *UAS* system. The GAL4, yeast activator, could able to initiate transcription in flies with bearing promoters with GAL4 binding sites (Fischer et al., 1988). Mutated GAL4 binding site was produced for high affinity binding (Webster et al., 1988). To bring out targeted gene expression, optimized GAL4 binding

site, upstream activation sequence *(UAS),* was designed in vector to subclone the sequences behind the GAL4 binding sites (Brand and and Perrimon, 1993).

3.6.5 Dissection of Adult Brains

Adult flies with desired crossed were anesthetized for 3 minutes inside ice-cooled 100 % EtOH. Adult males were selected and placed to ice cooled 1X PBS previous to dissection.

The properly dissected brains were put inside Eppendorf tubes containing 900 µl PBS. This step was not overrun the 1.5 hours.

3.6.6 Immunohistochemistry: adult brain antibody staining

Primary antibodies: α-GFP (rabbit), α-nC82 (mouse)

Secondary antibodies: Alexa 488 green (rabbit), Cy3 (mouse)

The dissected brains (in part *3.6.5*) were fixed with 100 µl 37 % Formaldehyde. The tube was incubated on shaker for 30 minutes at room temperature. Then, the solution was removed carefully. The brains were rinsed with 0.5% PBT quickly for three times. After that, the brains were washed 0.5% PBT on shaker for 20 minutes at room temperature. This step was repeated for 3 times.

The PBT inside of the tube was removed as much as possible. 5% FCS were activated at 42^{o}C for 10 minutes. Later, the brains were blocked with 500 µl Blocking mix including 5% FCS and 0.5% PBT for 1 hours on shaker at room temperature. The blocking mix was removed, the primary antibody mix (Anti GFP rabbit 1:1000 ratio and nc82 1:20 in 0.5% PBT in total 500 µl) was put and the tube was placed on shaker at 4^{o}C for two days.

The primary antibody mix was taken and the brains were rinsed with 0.5% PBT quickly for three times. The brains were washed with 0.5% PBT for 1 hour on shaker at room temperature. The step was done three times. The 0.5% PBT inside the eppendorf tube was discarded and secondary antibody (Alexa 488 rabbit 1:1000 and Cy3 mouse 1:200 in 0.5% PBT in total 500 µl) for overnight on shaker at 4^{o}C. The secondary antiboy mix was removed and the brains were rinsed three times with 0.5% PBT quickly. The

brains were washed for 20 minutes with 0.5% PBT on shaker at room temperature. The step was repeated for 3 times. The PBT was replaced with 50% glycerol as much as possible. And the tube was incubated for 30 minutes on the shaker at room temperature. The brains were taken and placed to slide. The slide was covered with coverslip. Vecta shield was put for preservation of brains in long time perion between slide and coverslip. And the brains were firstly analyzing under flourescence microscope and then they were analyzed on confocal microscope.

Table 3.5 Primary antibodies

Antibody	Concentration	Host	Source	Clonality
Anti-GFP	1:1000	rabbit	Rockland	polyclonal
Anti-nc82	1:20	mouse	DSHB	monoclonal
Anti-5HT	1:100-1:200	rat	Millipore	monoclonal

Table 3.6 Secondary antibodies

Antibody	Concentration	Host	Source	Fluorophor
Anti-rabbit	1:200	goat	Invitrogen	Alexa488
Anti-mouse	1:200	goat	Jackson ImmunoResearch	Cy3
Anti-rat	1:200	goat	Jackson ImmunoResearch	Cy3

3.6.7 Imaging Analysis

The Images were analyzed by Image J after receiving them with Zeiss LSM 510 confocal microscope.

3.6.8 cDNA Synthesis

1000 crossed male flies (maximum 5-6 days old) from each group were collected by anesthesizing. Before experiment, flies were stayed for 48 hours at 25°C room to get rid of anesthesizing affect. The flies were placed to 50 ml falcon tube. The tube was flipped to Liquid Nitrogen tank for 15 minutes. The heads were placed to 3 eppendorf tubes to put them into Liquid Nitrogen tank for 40 minute. Each tube was taken and the heads of the flies were cut with zing and they were homogenized firstly with 200 µl Trizol by pestle, then 800 µl Trizol added to mixture. The tubes were incubated for 4-5 minutes at the room remperature. The heads of each group were placed to 3 eppendorf tubes. 200 µl Chloroform was added to each tube and they were invered for 12-15 times. The tubes were centrifuged for 15 minutes at 4°C with 12000 rcf. 200 µl Chloroform again was added to tubes and the tubes were inverted for 12-15 times. Centrifugation was applied to tubes for 15 minutes at 4°C with 12000 rcf. The aqueous phase was taken out to put into fresh Eppendorf tubes seperately. 500 µl Isoproponal was added to each tube and tubes were inverted for 12-15 times. Centrifugation was done to tubes for 10 minutes at 4°C with 12000 rcf. The supernatant was discarded and the pellet was washed with 1 ml 70% EtOH. Tubes were centrifuged for 5 minutes at 4°C with 7500rcf. The washing step was applied twice. Then, the dry pellet was waited at room temperature until it become transparent. The pellets were dissolved with 50 µl ddH_2O. Tubes were waited on ice for a while and incubated for 10 minutes at 65°C.

- DNA Digesting

To each tube, 1 µl DNase were added for digestion of DNA. Tubes were incubated for 30 minutes at 37°C. The digestion reaction was stopped at 95°C for 10 minutes. The concentration of the RNA was measured for cDNA synthesis step.

- cDNA Synthesis

For each sample; 1 µg RNA, 1 µl Oligo (dT) Primer, 1 µl 10 mM dNTPs, and up to 10 µl ddH_2O,

were added. The mixture was incubated for 5 minutes at 65°C. The tubes were centrifuged briefly. 4 µl 5X First Strand Buffer, 2 µl 0.1 M DTT, 1 µl RNasae OUT were added to each tubes while mixing carefully. Tubes were waited on ice. 1 µl SuperScript

II (200U) was aaded to each tubes. Tubes were incubated at 42°C for 1,5-2 hours. Reaction was stopped at 70°C for 15 minutes. Tubes were placed on ice. 1 µl DNase free RNase were put to each tube. Tubes were incubated for 30 minutes at 37°C. The concentrations of cDNAs were measured on nanodrop. Each concentration were adjusted to 10 ng/ µl.

3.6.9 qRT-PCR

10 ng/ µl cDNA
12,5 µl SYBR Green MM
1 µl Primer sense
1 µl Primer anti
Up to 25 µl ddH_2O were added to each group. Each samples were replicated for 3 times.

As a reference primer, Rap2L was used due to its stability 0,275. The qPCR program was set as;

Table 3.8: qRT-PCR cycle

Holding Stage:	Initial Denaturation	95°C	5 minutes.
Cycling Stage:	Denaturation Hybridization Elongation	95°C. 57°C 72°C	15 seconds 30 seconds. 40x 30 seconds.
Melting Curve Stage;	Melting Curve	95°C 60°C 60-95°C	15 seconds 60 seconds 15 seconds. 70x

Analysis were done with;

Ratio=$(E_{target})^{\Delta Ct\ target(control-treated)}$ / $(E_{ref})^{\Delta Ct\ ref(control-treated)}$

Statics: Stutent T-Test Unpaired.

Table 3.9: Primers for cDNA synthesis

Name	Position	Sequence	TMº	GC%
CG9911 Exon 8-9 forward	+3499	CGACTCGTTCAAGCACATGT	64.0	50
CG9911 Exon 8-9 reverse	+3713	AGTGTGTGGGTCAGGTTCAA	63.2	50
CG9911 Exon 6-8 forward	+3186	CTGGTGCGGGGAAATAACATT	63.5	45
CG9911 Exon 6-8 reverse	+2739	GACTCTGGTGGCGATGTACC	64.6	60

3.6.10 Genomic DNA Isolation

1000 fly heads from each groups were one by one collected with zing in an 1.5 ml tube on ice. 500 µl Homogenization buffer were added to tubes and pestle was used to homogenized. The tubes were incubated at 70^{o}C for 30 minutes. 70 µl 8M KAc was added on each tube and tubes were briefly inverted 5-6 times. Incubation was done for 30 minutes on ice. The tubes were spinned for 15 minute at 4^{o}C with maximum rcf. Supernatant was taken and placed to new tube. Phenol was added as equal amount to supernatant. Tubes were centrifuged at room temperature for 5 minutes at 10000 rpm. The tubes were spun down for two minutes. Aqueous phase was transferred to new tubes and Phenol and Chloroform separately were added to each tube as half amount of this phase. Tubes were centrifuged for 5 minutes at room temperature. Spin down was applied to each pellet for 5 minutes. After that, the aqueous phase was again taken to new Eppendorf tubes and 100% EtOH as 2.5 volumes of it was added. Centrifugation was done for 5 minutes at 4^{o}C with maximum rcf. The pellet was washed with 70% EtOH. Centrifugation was repeated for 3 minutes. The supernatant was discarded and pellet was dried out for 5 minutes at 37^{o}C. 100 µl ddH_2O was used to resuspend the pellet to get DNA.

3.6.11 PCR

500 ng-1 µg DNA

1 µl dNTPs

1 µl Forward Primer

1 µl Reverse Primer

5 µl Thermo Polymerase Buffer

0.25 µl Taq DNA Polymerase

Up to 50 µl ddH_2O were put to PCR tubes for each groups seperately.

The program was arranged as;

Table 3.10: PCR cycle to amlify genomic DNA

Holding Stage:	Initial Denaturation	95°C	30 seconds 1x
Cycling Stage:	Denaturation Annealing Extension	95°C Optimized for Primers 68°C	30 seconds 30 seconds 40x 2 minutes
Melting Curve Stage;	Melting Curve	68°C 4°C	5 minutes 1x ∞

Table 3.11: Primers for PCR

Name	**Position**	**Sequence**	**TM°**	**GC%**
CG9911 intron 3-5 forward	+1430	TGTCTGGTCATGTTGCTGGT	64.4	50
CG9911 intron 3-5 reverse	+2498	ACTGGCGATCCATTTGACTC	64.0	50
Valium10-5' forward	+3539	GACTCCCATGGATAACTTCG	60.9	50
Valium10-3' reverse	+6684	GGATCCGGCTTACTAAAAGC	61.5	50
Hsp70 forward	+3737	CGTCTACGGAGCGACAATTC	64.6	55
Hsp70 reverse	+3880	TGCAGTTGATTTACTTGGTTGC	63.8	40.9
CG9911 exon 4 forward	+2622	CTGGAAAAGTGGTGCTAGGC	63.7	55

CG9911 exon 4 reverse	+2785	GATCCTCCAGCTGCTTCTTG	64.0	55
GAL80 forward	+23	GGTCTCAACCGTGCCTAATG	64.4	55
GAL80 reverse	+1004	CCGGGTCTAAAGGAGCTTG	64.3	55
GAL4 forward	+590	TGGCGACGGTTCTCTCTTATG	66.3	52.3
GAL4 reverse	+1573	AGAGGAGGCAATTGGTTGTG	64	50

3.6.12 Agarose Gel Electrophoresis

The PCR products were run on agarose gel electrophoresis which was prepared based on expected band size.

- For bans lower than 500 bp;

0.8% Agarose gel was prepared with 0.8g agarose inside to up to 100 ml TAE buffer. When agarose gel was getting cooler, 10 µl EtBr was added. The gel comb was put and gel was rested till completely semisolidified. The 1 kb DNA Ladder and samples with 4 µl DNA Loading Dye. The gel was run for 75 minutes at 90 voltage.

- For bands higher than 500 bp;

1.0% Agarose gel was made with 1.0g agarose mixing with up to 100 ml TAE buffer. After cooling down of gel, 10 µl EtBr was put. Gel comb was placed . Gel was rested till it became solidified. The 1 kb DNA Ladder was put on the first row of gel. The samples were loaded with 4 µl DNA Loading Dye. The gel was run for 60 minutes.

3.6.13 Capillary Feeder Assay (CaFe)

To determine body weight of male flies from experiment and control groups were seperately collected while paying attention not giving more than 5 minutes anesthesizing to flies. 100 male flies were placed inside the Eppendorf tubes which were already weighted before. And the tube was again measured on Precision Scale. The step was repeated for 7 times with total 700 male flies for each group to calculate optimum bodyweight.

Capillary Feeding Assay (CaFe) was used to determine food intake (Ja et al., 2007; Diegelmann et al., 2017)

For starvation assay: 20 male flies were put into small vial with food medium. This was done for each group till get 10 vial from each group at the day. They were flipped after 48 hours to vials containing 300 µl water on Whatmann filter paper. They were placed at the 25^{o}C room for 18 hours starvation. The same experiment set up was done three times at different days.

For ab-libitum assay: 8 male flies were put into small vial with food medium. The step was repeated for each group until 10 vial from each group were obtained in one day. The experimental set up was repeated for 3 times at different days.

This experimental set up was prepared for one diet. Repetation and again applied for different diets (in Table 3.12)

The flies were placed at 25^{o}C room to recover from CO_2 sedation for 2 days. CaFe assay consist of vial, sponge, short tips, long tips, 5 µl glass capilleries, food dye, diet solution, and Watmann paper. CaFe vials were covered with sponge to provide air acces and prevent from fly escaping. 6 short tips were placed. 6 long tips were placed on top of short tips. The diet composition was prepared with using same amount food dye (1 diet solution:1 food dye). The glass capillaries were loaded with same diet compositions and they were marked.

For ab libitum assay's flies;

The flies at each vial were flipped to vial only containing 300 µl water (autoclaved ddH_2O) on Whatmann paper. They was placed into CaFe Assay box. 3 eveporation control including 300 µl water on Whatmann paper was also put. Four 5 µl glass capillaries were presented per vial with the same diet compositions for each vials. In addition, 4 vial only containing autoclaved ddH_2O water were set to prevent flies from evaporation and to increase humidity. They were covered with a box to block the light acces. The CaFe assay box was placed at 25^{o}C for 24 hours.

After 24 hours, food solution meniscuses were measured with a caliper as milimeter for each vials. The same set up was done for starvation assay but the time period was changed from 24 to 3 hours.

Milimeter is converted to volume in µl based on capillary diameter as follows;

$$x[\mu l] = distance[mm]/14.6 \text{ (Capillary diameter)}$$

The intake was adjusted to the body weight to measure for one fly's consumption with dividing ,8 for ab-libitum assay and 20 for starvation assay, and bodyweight.

Table 3.12 The composition of CaFe Food Solutions

Number	Solution	Molecular Weight (g/mol)	Molar
1	5% Sucrose	342.30	0.17
2	5% Glucose	180.16	0.28
3	5% Fructose	180.16	0.28
4	5% Yeast	274.3	0.18
5	6% Arabinose	150.13	0.33
6	5% Mannose	180.16	0.28

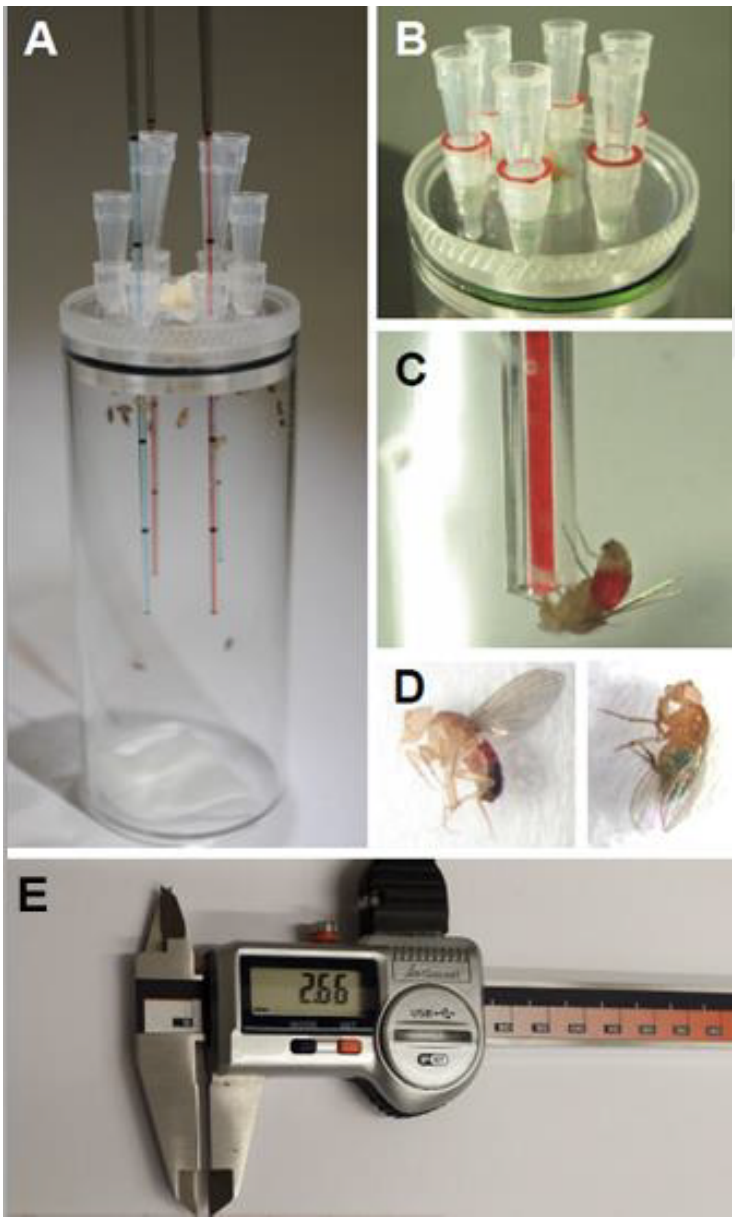


Figure 3.1 The Capillary Feeding Assay (CaFe Assay). Each vial contain four 5 µl capillary glass with either 8 or 20 male flies (A). The long tips were place to short tips to maintain glass capillary (B). Drosophila shows tendency to feed itself (C). The visible abdomen with red food on fly (C-D). The glass capillary measurement device in mm. The data could be directly transmitted to Excel sheet with USB extension (Diegelmann et al., 2017).

3.6.14 Statics Analysis

Two experimental groups comparision was done with Student t-test in Excel. Three group comparision was utilized based on the one-way ANOVA test and post-hoc Tukey-Kramer tests were used by Statistica 9.1 (StatSoft, Tulsa, OK, USA). Non-parametric sign test was choosen to understand food incidence to separate the outcome from random choice. P-value of <0.05 explained as one asterik (*), $p<0.01$ (**) and $p<0.001$ (***).

4. RESULTS

The aim of my thesis is to address the regulation of the serotonin transporter via insulin signaling by ERp44 protein during food intake. To understand this on the network level, Sert3-GAL4 targeted neurons which regulates carbohydrate intake, serotonin and serotonin transporter function were analyzed in the regulation of sugar intake. Sugars were eaten based on their caloric or taste features. And how the intake was selectively occurred for carbohydrate was investigated. Secondly, modulation of serotonin receptor function in sensory neurons was resolved. To reveal sensory receptor interaction with serotonin receptor was researched. And the selectivity of caloric and tasty sugar intake via insulin signaling was tested on cellular level. The regulatory mechanism between insulin signaling and serotonin transporter was tried to address with ERp44 ortholog protein in *Drosophila;* CG9911.

4.1 Serotonergic Neurons; SE1, IP and LP1, Stimulates Subset Specific Phenotype by Developmentally Reduced Serotonin Signaling

Feeding regulation by serotonin has been reported in many organisms (Song and Avery, 2012; Donovan and Tecott, 2013) including *Drosophila* (Vargas et. Al., 2010; Luo et. Al., 2012). Activation of all serotonergic neurons could cause not only limiting feeding but also inhibition of feeding in starved flies (Albin et. al., 2015). Single neuron might responsible from multiple neurotransmitter releasing (Gutierrez, 2009). So, I would like to research the serotonergic neurons driving by Sert3-GAL4 might results of limited carbohydrate intake (Kastenholz, 2019). The serotonin is important for regulation of the sucrose intake where Sert3-GAL4 neurons are activated. And the *UAS-Trh RNAi* line was utilized to destroy tryptophan hydroxylase in serotonin biosynthesis pathway (Coleman and Neckamayer, 2005). *UAS-Trh RNAi* line expressed in Sert3-GAL4 positive serotonergic clusters, LP1, SE1, and IP, the anti-serotonin expression would be eliminated precisely (Figure 4.1). The efficiency of the RNAi line was already shown (Albin et al., 2015). The sucrose consumption was measured ad-libitum fed flies tested for 24h and 18h pre-starved flies for 3h. So that, the Sert3-GAL4 phenotype, limiting carbohydrate intake, was confirmed through specifically

activated neurons with serotonin signaling. Ad-libitum fed and starved flies showed significantly reduction in sucrose intake (Figure 4.1). Developmentally decreased 5-HT signaling on the presynaptic cleft of IP, LP1, and SE1 neurons resulted in falling down the uptake of sucrose 51-46% in ad-libitum flies and 46-45% in pre-starved flies (Figure 4.1)

In conclusion, reducing the carbohydrate intake regulation of developmentally decreased 5-HT signaling was found on presynaptic cleft of IP, LP1 and SE1 neurons.

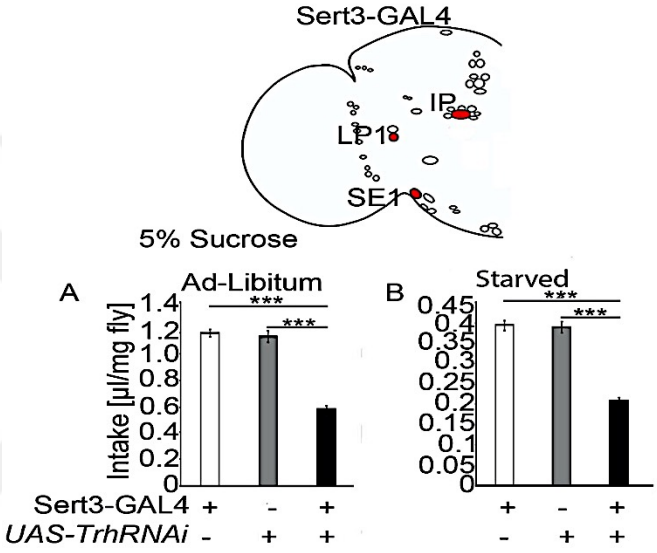


Figure 4.1. Reduction of sucrose intake in flies with subset specific decreased 5-HT signaling in SE1, LP1 and IP neurons. The Sert3-GAL4 driver line trigger the LP1, SE1 and IP (A-B) highlighted in red color. Food consumption was measured in two state; ad-libitum tested for 24h and 18h pre-starved flies tested for 3h with CaFe assay. Developmentally decreased 5-HT signaling because of disrupted trh enzyme showed reduction of sucrose intake in both states (A-B). Negatively regulated carbohydrate intake by serotonin signaling was obtained. in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica and n=20 in pre-starved flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

4.2 Over-expressed Sert Shows Subset Specific Phenotype on SE1, IP and LP1 Neurons

Sert mediated transport to pre-synaptic membrane level, function and efficiency of it are based on Sert is based on posttranslational modification of Sert in ER (Ramamoorthy et. al., 2010). The activity of transporter the amount of it on membrane which could result in different response for uptake of 5-HT, regulated by different pathways such as MAPK pathway or Ca^{++} pathway (Ramamoorthy et. al., 2007; Zhang

et. al., 2007, Oz et. al., 2010). Therefore, to get better resolution on whether Sert transporter activated on SE1, LP1, and IP serotonergic neurons by Sert3-GAL4 line might be affected by insufficient activity of Sert (Kastenholz, 2019). The reason of that the wild type *UAS-Sert::GFP* driver line overexpressing wild type Sert protein was used to increase activity and efficiency of Sert (Figure 4.2). Enhanced Sert function and amount on pre-synapse end up with reducing in 5-HT signaling in the synaptic cleft when the UAS-Sert::GFP driver line expressed on SE1, IP and LP1 neurons (Balmert S., master thesis 2017). Sucrose intake was again observed for ad-libitum fed flies tested for 24h and 18h pre-starved flies for 3h (Figure 4.3).

Hence, the reduction of sucrose consume was found as 63-66% for ad-libitum fed flies, and 20-25% for pre starved fed flies. Lastly, carbohydrate intake was negatively regulated as Sert3-GAL4 specific phenotype. So this limited carbohydrate intake phenotype might be prevented from post-translational modifications to affect the function of Sert on presynaptic cleft of IP, LP1 and SE1 neurons with over-expressed Sert.

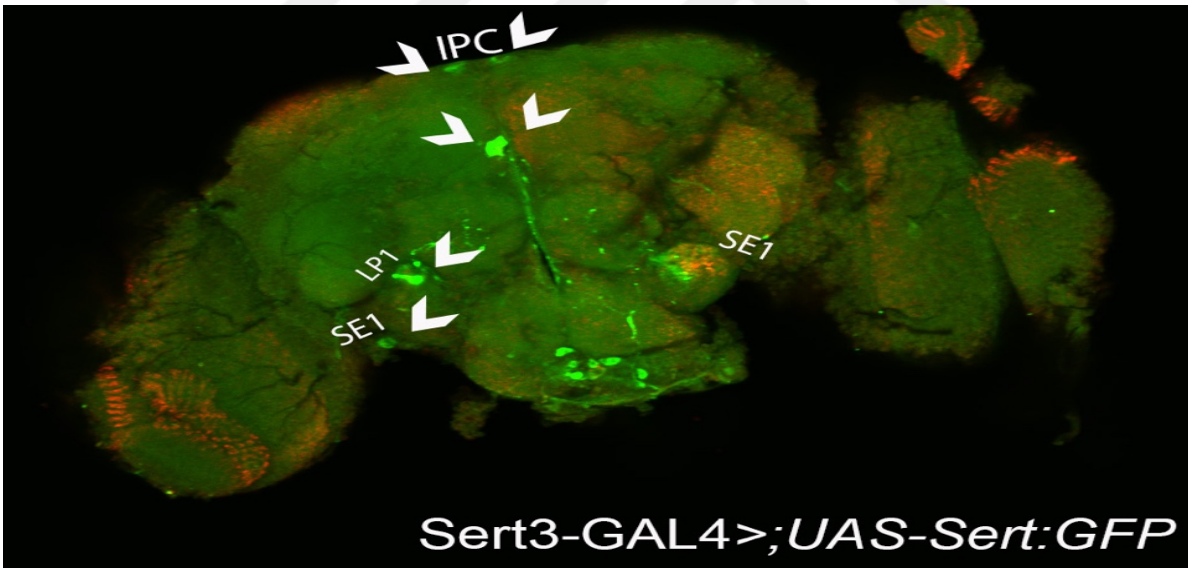


Figure 4.2. Over-expressed Sert on Sert3-GAL4 targeted neurons. Over expression of Sert by Sert:GFP (green) were expressed under the control of Sert3-GAL4 targeted neurons. Expression of wild type Sert localizes on the soma with stronger signal and stimulates projections on SE1, LP1, and IPC neurons. Anti-nc82 was used to visualize neuropil. n=6-10. Scale bar: 50 µm

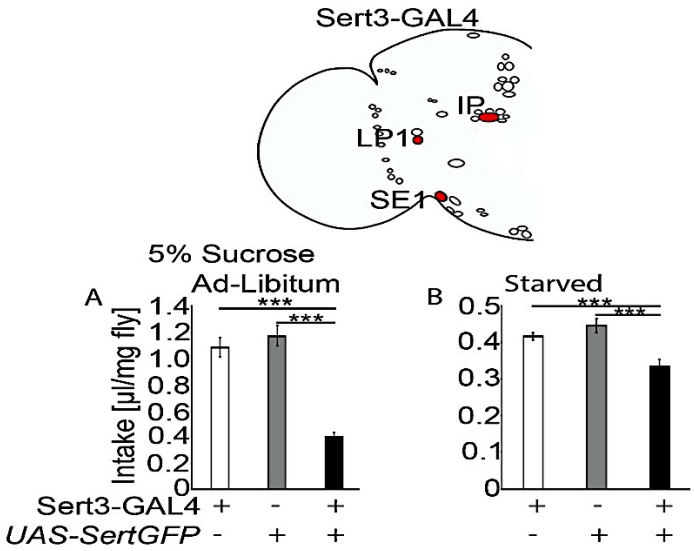


Figure 4.3. Over-expressed Sert decreases sucrose intake. Over expression of Sert under the control of Sert3-GAL4 targeted neurons via wild type Sert expression stimulates decreased sucrose intake measured ad-libitum tested for 24h and 18h pre-starved flies tested for 3h in CaFe assay (A-B). in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica and n=20 in pre-starved flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

4.3 A Small Subset of Serotonergic Neurons Regulate Caloric and Tasty Carbohydrate Intake by Early Developmental 5-HT Signaling

Previous studies in flies showed that of *dSert*^{10} or *dSert*^{16} mutations cannot express functional serotonin-transporter (dSert) in flies due to deletions in 1121 and 1192 positions leads to reduce or insufficient protein expression. Mutations results in both reduction of presynaptic uptake of serotonin while the increase of 5-HT signalling in the synaptic cleft (Kaiser, diploma thesis 2009) and the mutations reveals decreased carbohydrate intake (El Khadrawe, bachelor thesis 2017; Götz bachelor thesis 2018). The protein valuation is increased by serotonin (Ro et al, 2016) and the uptake of macronutrients; carbohydrates and protein is promoted by serotonin (Albin et al., 2015). Besides, the Sert3-GAL4 targets three serotonergic neurons per hemisphere (IP, LP1, SE1) and it negatively regulates the food approach (Xu et al., 2016). The Sert3-GAL4 driver line targeted serotonergic neurons, SE1, LP1, and IP, also negatively regulates the carbohydrate food intake (Kastenholz, PhD thesis 2019). So, our aim was to analyze whether the negative regulation of carbohydrate intake is whether sugar specific. To understand the modulation of subset specific serotonergic neurons on the selectively specific sugar consumption, the serotonin signalling was altered by

expression of *UAS-SERTDN: GFP* which disturb *dSert* function with dominant negative version under the control of the GAL4 driver lines. dSERT limits the uptake of serotonin in presynaptic level and determines the duration period of serotonin on post-synaptic receptors (Rudnick, 2006)

To research selectivity for sugar uptake, either caloric and tasty (5% Glucose, 5% Fructose), caloric but not tasty (5% Mannose) or not caloric but tasty (6% Arabinose) were fed to flies (Figure 4.3) The food intake was measured with Capillary Feeder (CaFe) assay (Diegelmann et al., 2017) under two metabolic conditions. Flies were fed ad-libitum and tested for 24 hours to intake foods based on taste to understand all circadian rhythmis done sufficiently, or pre-starved for 18 hours that would empty the crop to measure caloric intake based on internal needs. Then, tested for 3 hours to understand intake based on internal homeostasis (Dus M et al., 2011). Pre-starved flies choose strongly required nutrients. Although feeding time could not get longer in 3h testing, the flies could reach back to fed ad libitum state so that the 3h tests were done on the same day to prevent from daytime-dependent effects. So, comparison between the 24h testing and 3h testing will provide better resolution to understand intake based on the metabolic state or taste preference of the flies.

Moreover, both ab-libitum fed and pre-starved fed flies showed significantly reduction for all kind of sugar intake (Figure 4.4). Increased 5-HT signaling on presynaptic cleft of IP, LP1, and SE1 neurons activated via Sert3-GAL4 driver line in ad-libitum fed flies; caused a reduction in 36-40% glucose, 55-54% fructose, 45-42% mannose and 29-35% arabinose. Also, a decrease in pre-starved flies was observed in intake of 10-16% glucose, 76-75% fructose, 22-18% mannose and 25-29% arabinose uptake. Therefore, the metabolic-state independent was observed with reduction of arabinose intake in pre-starved contion. The palatability dependent regulation was shown due to reduction of all kind of sugar intake in ad-libitum fed in addition to carbohydrate limiting intake was observed. So, developmentally enhanced serotonin signaling on presynaptic cleft of IP, LP1 and SE1 neurons could sense the palatability of food.

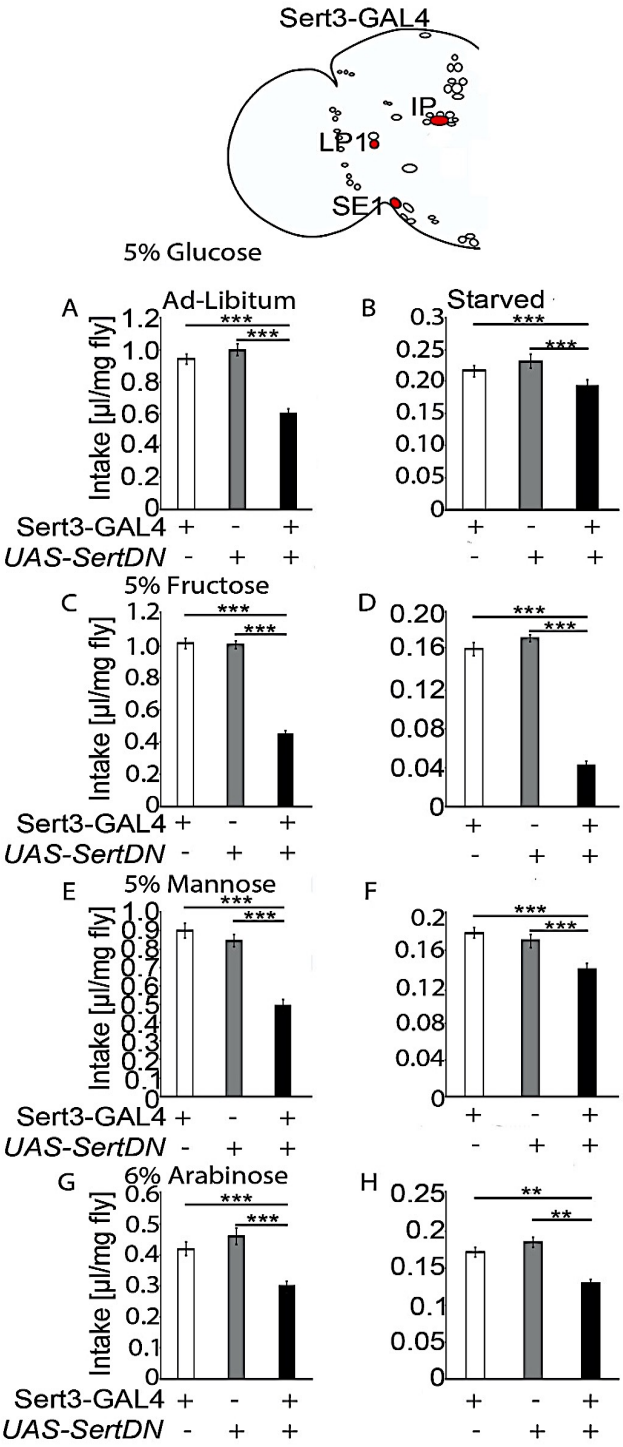


Figure 4.4 Taste specific regulation of carbohydrate intake by serotonergic neurons. The LP1, IP, and SE1 serotonergic neurons were targeted by Sert3-GAL4 driver line (A-H), the expression pattern highlighted in red color in the cartoon. The consumption of food was determined in two different states as ad-libitum fed flies testing for 24h and 18h pre-starved flies were tasted for 3h by using CaFe assay. Developmentally enhanced serotonin signaling due to dominant negative serotonin transporter with all tested sugar types in both ab-libitum state and pre-starved test groups were significantly decreased on LP1, IP, SE1 serotonergic neurons. The palatability and hedonic value dependent sugar intake by Sert3-GAL4 driver line was shown with significant reduction of sugars in ad-libitum flies (A-C-E-G). The internal state independent sugar intake was observed with significant decrease in pre-starved flies (B-D-F-H). in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica and n=20 in pre-starved flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

4.4 Blocked Transport of Serotonin by 5HT1B Receptor and 5HT2B Receptor Does Not Affect Sucrose Intake on IR60b Neurons.

Neurons targeted by the Sert3-GAL4 driver line negatively regulates the carbohydrate intake (Kastenholz, PhD Thesis 2019). Taste perception regulation by internal state with nutrient-specific uptake was demonstrated with specific nutrient intake (Steck et. al., 2018). Therefore, we aim to focus on whether taste neurons are modulated by Sert3-GAL4 targeted neurons. The IR60b receptor neurons limits sucrose intake in the pharynx (Joseph et al., 2017). The proximity between IR60b-GAL4 and Sert3-GAL4 targeted neurons were found in the lateral subesophageal zone (SEZ), but not a direct synaptic contact (Kastenholz, 2019). And therefore, there might be serotonin receptor on the surface to maintain serotonin signaling that might interacts with IR60b targeted neurons was trying to research. To do that, the *UAS-5HT1B RNAi* and *UAS-5HT2B* line were targeted. The 5-HT1B receptors negatively couples to activate adenyl cylase so that it reduce cAMP production. The 5HT1B RNAi induction via dsRNA formation for each 5-HT1B receptor positively regulates food intake by increasing body wall contraction and mouth hook movements in larvae (Majeed et. al., 2016; Yuan et. al., 2005) with IR60b targeted neurons whereas 5-HT2B receptors negatively regulates the food intake since 5-HT2B receptors positively modulates the adenyly cyclase to triggers cAMP production (Yuan et al., 2005). CaFe assay was performed for ad-libitum fed flies tested for 24h with 5% sucrose and 5% yeast intake for 5-HT1B (Figure 4.5). The knockdown of 5-HT1B receptors in IR60b neurons did not alter neither in sucrose intake nor 5% yeast between experimental and control groups (Figure 4.5). As 5-HT1B receptors, knockdown of 5-HT2B receptors were not alter significant change on IR60b neurons (Figure 4.6)

In summary, serotonin-dependent modulated IR60b neurons altered by the UAS-5HT1B RNAi and UAS-5HT2B RNAi lines did not affect the sucrose.

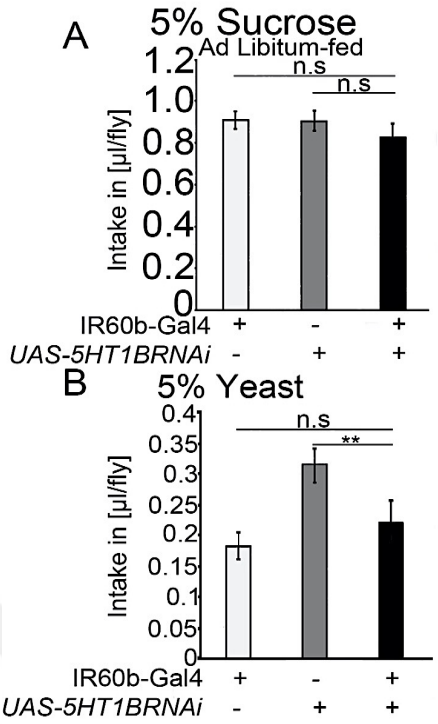


Figure 4.5 The knockdown of the 5-HT1B receptor on IR60b neurons did not affect food intake. There was no significant alteration between control and experimental groups for both 5% sucrose (A) and 5% yeast intake (B) in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

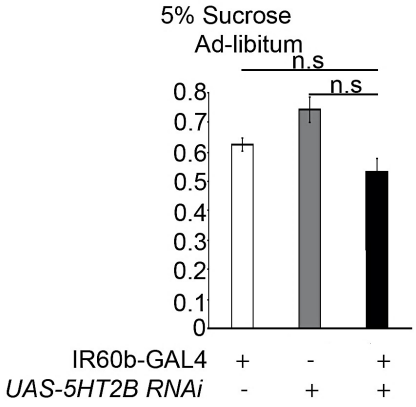


Figure 4.6 The knockdown of the 5-HT2B receptor on IR60b neurons did not affect food intake. No significant reduction between control and experimental groups were observed for both 5% sucrose in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

4.5 InR Signaling in Serotonergic Neurons Regulates Internal Homeostasis and Palatability Dependent Carbohydrate Intake.

Internal homeostasis is needed to be in balance with food intake (Williams et al., 2010). CNS regulates neurons responsible for food intake via insulin/insulin-like signaling (Williams et. al., 2010, Qui et. al., 2014). DILPs are released to activate

insulin signaling to balance sugar level inside hemolymph (Haselton et. al., 2010). The modulation of IP and LP1 neurons with InR expression was detected in Tph-GAL4, an enzyme to produce 5-HT and this line targets around 26 neurons per hemisphere (Park et al., 2006). The up and downregulation of InR signaling via Sert3-GAL4 targeted neurons were shown as subset specific phenotype observing in increased serotonin modulation via $Sert^{DN}$ expression in Sert3-GAL4 targeted neurons (Kastenholz, PhD thesis 2019). So, to know whether the carbohydrate intake is sugar specific in Sert3-GAL4 targeted neurons, we used the dominant receptor insulin-like receptor variant, *UAS-InR^{DN}*, line to block insulin signaling. *UAS-InR^{DN}* is generated by changing one amino acid, lysine, to alanine at 1409 position and this single point mutation caused non-functional receptor such as dimer or homodimer endogenous InR (Kastenholz, PhD thesis 2019). Two metabolic conditions, ad-libitum tested for 24h or 18h pre-starved flies tested for 3h, were used to comprehend the regulation subject to internal homeostasis state and/or palatability and hedonic value of food (Figure 4.7). As in the 4.1, CaFe assay was performed with similar sugars; caloric and tasty (5% Glucose, 5% Fructose), caloric but not tasty (5% Mannose) or not caloric but tasty (6% Arabinose). Subset specific phenotype to limit carbohydrate intake was verified with insulin signaling. Blockage of InR signaling in presynaptic cleft of IP, LP1, and SE1 neurons activated via Sert3-GAL4 driver line resulted in significant reduction of 57-53% in glucose uptake, 26-30% in fructose uptake, 40-38% in mannose uptake and 23-16% in arabinose uptake for ad-libitum fed flies. In addition to that, diminished in the uptake of 50-34% glucose, 39-37% fructose and 48-44% mannose for pre-starved flies (Figure 4.7). However, there was no significant change in uptake of arabinose for pre-starved flies.

Conclusively, both metabolic-state and palatability and hedonic value dependent regulatory mechanism (Dus M et al., 2011). was observed on presynaptic cleft of IP, LP1 and SE1 neurons.

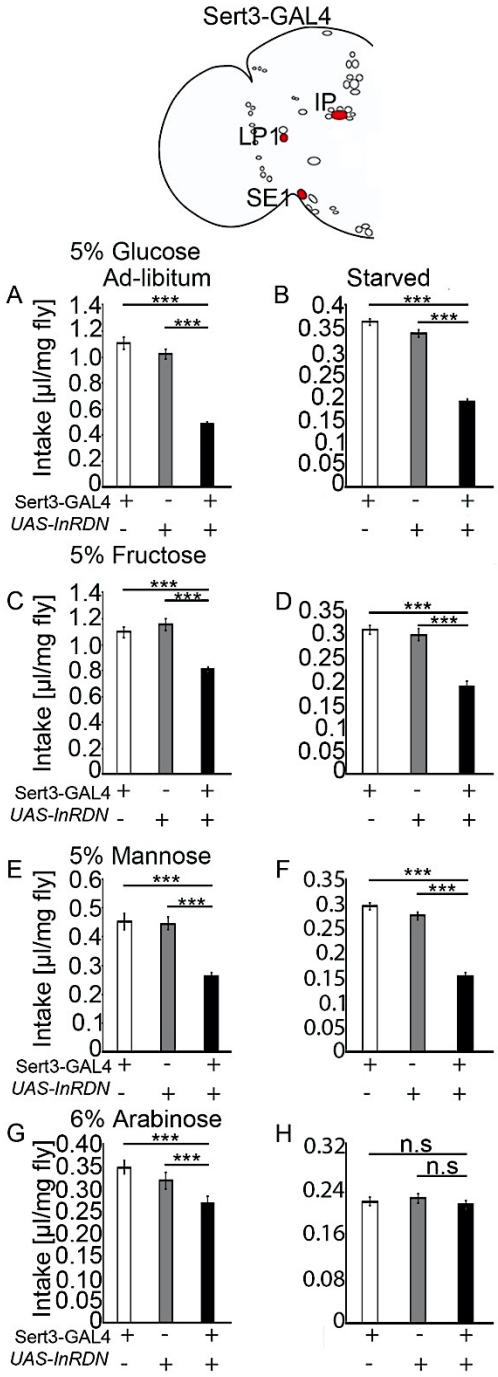


Figure 4.7 Metabolic state and taste specific regulation of carbohydrate intake by Sert3-GAL4 targeted neurons. The LP1, IP, and SE1 serotonergic neurons which were shown in red color, by targeted with Sert3-GAL4 driver line (A-H). Ad-libitum fed flies testing for 24h and 18h pre-starved fed flies testing for 18h were measured to understand consumption of carbohydrate by using CaFe assay. Developmentally blocked insulin signaling because of the dominant negative insulin receptor with all sugar types in ad-libitum state (A-C-E-G), and in uptake of glucose, fructose and mannose for pre-starved fed flies (B-D-F) were significantly cut down. There was no significant alter in uptake of arabinose for pre-fed flies (H). in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica and n=20 in pre-starved flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

Table 4.1 SE1, LP1, and IP neurons show sugar specific regulation

Targeted Constructs	*UAS-Sert^{DN}*	*UAS-InR^{DN}*
Pre-fed, 24h test		
Glucose	decrease	decrease
Fructose	decrease	decrease
Mannose	decrease	decrease
Arabinose	decrease	decrease
Pre-starved, 3h test		
Glucose	decrease	decrease
Fructose	decrease	decrease
Mannose	decrease	decrease
Arabinose	decrease	no effect

4.7 CG9911 protein function in SE1, LP1, and IP neurons targeted by Sert3-GAL4

Subset specific phenotype was observed with Sert3-GAL4 targeted neurons for both InR signaling and serotonin signaling (Kastenholz, PhD thesis 2019). Thereof, the possible interaction was tried to figure out. Endoplasmic reticulum is important for synthesis and properly folding of proteins and it is regulated by stress response (Li et al., 2014). Stress response triggers unfolded protein responses (UPRs) (Nyirimigabo et al., 2019). UPR stimulates protein disulfide isomare (PDI) family. One of the PDI protein is ERp44. Blood glucose level is reduced and increased the lipid synthesis disorders in ERp44 knockout mouse (Nyirimigabo et. al., 2019). ERp44 provides proper glycosylation of Sert at the Cys200 and Cys209 sites in post-translational modification (Li et. al., 2014).. In gestational diabetes mellitus (GDM) patients, ERp44 prevent glycosylation of Sert on Asp208 site which is between Cys200 and Cys209 (Li et. al., 2014). So, I would like to research the ERp44 protein is the responsible for subset specific phenotype, limiting carbohydrate intake. To do this, *UAS-CG9911 RNAi* line which produce dsRNA hairpin structure to destroy function of CG9911 protein, ortholog of ERp44 in Drosophila, was used on Sert3-GAL4 targeted neurons. CaFe assay was performed with ad-libitum tested for 24h and 18h pre-starved flies tested for 3h. The reason for this, internal homeostasis state regulation and palatability of food regulation would be understood. 5% Sucrose, 5% Glucose, 5% Fructose and 5% Yeast were applied (Figure 4.8).

Both for ad-libitum fed tests and pre-starved fed flies tests resulted in significant decrease of carbohydrate intake (Figure 4.8). The ad-libitum fed flies showed

decreased intake of 50-48% sucrose, 35-41% glucose, 36-41% fructose and the pre-starved flies diminished the intake of 38-42% sucrose, 29-31% glucose, and 34-35% fructose. As in serotonin and insulin regulation, there was no significant change in the consumption of yeast. The ad-libitum state yeast intake increased 4-2%. The pre-fed flies show reduction for yeast intake as 8-10%. Also, molecular weight of the experimental group significantly higher than the control groups as 9-18% (Figure 4.9). Based on this result, limited carbohydrate intake was regulated with CG9911 RNAi construction. It could provide positive regulation for carbohydrate intake in SE1, LP1 and IP neurons while reflecting the same feeding phenotype with the insulin and serotonin signaling.

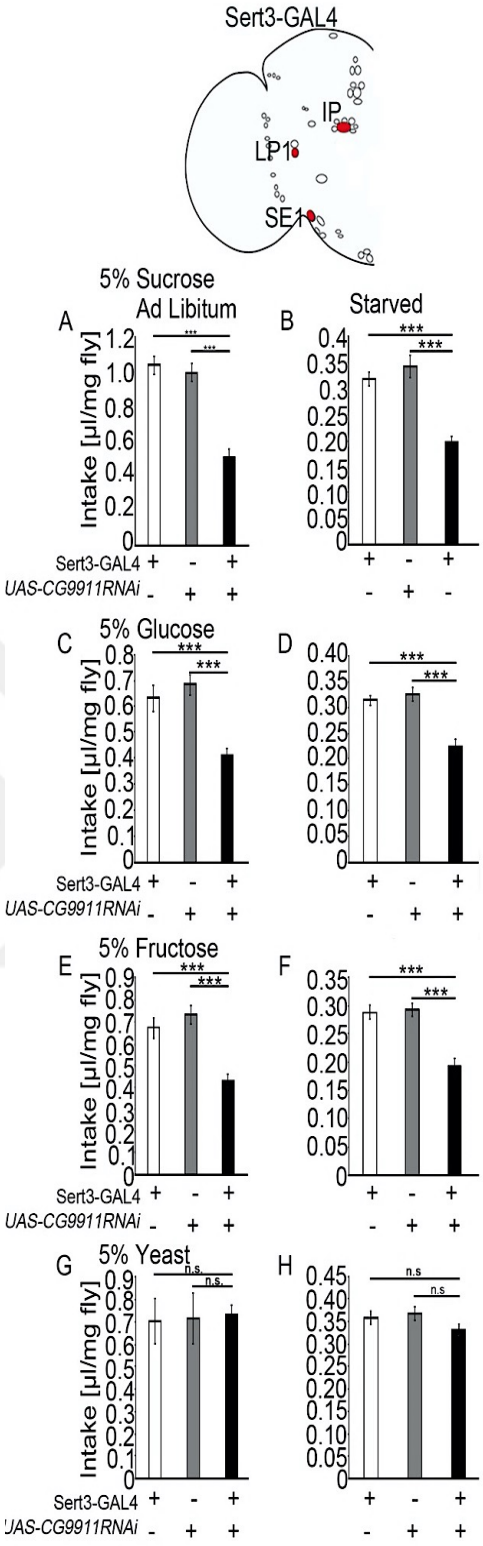


Figure 4.8 Limited carbohydrate intake by CG9911 on Sert3-GAL4 targeted neurons. The LP1, IP, and SE1 serotonergic neurons which were shown in red color, by targeted with Sert3-GAL4 driver line (A-H). Two metabolic state, ad-libitum and starvation, were used. Developmentally blocked of CG9911 protein gave the subset specific phenotype. It reduces the carbohydrate intake for both metabolic states (A-F)while there was no significant change in uptake of yeast in both metabolic state (G-H). in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica and n=20 in pre-starved flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P <$ 0.05, ** $P <$ 0.01, *** $P <$ 0.001 based on one-way ANOVA and post-huc Tukey test.

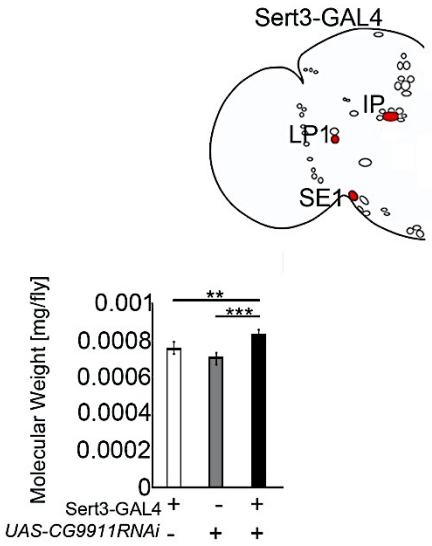


Figure 4.9. Molecular weight comparison of experimental group and control groups. 100 flies were collected and weighted. The step was done for 7 times. The values were averaged. The experimental group showed significantly higher molecular weight than control groups. Body weight was shown as µg/fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

4.7 Quantitative confirmation of knockdown *UAS-CG9911 RNAi* line

Since the possible interaction could be solved between insulin signaling and Sert, the construct has to be verified whether it is actually working. To confirm the *UAS-CG9911 RNAi line* is working, firstly, Appl (amyloid precursor protein like)-GAL4 driver line was utilized. It targets almost all serotonergic neurons in the CNS. The ad-libitum fed flies were tested for 24 hours with CaFe assay to measure 5% sucrose intake (Figure 4.10). The significant reduction was again showed (Figure 4.11). The sucrose intake was reduced as 72-44% between control groups and experimental group (Figure 4.10).

Secondly, quantitative polymerase chain reaction was used to verify *UAS-CG9911 RNAi line*. QPCR provides detection, characterization and quantification of nucleic acids based on cDNA of the sample. The primers were designed for spanning the exon 6 and 7 which are not affected by dsRNA hairpin structure of RNAi line. And the housekeeping primer was selected as Rplp0. Experimental group was compared with control groups separately by using Student T-test.

Significant reduction was not observed in differences between experimental group and the control groups (Figure 4.11). Unexpectedly, experimental group showed the increase as 2-20%.

In summary, although the construct function was obtained with 24h CaFe assay in a behavioral manner, it was not verified with qPCR method.

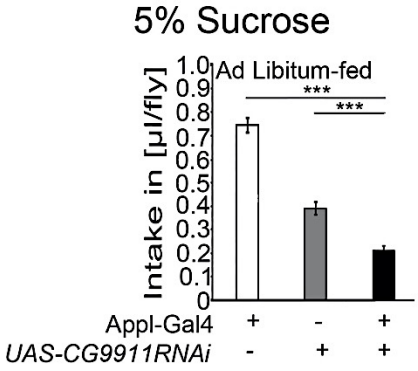


Figure 4.10 Limited carbohydrate intake by CG9911 on Appl-GAL4 targeted neurons. Ad-libitum metabolic state was used. Developmentally blocked of CG9911 protein gave the subset specific phenotype on Appl-GAL4 targeted neurons. It reduces the carbohydrate intake in 24h CaFe assay. n=8 in ad-libitum flies with 10 replica for each group. Intake was shown as µl/mg of a fly ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ based on one-way ANOVA and post-huc Tukey test.

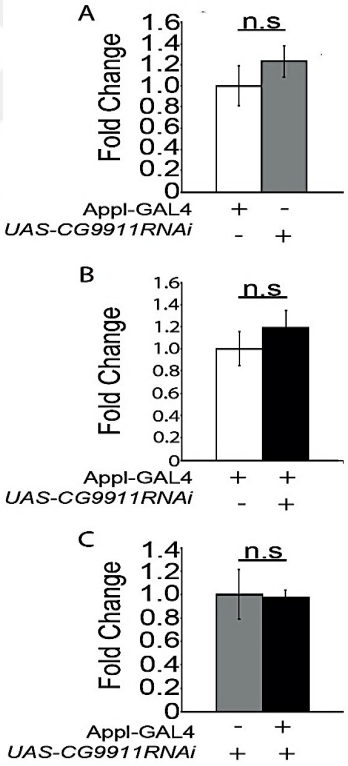


Figure 4.11 Quantitative polymerase reaction analysis of CG9911 RNAi expression. The expression of CG9911 was normalized to housekeeping Rplp0. The GAL4 control groups had higher expression than UAS control group but there was no significancy. The expression of CG9911 RNAi was higher than the control groups (B-C). Expression differences was shown as fold change of a gene ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

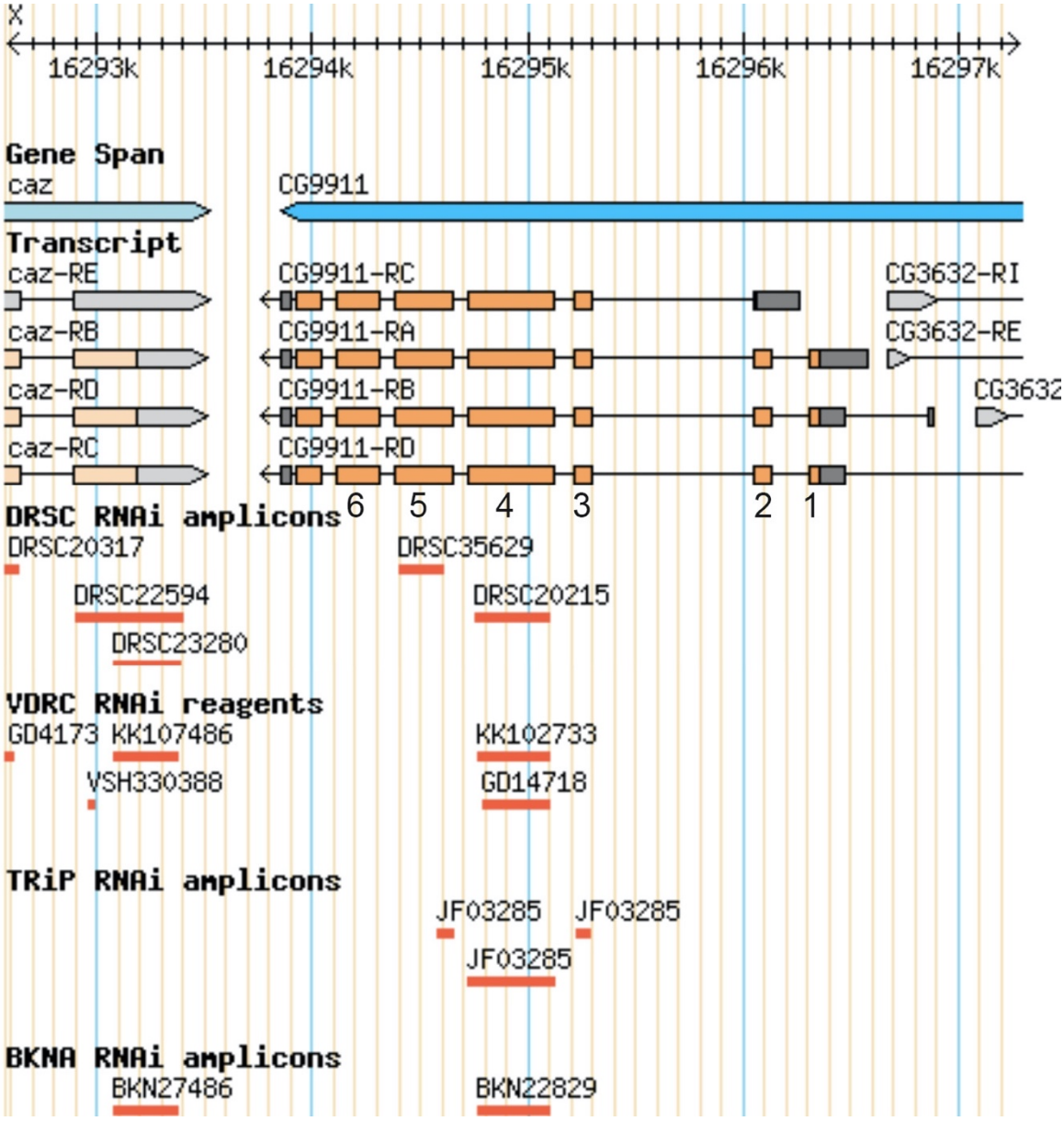


Figure 4.12 The genomic DNA, transcripts and RNAi amplicons of CG9911. The exon (orange) were shown with numbers. The introns were seen as grey box and the black line between each exon. The gene is reversely mapped. JF03285 TRiP RNAi amplicons was used to disrupt CG9911 function by generating ds RNA hairpin structure.

Next, the VALIUM10 vector contained the ds RNAi to knockdown the CG9911 function (Perkins et al., 2015). To confirm the insertion of the construct into the genome of flies, the PCR was performed to genomic DNAs and cDNAs of flies so that if intron is observed in PCR of cDNA, the ds RNA hairpin structure could not be studied and there would be intron contamination. gDNA confirmed the cDNA with size of exon and intron. Primers were designed to target exon 6-7 and intron 3-5 (Figure 4.13).

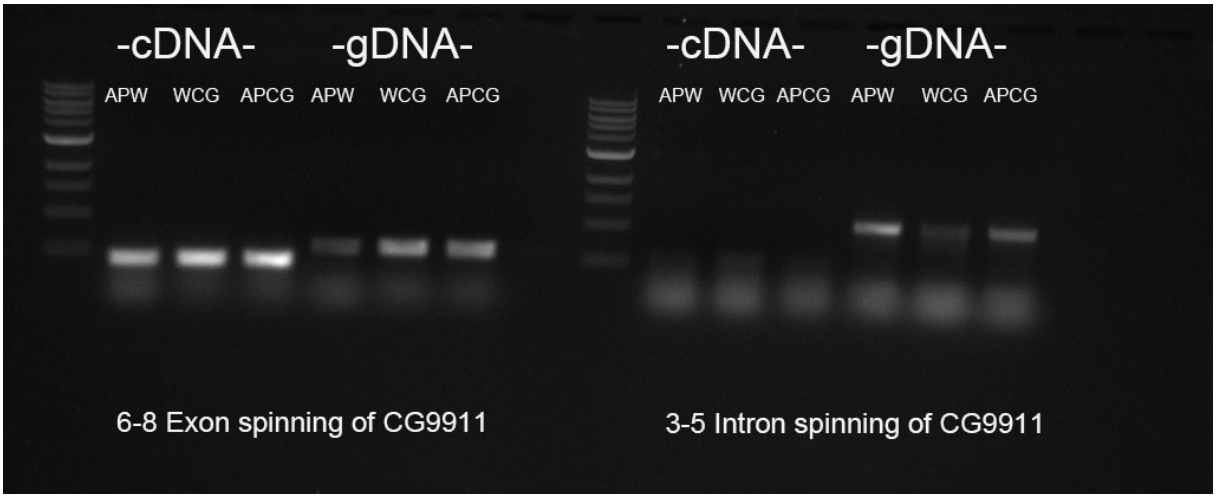


Figure 4.13 PCR results for targeting exon 5-7 and intron 3-5 of cDNA and gDNA of CG9911. 1 kb DNA ladder was used. The primers were targeted the exon 5-7 on the left side and intron 3-5. The cDNA was utilized to confirm there was no intron that could disrupt the ds RNA hairpin structure with 1000 bp DNA fragment. The genomic DNA confirmed the size of exon in addition to intron size inside the targeted region with 1500 bp DNA fragment. Based on that, the construct is actually inserted to the flies. Since all flies express the CG9911 gene to indicate UPR in ER stress, all groups showed positive in the cDNA and gDNA. Only introns were not seen in cDNA of the each group so there was no intron contamination.

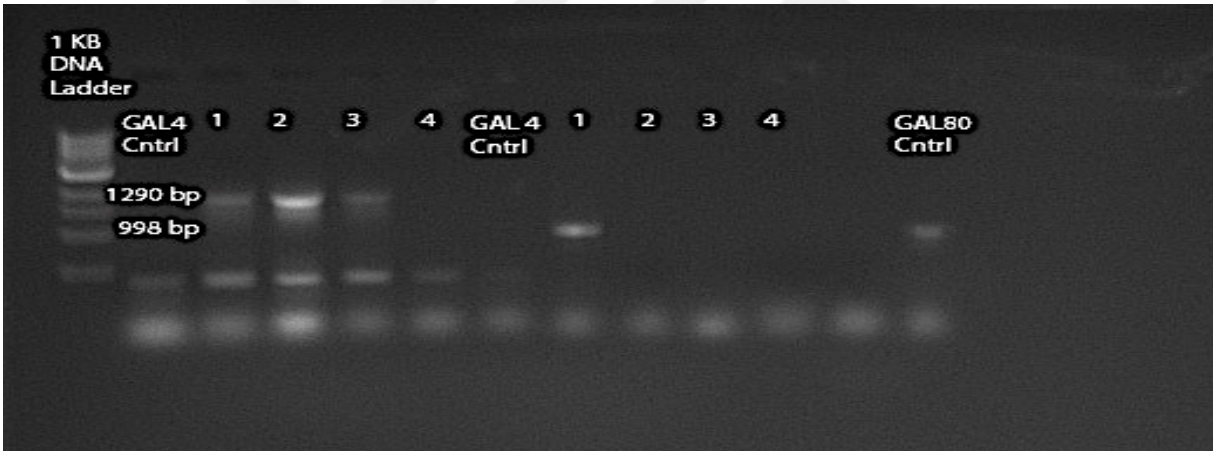


Figure 4.14 PCR results to verify stable collection of w1118;Sert3-GAL4,tubGAL80ts. 1 kb DNA Ladder was utilized. The temporary affect of serotonin signaling wanted to research so that Sert3-GAL4, tubGAL80ts stable line needed to generated. The different stock collections were confirm with the GAL4 and GAL80 targeted primers. Only stock one gives the band for both GAL sequence wih 998 bp for GAL80, 1290 bp to GAL4.

4.8 Tph-GAL4 targeted neurons projects through Thoracic ganglia

To understand how serotonergic neurons project in TPH-GAL4 driver line, serotonin and GFP double immunostaining were performed. Both brain and thoracic ganglia were observed in TPH-GAL3, UAS-mCD8::GFP. For serotonin staining, 12 cluster have been found (Giang et al., 2011) and matched in Figure 4.15 (Serotonin staining=magenta).

Co-localization was observed between Serotonin and GFP staining. GFP staining was seen from posterior to anterior in the brain. Apart from the serotonergic neurons, there were non-serotonergic neurons were marked with white arrows (Figure 4.15). In ventral nerve cord the serotonergic neurons were GFP positive in Pro, Meso and Meta of thoracic ganglia (Figure 4.15) as descrived by Valles and White (1988).

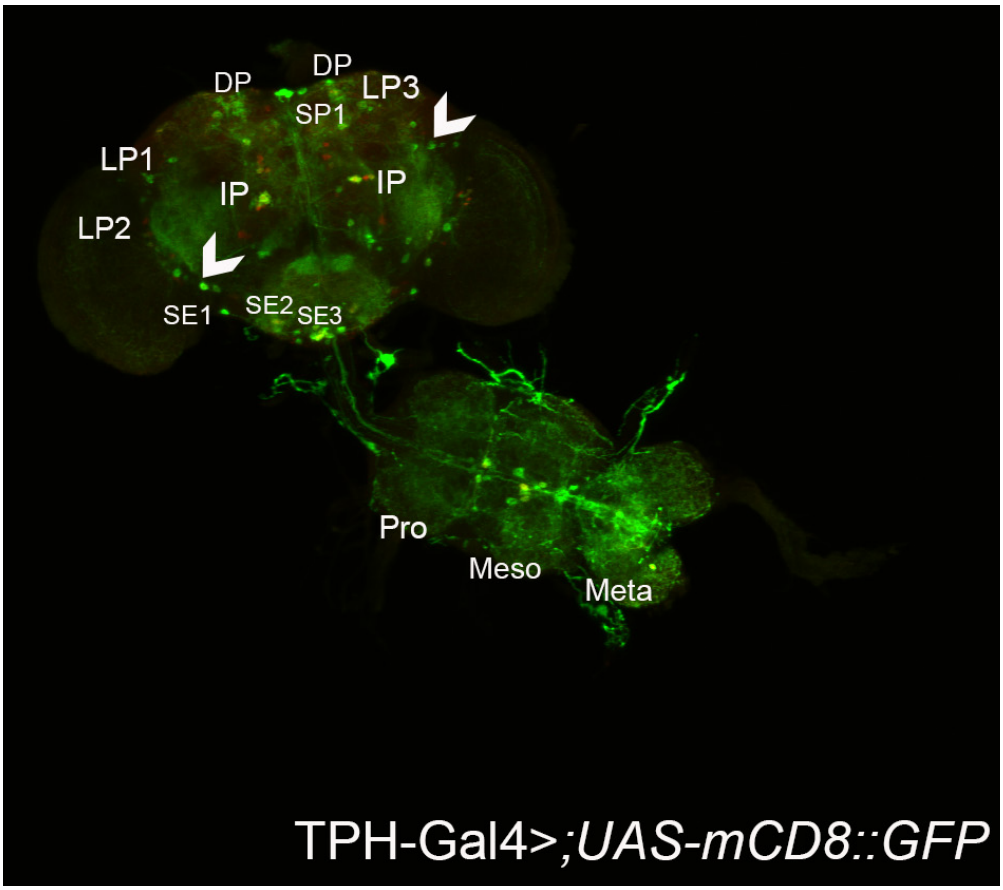


Figure 4.15 Co-localized serotonin and GFP signaling on TPH-GAL4 targeted neurons. Co-localization of serotonin (magenta) and GFP staining in adult brain. Positive GFP neurons exist in many of serotonin clusters. The serotonin and GFP co-localized in Pro, Mesa and Meta of the thoracic ganglia (magenta=serotonin, green=GFP; scale bar 50μm).

5. DISCUSSION and FUTURE PERSPECTIVES

5.1 A Small Serotonergic Subset Specifically Regulates Sucrose and Glucose Intake whereas Developmentally Enhanced Serotonin Signaling Limits Palatable Food Intake of Glucose, Fructose, Mannose and Arabinose.

Our results showed the developmentally decreased or increased serotonin signaling on Sert3-GAL4 targeted neurons, modulates negatively carbohydrate intake in adult flies. Additionally, how the regulation of food intake based on hedonic value or/and internal needs is still unknown. Here, our findings could verify that the developmentally enhanced serotonin signaling negatively modulate sugar intake based on their palatable (hedonic) value.

The serotonergic neurons activated by Tph-GAL4 and Sert3-GAL4 with developmentally raised serotonin signaling negatively intake the sucrose in fed flies (Kastenholz, PhD thesis 2019). The same phenotypic behavior was observed with temporary neural activated serotonin signaling in pre-starved flies using the Trh-GAL4 driver (Pooryasin and Fiala, 2015; Ro et al., 2016; He, unpublished 2018). The Trh-GAL4 triggered serotonergic neurons limits sucrose and sucrose-yeast intake (Pooryasin and Fiala, 2015; Albin et al., 2015; Ro et al., 2016). The administration of the serotonin to cockroaches diminishes the sucrose intake (Cohen, 2001). The treatment with serotonin reuptake inhibitors (SSRI) indirectly leads to decrease of sucrose intake (Götz, bachelor thesis 2017). Also, the $dSert^{10}$ and $dSert^{16}$ mutation on *Sert* gene provide non-functional Sert transcripts that reduced the sucrose intake (El Khadrawe T., bachelor thesis 2017). The carbohydrate intake causes the serotonin releasing to presynaptic cleft of rats (Fernstrom and Wurtman, 1972). So, the serotonin signaling negatively regulates carbohydrate intake.

It was supposed that lower serotonin promotes food intake (Fernstrom and Wurtman, 1972) and the developmentally decreased the serotonin signaling should provide enhancement in carbohydrate intake. However, results with developmentally decreased serotonin signaling by knowdown of Trh enzyme on SE1, IP and LP1

neurons (Figure 4.1) The *UAS-Trh RNAi* construct was already verified as functional (Albin et al., 2015). The developmentally increased Sert on Sert3-GAL4 targeted neurons by *UAS-Sert:GFP* to produce more Sert to prevent it from an unmodified Sert function in post-translational level (Figure 4.2; Figure 4.3) showed the opposite effect. The ad-libitum fed and the pre-starved fed flies gave the same phenotype that decreased the sucrose intake. Serotonin concentration is dependent on presynaptic serotonin release, serotonin transporter amount and functionality, and/or postsynaptic receptor densities during developmental alteration of serotonin signaling (Rudnick et al., 2006). So, there could be neuronal adaptations to developmentally altered serotonin signaling since it´s included in the development of neurons (Daubert and Condron, 2010) that might be due to serotonin-receptor composition on targeted neurons.

In addition to that, developmentally enhanced serotonin signaling via $Sert^{DN}$ construct showed the carbohydrate limited intake on SE1, LP1 and IP neurons (Kastenholz, PhD thesis 2019). Therefore we wanted to observe whether the intake is dependent on the internal state of the organism or the palatability of the food or both. The maximal caloric, and tasty (glucose), the maximal tasty and caloric (fructose), minimal tasty, but highly caloric (mannose) and the maximal tasty but minimal caloric (arabinose) were utilized. All the sugars have different caloric and taste level. The ad-libitum state would measure the hedonic value (palatability) of the food. The pre-starved state would observe the internal state of the food. The result indicated, all the sugar intake was reduced in ad libitum state (Figure 4.4). That provides the palatability of food could be modulated by serotonin signaling. The level is almost same as glucose and fructose intake 0.5 µl/mg. That makes a fly to reach the required palatable level. However, internal state might be not regulated by since the reduction of arabinose level in pre-starved condition was also observed.

We used the *UAS-$Sert^{DN}$* construct to reduce serotonin reuptake in the presynapse (Xu et al., 2016) and the some serotonin levels (Balmert S., master thesis 2016). However, it might have lower number localized Sert on membrane so that reduce the uptake of the serotonin. To verify the functionality of $Sert^{DN}$, the subcellular localization by Western Blot analysis could be performed to separate cytosolic proteins and membrane proteins depending on the location.

Although the results from different studies for the food intake regulation by serotonin signaling with *UAS-Sert*^{DN} show different intake levels for cabrohydtrate consumption as 0.6 µl/mg, 0.5 µl/mg, 0.25 µl/mg in (Figure 4.4). This could be the effect of the standard food that I used to maintain the flies. The standard food differs in the nutrient composition based on carbohdyrate to amino acid ratio. The different food compositions could show different level of nutrient uptake (Grangeteau et al., 2018; Piper et al., 2014). It might be prevented with different food compositions (Piper et al., 2014). Nevertheless, there is still relevance between the experimental group and control groups in the same conditions so the results remain consistent. Also, the temporary enhanced serotonin signaling should be tested on Sert3-GAL4,tubGAL80ts targeted neurons. Sert3-GAL4,tubGAL80ts stock was generated (Figure 4.14) to understand the CG9911 dependent food intake behavior could be adult behavior. It needs to be further researched to verify adult behavior by CaFe assay.

Also, the Tph-GAL4 projections were observed to the thoracic ganglia (Figure 4.15). The possible projection could be also observed by Sert3-GAL4 neurons so that, the limited carbohydrate intake phenotype could be related with those projections. That can be further researched with immunohistochemistry approach on ventral nerve cord, crop and intestine.

Overall, the serotonin signaling might regulate the palatable sugar intake (Dus et al., 2011). The taste perception of carbohydrates might be modulated by internal state (Steck et al., 2018). So, there chould be a modulator for internal state like insulin (Baker and Thummel, 2007).

5.2 The IR60b Targeted Neurons Could Not Modulated by the 5-HT1B and 5-HT2B.

The IR60b neurons which project into the proximity distance to SE1 neurons (Hastenholz, 2018), specifically stimulated by sucrose intake even in the low concentrations (Joseph et al., 2017). The intake specificity could be the result of modulation of Sert3-GAL4 neuronal activation by IR60b taste neurons. So, possible serotonin receptor interacting with IR60b taste neurons was tried to understand by using 5-HT1B and 5HT2B receptors. 5-HT1B receptors promote food intake with the

negatively couple to adenylyl cyclase (Majeed et. al., 2016; Yuan et. al., 2005). 5-HT2B receptors positively linked to adenylyl cyclase (Yuan et al., 2005) therefore it limits food intake. Despite the fact that there was a decrease in the level of sucrose uptake for both receptors (Figure 4.5, Figure 4.6), the decrease was not significant. This might be because there might be other serotonin receptor that plays role in IR60b neuron interaction. So that, other receptors can be further analyzed.
Here, our findings demonstrated the 5-HT1B and 5-HT2B neurons are not responsible from to modulate the IR60b targeted neurons.

5.3 Insulin Signaling Regulates Palatable and Internal State based Sugar Intake

Our result showed that the downregulated insulin receptor signalling on Sert3-GAL4 targeted neurons could modulate both internal state of fly and palatable sugar intake in adult flies.
The developmentally down- and up-regulated insulin signalling showed the similar phenotype that reveals indirect regulation of insulin signalling on SE1, LP1 and IP neurons (Kastenholz, PhD thesis 2019). The palatable sugar intake could be sensed by developmentally enhanced serotonin intake (Figure 4.3). Developmentally diminished insulin signalling can regulate both metabolic state and the palatable food intake (Figure 4.7). The construct of *UAS-InRDN* was already confirmed as functional (Siegrist, et al., 2010). The insulin level and the insulin release fluctuate during the day (Malherbe et al., 1969; Jarrett et al., 1972). The neuronal level differences could be occurred based on insulin signalling to affect signaling strength,or modulators. So that, downstream elements as modifiers or regulatory molecules can be affected as ERp44.

5.4 The CG9911 is positive regulator for food intake in Sert3-GAL4 Targeted Neruons

The indirect regulation of serotonin transporter by insulin signaling was observed (Kastenholz, 2019). To understand how this regulation occurred, ERp44 ortholog CG9911 was used to find out the function of it in food intake. Sert and insulin is maturated by folding in the endoplasmic reticulum. The endoplasmic reticulum stress could be occurred with unproper folded proteins (Nyirimigabo et. al., 2019). To understand how the food intake is regulated by CG9911 function on SE1, LP1 and IP neurons, the ad-libitum and pre-starved CaFe assay used for 5% Sucrose, 5% Glucose, 5% Fructose and 5% Yeast. The carbohydrate intake was again limited by developmentally decreased CG9911 level as subset specific phenotype in both states (Figure 4.8; Table 5.1). The yeast intake alteration was not observed (Figure 4.8). The CG9911 protein could positively regulate carbohydrate intake.

Table 5.1 Limited carbohydrate uptake on LP1, SE1, and IP neurons

Targeted constructs	***UAS-SERT^{DN}***	***UAS-INR^{DN}***	***UAS-CG9911RNAi***
Pre-fed, 24h test			
Sucrose	decrease	decrease	decrease
Yeast	no effect	no effect	no effect
Glucose	Decrease	decrease	decrease
Fructose	decrease	decrease	decrease
Pre-starved, 3h test			
Sucrose	decrease	no effect	decrease
Yeast	No effect	no effect	no effect
Glucose	decrease	decrease	decrease
Fructose	decrease	decrease	decrease

Apart from that, the body weight of the experimental group was higher than the control groups (Figure 4.9). That might be because of the flies were not hungry to take the food, or there might be a possible developmental alteration for these flies. This needs

to be further analyzed by short time alteration of CG9911 on Sert3-GAL4 neurons by tubGAL80^{ts}.

So, the ERp44 could be the potential protein that limits carbohydrate intake on Sert activated subset specific serotonergic neurons by insulin signaling. So, the construct was tried to be verified.

Firstly, a different GAL4 driver was used to alter the CG9911 function: the Appl-GAL4 driver line. It targets the sensory neurons, mushroom body, antennal lobe, ventral nerve cord, Malpighian tubule, salivary gland and fat body (Legan et al.,2008; Haddadi et al., 2016). The 5% sucrose limited intake was confirmed with developmentally knockdown CG9911 protein. On Appl-GAL4 targeted neurons (Figure 4.10). The construct knockdown was tried to be understood with qPCR. However, there was no significant knockdown was observed after qPCR (Figure 4.11). That might be caused by several reasons. The protein knockdown level might not be understood by qPCR. Also, he construct might be unfunctional due to contaminated intron or construct is not there. The PCR was done with cDNA and gDNA of experimental and control groups based on primers targeting unaffected dsRNA hairpin structure (Figure 4.12) to destroy protein function (Figure 4.13). But the construct neither intron contaminated nor was not there. So, the second possibility could be because the primers were not sufficiently targeted to show knockdown level so it might be repeated with other designed primers. Thirdly, the CG9911 could be not responsible for gene expression, it might responsible for epigenetic regulation that could not be detected by qPCR. In addition to that, the Appl-GAL4 could not target the serotonergic neurons that might not regulate the food intake so that the knockdown expression level with this construct could not be measured. Tph-GAL4 that targets 26 serotonergic neurons per hemisphere (Park et al., 2006) including Sert3-GAL4 targeted neurons could be used to understand the knockdown expression level. Also, the neuroanatomical approach could be used to understand the effect of the construct on Sert3-GAL4 targeted neurons as changing the morphology of the serotonergic neurons, decrease the arborization or cause the decrease in neuronal survival. Finally, subcellular localized western blot could be performed to understand the location and function of the protein on serotonergic neurons.

Overall, our data indicated that CG9911 might be the potential protein, that is responsible for the food intake phenotype on subset specific neurons, SE1, LP1 and IP, through insulin signalling.

6. REFERENCES

Albin S. D., Kaun K. R., et al. (2015) A subset of serotonergic neurons evokes hunger in adult *Drosophila. Curr. Biol*. 21; 25 (18): 2435-40.

Alekseyenko O. V., Chan Y. B., et al. (2014) Single serotonergic neurons that modulate aggression in *Drosophila melonagaster. Curr. Biol*. Nov 17; 24(22): 2700-7

Amrein H., Thorne N (2005) Gustatory perception and behavior in *Drosophila melonagaster*. *Curr. Biol*. Sep 6; 15(17): R673-84.

Apostolatos A., Song S., et al. (2012) Insulin promotes neuronal survival via the alternatively spliced protein kinase CδII isoform. *J Biol Chem.* Mar 16; 287(12): 9299-9310.

Baker K. D., and Thummel C. S. (2007) Diabetic larvae and obese flies emerging studies of metabolism in *Drosophila. Cell Metab.* Oct; 6(4):257-66.

Balmert S (2016) Oppositional manipulation of serotonin transporter activity in the serotonergic nervous system and its effect on ethanol-related behavior in *Drosophila melanogaster.* Master thesis. University of Cologne.

Bay C. M. (1978) The control of enzyme secretion from fly salivary glands. *J Physiol.* Jan; 274: 421-435.

Becher P. G., et al. (2012) Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Behavioral ecology.* Vol 26, Issue 4. 822-828.

Becnel J., Johnson O., et al. (2011) The serotonin 5-HT7Dro receptor is expressed in the brain of Drosophila and is essential for normal courtship and mating. *PLoS One*. 6 (6): e20800.

Belfore A., Frasca F., Pandini G. (2009) Insulin receptor isoforms and insulin receptor/insulinlike growth factor receptor hybrids in physiology and disease. *Endocrine Reviews* 30 (6): 586-623.

Belgardt B. F., Okamura T., and Brüning J. C. (2009) Hormone and glucose signaling in POMC and AgRP neurons. J Physiol. Nov 15; 587 (Pt 22): 5305-5314.

Bernays E. A. (1985) The feeding and breeding sites of *Drosophila obscura* Fallen and *Drosophila subobscura* Collin. *J. nat. Hist.* 12, 137-151.

Boer P. H., and Hickey D. A. (1986) The-alpha amylase gene in *Drosophila melanogaster*: nucleotide sequence, gene structure and expression motifs. *Nucleic Acids Res.* 14:8399-8411.

Boyd F. T. J., Clarke D. W., Muther T. F., and Ralzada M. K. (1985) Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. *J Biol Chem.* 15; 260(29): 15880-4.

Brand A. H., and Perrimon N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development.* 118:401-415.

Brown L. M., Clegg D. J., Benolt S. C. , and Woods S. C. (2006) Intraventricular insulin and leptin reduce food intake and body weight in C57BL/6J mice. *Physiol. Behav.* Dec 30; 89(5): 687-91.

Buchon N., Osman D., et al. (2013) Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila. Cell Rep.* 3:1725-1738.

Cameron P., Hiroi M., et al (2010) The molecular basis for water taste in *Drosophila. Nature.* 105(44)16871-16875.

Chaudhari N., et al. (2014) A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. *Frontiers in Cellu Neurosci.* 8(213): 1-15

Cheng C. M., et al. (2003) Insulin-like growth factor 1 is essential for normal dendritic growth. *J Neurosci Res.* 73:1-9.

Chiu S. L., and Cline H. T. (2010) Insulin receptor signaling in the development of neuronal structure and function. *Neural dev.* 5:7.

Chyb S., et al. (2003) *Drosophila* Gr5a encodes a taste receptor tuned to trehalose. Proc. Natl Acad Sci USA. 100(Suppl 2)14526-142530.

Cohen R. W. (2001) Diet balancing in the cockroach rhyparobia madera: does serotonin regulate this behavior. *J Insect Behav.* 14:99-111.

Coleman J. A., Green E. M., Grouaux E. (2016) X Ray structures and mechanisms of the human serotonin transporter. *Nature.* 532(7599):334-9.

DahanukarA., Lel Y. T., Kwon J. Y., Carlson J. R. (2007) Two Gr genes underlie sugar reception in *Drosophila. Neuron.* 56:503-516.

Das G., et al. (2014) *Drosophila* leanrs opposing components of a compound food stimulus. *Curr Biol.* 24(15):1723-30.

Deutsch J. A., et al. (1989) Unlearned specific appetite for protein. *Physiology and Behavior.* 46:619-624.

Diegemann S., et al. (2017) The capillary feeder assay measures food intake in *Drosophila melanogaster. J Vis Exp.* Mar 17;(121)

Dus M., Min S., et al. (2011) Taste-independent detection of the caloric content of sugar in *Drosophila. Proc Natl Acad Sci USA.* 108(28): 11644-9.

El Khadawre T. (2017) Dissecting the function of serotonin transporter mutants (dSert) and their relationship to tyramine beta hydroxylase mutants in food intake. Bachelor thesis. University of Cologne.

Fabre A., Felton A. M. (2009) Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behavioral Ecology.* 20(4)1;685-690.

Fernandez R., et al. (1995) The *Drosophila* insulin receptor homolog: a gene essential for embryonic development encodes two receptor isoforms with different signaling potential. *EMBO J.* 14(14):3373-84.

Fernstrom J. D., Wurtman R. J. (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science.* 178(4059):414-6.

Figlewicz D. P., et al. (1994) Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res.* May 2;644(2):331-4.

Fujii S., et al. (2015) *Drosophila* sugar receptors in sweet taste perception, olfaction and internal nutrient sensing. *Curr Biol.* Mar 2; 25(5): 621-627.

Fujita M., Tanimura T. (2011) *Drosophila* evaluates and learns the nutritional value of sugars. *Curr. Biol.* May 10;21(9):751-5

Gade G., Auerswald L. (2003) Mode of action of neuropeptides from the adipokinetic hormone family. *Gen Comp Endoc.* 132:10-20.

Gasque G., Conway S., et al. (2013) Small molecule drug screening in *Drosophila* identifies the 5-HT2A receptor as a feeding modulation target. *Sci Rep;* 3:srep02120.

Gelperin A. (1972) Neural control systems underlying insect feeding behavior. *Am. Zool.* 12, 489-496.

Giang T., et al. (2011) The serotonin transporter expression in *Drosophila melanogaster. J Neurogen.* 25:17-26.

Gordon M. D., Scott K. (2009) Motor control in a *Drosophila* taste circuit. *Neuron.* Feb 12; 61(3): 373-84.

Gosby A. K., et al. (2011) Testing protein leverage in lean humans: a randomized controlled experimental study. *PLoS.* October 12h; 6(10): e25929.

Götz J. (2018) Investigation of food intake in *Drosophila melanogoster* with altered serotonin and octopamine concentrations. Bachelor thesis. University of Cologne.

Govind S., et al. (2001) Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin. *J Cell Biol.* 152:579-594.

Grangeteau C., et al. (2018) Yeast quality in juvenile diet affects *Drosophila melanogaster* adult life traits. *Sci Rep.* 2018 Aug 30; 8(1): 13070.

Griffioen-roose S., et al (2014) Human protein status modulates brain reward responses to food cues. *Am J. Clin Nutr*. Jul;100(1): 113-22.

Hardie D. G. (2011) AMP-activated protein kinase- an energy sensor that regulates all aspects of cell function. *Genes Dev.* 25, 1895-1908.

Hardie D. G. (2012) Organismal carbohydrate and lipid homeostasis. Cold Spring Harbor Laboratory Press. 4:a006031. DOI: 101101/cshperpect.a006031.

Haselton A. T., Fridell Y. W. (2010) Adult *Drosophila melanogaster* as a model for the study of glucose homeostasis. *Aging.* Aug; 2(8): 523-6.

He J. (2007) Dissecting neural mechanisms of appetitive behavior mediated by serotonergic neurons in the adult *Drosophila melanogaster*. PhD thesis. University of cologne.

Herb A. (2005) Herstellung von genetischen werkzeugen zur manipulation der serotoninkonentrationin der fliege *Drosophila melanogaster*. Diploma thesis. University of Wuerzburg.

Huetteroth W., et al. (2015) Sweet taste and nutrient value subdivide rewarding dopaminergic neurons in *Drosophila. Curr Biol.* 25 (6), 751-758. DOI: 10.1016/j.cub.2015.01.036

Illius A. W., Tolkamp B. J., and Yearsley J. (2002) The evolution of the control of food intake. *Proceedings of the Nutrition Society*. 61, 465-472. DOI: 10.1079/PNS2002179.

Itskov P. M., Riverio C. (2013) The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in *Drosophila. Frontiers in Neuroscience.* 7:12

Ja W. W., et al. (2007) Prandiology of *Drosophila* and the CaFe assay. *Proc Natl Acad Sci USA.* May 15;104(20): 8253-6.

Jauch-chara, et al. (2012) Intranasal insulin suppress food intake via enhancement of brain energy levels in humans. *Diabetes.* Sep; 61(9): 2261-8.

Jiao Y., Moon S. J., Montell C. (2007) A *Drosophila* gustatory receptor required for the responses to sucrose, glucose, and maltose identified by mRNA tagging. *Proc Natl Acad Sci USA.* 104:14110-14115.

Johnson O., Becnel J., Nichols C. D. (2011) Serotonin receptor activity is necessary for olfactory learning and memory in *Drosophila melanogaster. Neurosience.* Sep 29; 192: 372-81.

Joseph R M., Sun J. S., et al. (2017) A receptor and neuron that activate a circuit limiting sucrose consumption. *eLife*. Mar 23;6 pii:e24992.

Kaiser A (2009) Einfluss von veranderter dSert function auf alcohol-induziertes verhalten bei *Drosophila melanogaster*. Diploma thesis. University of Cologne.

Koh T. W., et al (2014) The *Drosophila* IR20a clade of ionotropic receptors are candidate taste and pheromone receptros. *Neuron.* Aug 20; 83(4): 850-865.

Könner A. C., et al. (2011) Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell Metab.* Jun 8; 13(&): 720-8.

Lee Y., Moon S. J., Montell C. (2009) Multiple gustatory receptors required for the caffeine response in *Drosophila. Proc Natl Acad Sci USA.* Mar 17; 106(11): 4495-500.

Lee P. T., et al. (2011) Serotonin-mushroom body circuit modulating the formation of anesthesia-resistant memory in *Drosophila. Proc Natl Acad Sci USA.* Aug 16; 108(33): 13794-9.

Lee K. P. (2015) Dietary protein: carbohydrate balance is a critical modulator of lifespan and reproduction in *Drosophila melanogaster*: a test using a chemicall defined diet. *J Insect Physiol.* Apr; 75:12-9.

Lemaitre B., Miguel-Aliaga I. (2013) The digestive tract of *Drosophila melanogaster. Annu Rev Genet.* 47:377-404.

Li Y., et al (2014) GDM-associated insulin deficiency hinders the dissociation of Sert from ERp44 and down-regulates the placental 5-HT uptake. *PNAS.* DOI:10.1073/pnas.1416675112.

Li Y., et al. (2016)Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila. Sci Rep.* 6:35359.

Loh K., et al. (2017) Insulin controls food intake and energy balance via NPY neurons. *Mol Metab*. 6 (6). 574-584. DOI: 10.1015/j.molmet.2017.03.013

Luo J., Becnel J., Nichols C. D., Nassel D. R. (2012) Insulin producing cells in the brain of adult *Drosophila* are regulated by the serotonin 5-HT1A receptor. *Cell Mol Life Sci.* Feb; 69(3): 471-84.

Masek P., Keene A. C. (2013) *Drosophila* fatty acid taste signals through the PLC pathway in sugar sensing neurons. *PLoS Genet.* 9(9): e1003710.

McKenzie J. A., Parsons P. A. (1972) Alcohol tolerance: an ecological parameter in the relative success of *Drosophila melanogaster* and *Drosophila simulans. Oecologia*. 10, 373-383.

Miguel-Aliaga I., Thor S., Gould A. P. (2008) Postmitotic specification of *Drosophila* insulinergic neurons from pioneer neurons. *PLoS Biol.* 6(3): e58.

Minton E. A., and Khale L. R. (2014) Belief systems, religion, and behavioral economics. *New York: Business Expert Press LLC.*

Montell C (2009) A taste of *Drosophila* gustatory receptors. *Curr. Opin. Neurobiol.* Aug; 19(4): 345-353.

Nassel D. R., Vanden Broeck J. (2016) Insulin/IGF signaling in *Droophila* and other insects: factors that regulate production, release and post-release action of the insulin-like peptides. *Cell Mol Life Sci.* Jan; 73(2): 271-90.

Neumann J. and Morgenstern O. (1944) Theory of games and economic taste. *Princeton Univ. Press*

Nichols D. E., Nichols C. D. (2008) Serotonin receptors. *Chem Rev.* May; 108(5): 1614-1641.

Nyirimigabo E., et al. (2019) The role of ERp44 in glucose and lipid metabolism. *Elsevier.* 671(2019) 175-184.

Ogueta M., et al. (2010) The influence of Adh function on ethanol preference and tolerance in adult *Drosophila melanogaster. Chem Senses*. 35: 813-822.

Ohtsu T., Kimura M. T., Hori S. H. (1992) Energy storage during reproductive dispause in the *Drosophila melanogaster* species group. *J comp physio B*. 162: 203-208.

Olds W. H., and Xu T. (2014) Regulation of food intake by mechanosensory ion channels in enteric neurons. *eLife.* Oct 6;3.

Ong C., Yung L. L., et al. (2014) Drosohila melanogaster as a model organism to study nanotoxicity. *Nanotoxicology.* 9(3), 396-403. DOI: 10.3109/17435390.2014.940405.

Owald D., et al. (2010) A Syd-1 homologue regulates pre- and postsynaptic maturation in *Drosophila. J Cell Biol*. Feb 22; 188(4): 565-79.

Oz M., Libby T., et al. (2010) Realtime, spatially resolved analysis of serotonin transporter activity and regulation using the fluorescent substrate, ASP+. *Journal of Neurochemistry.* 114, 1019-1029.

Pang X., et al. (2018) ERp44 depletion exacerbates ER stress and aggravates diabetic nephropathy in db/db mice. *Elsevier.* 504 (2018):921-926.

Park J., et al. (2006) Mitochondrial dysfunction In *Drosophila* PINK1 mutants is complemented by parkin. *Nature.* 29; 441(7097): 1157-1161.

Piper M. D., Blanc E., Leitao-Gonçalves R., Yang M., He X, et al. (2014) A holidic medium for *Drosophila melanogaster. Nat Methods.* Jan;11(1): 100-

Pool A.-H., Scott K. (2014) Feeding regulation in *Drosophila. Current opinion in neurobiology.* 29, 57-63. DOI:10.1016/j.conb.2014.05.008

Pooryasin A., Fiala A. (2015) Identified serotonin-releasing neurons induce behavioral quiescence and suppress mating in *Drosophila. J Neurosci.* Sep 16;35(37): 12792-812.

Qi W., Yang Z., Lin Z., Park J., Suh G. S. B., Wang L (2015) A quantitative feeding assay in adult *Drosophila* reveals rapid modulation of food ingestion by its nutritional value. *Mol Brain.* 8;87.

Qian Y., et al. (2017) Sleep homeostasis regulated by 5HT2B receptor in a small subset of neurons in the dorsal fan shaped-body of *Drosophila. eLife*. 6:e26519.

Rahul D., Dobens L. L. (2015) Conservation of gene and tissue networks regulating insulin signaling in flies and vertebrates. *Biochemical Society Transactions.* Oct 09; 43(5): 1057-1062.

Ramamoorthy S., Samuvel D. J., Buck E. R., Rudnick G., Jayanthi L. D. (2007) Phosphorylation of threonine residue is required for acute regulation of serotonin transporter by cyclic GMP. *J Biol Chem.* 282, 11639-11647.

Ramamoorthy S., Shippenberg T. S., Jayanthi L. D. (2010) Regulation of monoamine transporters: role of transporter phosphorylation. *Pharmacol Ther.* 129, 220-238.

Root C. M., Ko K. I., et al. (2011) Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. Apr 1; 145(1): 133-44.

Rudnick G (2006) Serotonin transporters-structure and function. *J Membr Biol.* 213(2): 101-110.

Rudnick G., Kramer R., Verrey F. (2013) The SLC6 transporters: perspectives on structure, functions, regulation, and models for transporter dysfunction. *Pflüfers Archiv-European Journal of Physiology*. 466,25.

Rulifson E. J., Kim S. K., Nusse R. (2002) Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science*. May 10; 296(5570): 1118-20.

Saudou F., Boschert U., Amlaiky N., Plassat J. L., Hen R. (1992) A family of *Drosophila* serotonin receptors with distinct intracellular signaling properties and expression patterns. *EMBO J.* Jan; 11(1): 7-17.

Scott K. (2005) Taste recognition: food for thought. *Neuron*. Nov 3; 48(3): 455-64.

Silva B., Goles N. I., Varas R., Campusano J. M. (2014) Serotonin receptors expressed in *Drosophila* mushroom bodies differentially modulate larval locomotion. *PLoS One*. Feb 25; 9(2): e89641.

Sitaraman D., LaFErriere H., Birman S., and Pages T. Z. (2012) Serotonin is critical for rewarded olfactory short-term memory in *Drosophila*. *Journal of Neurogenetics*. Jun; 26(2): 238-44.

Slone J., Daniels J., Amrein H (2007) Sugar receptors in *Drosophila. Curr Biol*.Oct 23; 17(20): 1809-1826.

Sohn J. W. (2015) Network of hypothalamic neurons that control appetite. *BMB Rep.* Apr; 48(4): 229-233.

Sorensen A., Mayntz D., Raubenheimer D., Simpson S. J. (2008) Protein-leverage in *mice*: the geometry of macronutrient balancing and consequences for fat deposition. *Obesity Res Jour.* 16(3): 5666-571.

Stafford J W., et al. (2012) Integration of taste and calorie sensing in *Drosophila*. *The Journal of Neuroscience.* 32 (42), 14767-14774. DOI: 10.1523/JNEUROSCI.1887-12.2012

Steck K., Walker S. J., et al. (2018) Internal amino acid state modulates yeast taste neurons to support protein homeostasis in *Drosophila*. 1eLife 7:e31625.

Stewart S., Koh T. W., Ghosh A. C., Carlson J. R. (2015) Candidate ionotropic taste receptors in the *Drosophila* larva. *Proc Natl Acad Sci USA.* Apr 7; 112(14): 4195-4201.

Sutton S. R., and Barto A. G. (1998) Reinforcement and learning: an introduction. *MIT Press*

Tolman E. C. (1948) Cognitive maps in rats and men. *Psychol. Rev.* 55. 189-208.

Valles A. M., White K. (1988) Serotonin-containing neurons in *Drosophila melanogaster*: development and distribution. *J Comp Neurol.* 268: 414-428.

Vargas M. A., Luo N., Yamaguchi A., Kapahi P. (2010)A role for s6 kinase and serotonin in postmating dietary switch and balance of nutrients in *D. melanogaster. Curr Biol Jun.* 8;20(11): 1006-11.

Vleguels R., Verlinden H., Broeck J. V. (2014) Serotonin, serotonin receptors and their actions in insects. *Neurotransmitter*. 2:e314.

Wan Q., et al. (1997) Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. *Nature*. 3888: 686-690.

Watanabe S., et al. (2017) Structural basis of pH-dependent client binding by ERp44, a key regulator of protein secretion at the ER-Golgi interface. *PNAS.* DOI:10.1073.

Who (World Health Organization) (2000) Obesity: preventing and managing the global epidemic. Geneva, Switzerland: *World Health Organization*.

Williams K. W., Margatho L. O., Lee C. E., et al. (2010) Segregation of acute leptin and insulin effects in distinct populations of arcuate proopioelanocortin neurons. *J Neurosci.* Feb 17; 30(7): 2742-9.

Whitworth E. J., Hinson J. P., Vinson G. P. (2002) Neuropeptides and adrenocortical proliferation in vitro. *Endocr Res*. Nov; 28(4): 677-81.

Wong D. T., Perry K. W., Bymaster F. P. (2005) Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat Rev Drug Discov.* Sep; 4(9): 764-74.

Wood S. C., Lotter E. C., McKay L. D., Porte D. Jr. (1979) Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature*. Nov 29; 282 (5738): 503-5.

Wu Q., Zhang Y., Xu J., and Shen P. (2005) Regulation of hunger driven behaviors by neural ribosomal S6 kinase in *Drosophila. Proc Natl., Acad. Sci. UAS. Sep 13*; 102(37): 13289-13294

Xu L., He J. Z., et al. (2016) A signle pair of serotonergic neurons counteracts serotonergic inhibition of ethanol attraction in *Drosophila. PLoS One.* 11(12): e0167518.

Xu L (2013) Two opposing serotonergic neuronal circuits modulate ethanol preference of *Drosophila melanogaster*. PhD thesis. Univerwsity of Cologne.

Yarmolinsky D. A., Zucker C. S., Ryba N. J. P (2009) Review: Common sense about taste: From mammals to insects. *Cell.* 139 (2), 234-244.

Zahniser N. R., Doolen S. (2001) Chronic and acute regulation of Na+/Cl- dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. *Pharmacol Ther* Oct; 92(1): 21-55.

Zhang Y., Xi Y. (2015) Fat body development and its function in energy storage and nutrient sensing in *Drosophila melanogaster. J Tissue Sci Eng* 6;1.

Zhang Y.W., Gesmonde J., Ramamoorthy S., Rudnick G. (2007) Serotonin transporter phosphorylation by cGMP-dependent protein kinase is altered by mutation associated with obsessive compulsive disorder. *J. Neurosci.* 27, 10878-10886.

Zhu J., Park K. C., Baker T. C. (2003) Identification of odors from overripe mango that attract vinegar flies, *Drosophila melanogaster. Journal of Chemical Ecology.* 29: 899-909.

CURRICULUM VITAE

PERSONAL DETAILS

- **Name-Surname:** Merve TORUN
- **Phone:** +90 553 590 35 19
- **E-mail:** mervetorun94@gmail.com/
- **Birth Date:** 28/12/1994
- **Address:** Gazi mahallesi 20 Sokak Numara:1 Kat:2 D:8
  35410/ Gaziemir/İzmir/Turkey

EDUCATION

- **2017-Present:** International Biomedicine and Genome Institute/İzmir/ Molecular Biology and Genetics (M.Sc.).
- **2013-2017:** Izmir Institute of Technology/ Undergraduate-Molecular Biology and Genetics (B.Sc.)
- **2012-2013:** Izmir Institute of Technology/ Prep School (English)
- **2008-2012:** Gaziemir High School (As best ranking student)

BASIC MOLECULAR BIOLOGY TECHNIQUES APPLIED EXTENSIVELY

- **Molecular Biology Laboratory (IZTECH-Lab Course):** DNA extraction, plasmid DNA preparation, restriction enzyme digests, PCR, agarose gel electrophoresis, purification, cell fractionation by differential centrifugation, spectrophotometer, hemocytometer, colony PCR, competent cell preparation, RT PCR, protein isolation, RNA isolation, and Western Blotting.
- **Microbiology Laboratory (IZTECH-Lab Course):** aseptic and sterile techniques, optical microscopy, bacterial staining, plating methods (streak, spread, pour, replica), enumeration and identification of bacteria, use of biological safety cabinets, media and buffer preparation, water sample analysis by membrane filtration, use of selective media, antibiotic assay (MIC), usage of hoods
- **Cell Biology Laboratory (IZTECH-Lab Course):** Basic cell culture techniques
- **Computer skills:** C programming, JAVA, KNIME platform usage and also extensive use of Word, Excel, Access and PowerPoint.
- **Biochemistry Laboratory (IZTECH-Lab Course):** Protein purification, Buffer and Solution preparation, Bradford Assay, affinity chromatography and size exclusion chromatography, SDS-PAGE gel electrophoresis, RNA Isolation, TLC chromatography.
- **Research Skills:**
  - **Noncoding RNA Laboratory (Asst. Prof. Dr. Bünyamin Akgül's Laboratory):** Competent cell preparation, transformation, transfection, basic cloning steps (as DNA isolation, TA cloning, and gel extraction), qPCR, primer designing, phenotype observation, basic cell culture techniques and basic flow cytometer usage.
  - **Bioinformatics Laboratory (Asst. Dr. Alessio Zippo's Laboratory):** stem cell culture techniques, cloning techniques, JAVA learning.

- **Molecular Microbiology (Prof. Dr. Gerhard Braus' Laboratory):** cloning techniques, Southern Blotting, Transfection, yeast plating techniques, phenotype analysis under SEM.
- **Immunology Laboratory (Asst. Prof. Dr. Ayten Nalbant's Laboratory):** PMBC isolation, Western Blotting, T cell sorting,
- **Cancer Laboratory (Asso. Prof. Dr. Ralph Meuwissen's Laboratory):** Cell Culture Techniques, Western Blotting, Flow Cytometer usage, IHC staining, PDX modelling, XTT asay, Immunoflourescence staining.
- **Neurophysiology Laboratory (Prof. Dr Henrike Scholz):** IHC, Brain, VNC, and Crop dissection of Drosophila Melanogaster, PCR, Agarose Gel Running

SEMINARS, COURSES & CONGRESS

- -Bioinformatics Course in Middle East Technical University 2014- Ankara, Turkey
- -Turkey Symposium Series: Catalysis and Sensing for Health (IZTECH) 2015-Izmir, Turkey
- -IV. International Congress of the Molecular Biology Association of Turkey 2015- Ankara, Turkey
- -II. Biology Congress in 2017-Kayseri, Turkey
- -11th Aykut Kence Evolution Congress in 2017- Ankara, Turkey
- Certificate of experimental animal course, Dokuz Eylül University, 2017, İzmir, Turkey

LANGUAGE

- **Turkish:** Advanced
- **English:** Upper-Intermediate
- **Japanese:** Pre-Intermediate

CLUBS and MEMBERSHIPS

- YABITO (was **chairman** from August, 2014 to November, 2015 and **editor** of club from November, 2015 to present)
- TEMA (membership from 2016 to 2017)
- IYTE HayDos (membership from September, 2012 to July, 2017)

HOBBIES and ACTIVITIES

- Rafting
- Zip line
- Making Ceramic
- Painting
- Volleyball (was found as registered player in Gaziemir Municipality from 2002 to 2011)
- Basketball (was found as player in EFES from 2008 to 2010)

REFERENCES

1. Assoc. Dr. Duygu SAĞ WİNGENDER

Izmir Biomedicine and Genome Institute

Basic and Translational Research Program

Balçova/Izmir/Turkey

Phone: +902322994100 (5021)

E-mail: duygu.sag@ibg.edu.tr

2. Prof Dr. Vasfi Karatosun

Department of Medicine

Dokuz Eylül University

Balçova/İzmir/Turkey

Phone: +90 232 422 67 67

E-mail: vasfi.karatosun@gmail.com

3. Prof. Dr Bünyamin AKGÜL

Department of Molecular Biology and Genetics

Izmir Institute of Technology

Urla/İzmir/Turkey

Phone: +90 232 750 73 16

E-mail: bunyaminakgul@iyte.edu.tr

4. Prof. Dr. Köksal Alptekin

Department of Medicine

Dokuz Eylül University

Balçova/İzmir/Turkey

Phone: +90 232 422 42 52

E-mail: koksal.alptekin@deu.edu.tr

5. Prof. Dr. Gerhard BRAUS

Georg August University Göttingen

Institute for Microbiology and Genetics Department of Molecular Microbiology and Genetics

Grisebachstr. 8 37077 Göttingen/Germany

Phone: +49- (0) 551-39 33771

E-mail: gbraus@gwdg.de

6. Prof. Dr. Anne FRARY

Department of Molecular Biology and Genetics

Izmir Institute of Technology

Urla/İzmir/Turkey

Phone: +90 232 750 73 05

E.mail:annefrary@iyte.edu.tr