

The elusive sixth sense – lipid perception in *Drosophila*

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Abstract

Sense of taste is instructive for food perception and is encoded by five principle sensory inputs: sweet, bitter, salty, sour, and umami. However, there is mounting evidence that dietary fat is also palatable, and that many species including humans are able to discriminate the quality of free fatty acids in dedicated experimental environments. Multiple lipid-taste receptors are suggested to be capable of binding subsets of fatty acids and initiating cellular signals in specific sensory cells. Nevertheless, the structure and composition of dietary lipids is complex by nature and food consists of many other tasty macronutrients. To date, it remains an open question if or how dietary lipids induce taste sensation instructive for the feeding behavior of animals.

We recorded the feeding behavior of adult *Drosophila melanogaster* utilizing different established feeding assays. Using lipid defined diets and the yeast strain BY4741, we found that flies are able to detect the fungal sterol ergosterol and specific saturated fatty acid species, the latter bound in more complex nutritional lipids. Moreover, we show that the neuronal expression of the CD36-member SNMP1 together with the neuronal insulin signaling activity are essential to define the perception of one selected tasty lipid. We provide evidence that the sugar and protein content in the food is instrumental to modulate insulin signaling, and propose that the sugar:protein ratio is one determining factor instructive for the feeding response of fruit flies to given dietary lipid profiles.

Introduction

One way to advertise and sell industrially processed food is to elevate olfactory and gustatory cues known to attract consumers. Conventional product design strategies aim to address the five commonly known taste sensations: bitter, sweet, salty, sour and umami (savory). Especially the extensive use of artificial sweeteners (eg. Sucralose) or amino acids (eg. Glutamate) aims to create a positive experience or mild addiction after consumption of food products. Many diets include substantial amounts of fat, and it is of no secret that different ratios between sugars and dietary lipids improves the perceived quality of sweet dishes.

There is mounting evidence that animals and humans are able to taste the quality of dietary lipids. For instance, fruit flies and mice are able to discriminate provided free fatty acids added to water¹⁻³. Receptors belonging to the CD36 protein families are identified in rodent taste bud cells^{4,5} and genetic

ablation of CD36 result in mice with impaired fatty acid perception^{6,7}. Insects encode CD36 proteins⁸. In *Drosophila melanogaster*, CD36 is known to have a role in pheromone perception^{9,10}.

Wild *Drosophila* feed on rotting fruit, a composite diet that consists of plant material and microbes. It was shown that many different microbial species are associated with flies^{11,12}. However, it remains open, if or how wild fruit flies would select optimal food in rotting plant materials infested by a wide variety of microbes. In the laboratory microbial communities are strongly reduced^{13,14} and the yeast *Saccharomyces cerevisiae* is used as a principal food resource. *Saccharomyces* lipid production is well understood and it was shown that the lipid composition of these yeast species changes with cultivation temperatures^{15,16}. For instance, at low temperatures yeast produce large lipid droplets with high triacylglyceride (TAG) yields¹⁷. Optimal cultivation results in yeast with rather small lipid droplets and low TAG levels¹⁶. Many yeasts have a similar optimal temperature range to flies and therefore, share the same biotope. However, feeding on yeast or plant material does not provide fruit flies immediately with free fatty acids and ingested dietary lipids belong mainly to membrane lipids, such as sterols or glycerophospholipids, or storage lipids. In mammals is the presence of oral lipases reported but their contribution in the oral degradation of dietary lipids remains speculative at best¹⁸. Only later, in the intestine dietary lipids are degraded into fatty acids and sterols¹⁹. Thus, potential lipid taste receptors need to detect fatty acids or sterols bound in more complex lipids.

It was shown that dietary lipids directly modulate the insulin production in vertebrates and invertebrates^{20,21}. Systemic insulin signaling relies on insulin levels in circulation and the presence of the insulin receptor (InR) on respective cell types. In *Drosophila*, insulin producing cells (IPCs) are localized in the brain. IPCs in mature adult flies produce three insulin-like peptides (DILPs) which secrete their hormones into circulation²² but also target distinct regions in the brain. One CNS region with IPC projections is the subesophageal ganglion (SEG) known to regulate the feeding behavior of flies^{23,24}. The SEG region is also targeted by gustatory neurons^{25,26}, and it was shown that some gustatory neurons express metabolic receptors, such as the insulin receptor²⁷⁻³⁰. The cellular insulin signal cascade is functionally and structurally conserved³¹. Two central enzymes in the cascade are the two kinases MAPK and Akt. MAPK is instructive for the 'mitogenic' signaling, ultimately modulating katabolic turnover³². Akt regulates the 'metabolic' signaling branch required to control the cellular uptake rate of sugars and anabolic turnover. *Drosophila* rely on one dAkt gene and produces three different protein isoforms^{17,33,34}. Although the precise role of the dAkt¹²⁰ is not understood, the phosphorylation state of dAkt⁶⁶ and dAkt⁸⁵ responds directly to dietary signals^{17,23,33}.

It was shown that *Drosophila* demonstrate temperature-dependent feeding behavior^{35,36}. Low temperatures facilitate a behavioral switch and *Drosophila* avoid feeding on yeast while preferring plant material^{35,36}. Phytosterols are different from fungal counterparts and give rise to another set of steroid hormones³⁷. Plant fatty acids are rather long and unsaturated^{38,39}. Thus, *Drosophila* kept on plant food adopt a very different lipid profile with respect to yeast fed animals. The ingestion of plant material improves the cold response of flies, results in downregulation of the Insulin signal cascade and ensures survival^{20,21,35,40}.

Other than dietary lipids, sugars and protein yields are known to modulate insulin signaling^{21,41,42}. In addition, sugar and protein ratios modulate the feeding behavior of adult flies^{43,44} and can drive physiological changes⁴⁵⁻⁴⁷. It remains largely unclear how strong protein/sugar ratios vary in seasonal microbe infested rotting fruits or similar *Drosophila* diets. One way, flies possibly could identify optimal food is through the perception of dietary lipids.

Here we show that flies are able to taste dietary lipids supplemented to sugar and protein rich food. We identified the neuronal expression of the CD36 protein family member SMNP1 as one molecular key

to lipid detection. We found that SMNP1 is likely not an exclusive taste receptor for TAGs or the fungal sterol ergosterol. Instead, we suggest that neuronal SMNP1 modulates the perception of lipids regulating neuronal fatty acid traffic. In addition, we demonstrate that lipid taste is directly linked to the activity of neuronal insulin signaling. We propose that the extent and quality of the lipid taste is modulated by present dietary protein/sugar ratios, which regulate the intensity of the insulin response. We speculate that dietary lipid perception could be one way for fruit flies to select optimal food in response to environmental challenges, such as temperature.

Results

Flies discriminate between dietary lipid species

Dietary fats comprise fatty acids and/or sterols. Whereas dietary sterols are directly accessible, fatty acids are bound in more complex lipids. To test if adult flies discriminate between fatty acids or sterols, we measured the consumption of lipid-free food (YAF) supplemented with different synthetic lipids. In our assay flies were given the choice between lipid-free YAF and additional YAF diets supplemented with one candidate lipid at three different concentrations. However, even the highest tested lipid yield in our assay represented likely only a fraction of the same lipid in yeast or other food types^{48,49}. At first, we assessed the feeding response of flies to ergosterol, the main yeast sterol. We found that *Drosophila* are repelled by low, but not high concentrations of ergosterol (Figure 1A). To test if fruit flies are able to detect other sterol species, we repeated the experiment with the phytosterol stigmasterol. Stigmasterol did not induce any directed feeding behavior indicating that *Drosophila* do not taste all sterols (Figure 1B). Another important dietary lipid class are triacylglycerides (TAG). TAGs are formed by three fatty acids bound to a glycerol backbone, and are mainly found stored in cellular lipid droplets. Dietary TAGs are less toxic compared to respective free fatty acids⁵⁰, but are efficiently degraded in the intestine. We tested the feeding response of flies to YAF supplemented with different TAG species composed from fatty acids including octanoic acid (TAG^{C24:0}), capric acid (TAG^{C30:0}), lauric acid (TAG^{C36:0}), myristic acid (TAG^{C42:0}), palmitic acid (TAG^{C48:0}), palmitoleic acid (TAG^{C48:3}) and stearic acid (TAG^{C54:0}). Food supplemented with TAG^{C24:0} or TAG^{C54:0} repels flies if compared to lipid free food baits (Figure 1C,D). In contrast, TAG^{C42:0} is somewhat attractive to adult flies (Figure 1E) and *Drosophila* show indifferent feeding behavior to YAF supplemented with TAG^{C30:0}, TAG^{C36:0}, TAG^{C48:0} or TAG^{C48:3} (Figure 1F and Figure S1A, B).

Taken together, adult flies are able to taste individual lipids. However, it remains open if flies can taste lipid species in real food that has typically a complex lipid mixture.

Neuronal CD36 is essential to taste TAGs

Our next aim was to identify the potential receptor responsible to detect dietary TAGs. In vertebrates, evidence is accumulating that members of the CD36 protein family expressed in lingual taste buds of rodents are responsible for fatty acid detection⁷. In *Drosophila*, the CD36 family member SMNP1 represents a suitable candidate involved in taste regulation. To validate the involvement of SMNP1 in lipid detection we decided to knock out this protein in all neurons (SMNP1^{KD}). First, we tested SMNP1^{KD} flies on YAF supplemented with ergosterol. We found that food supplemented with ergosterol shows no changes in the feeding behavior of SMNP1^{KD} (Figure 2A). Thus, the neuronal presence of SMNP1 is modulating sterol perception. Next, we decided to test the response of flies to TAG^{C42:0} and TAG^{C54:0}. We found that SMNP1^{KD} are unable to detect TAG^{C42:0} (Figure 2B). However, TAG^{C54:0} food is attractive to SMNP1^{KD} flies - a result contrasting our findings with wild type animals (Figure 1D,2C). We deem it unlikely that SMNP1 represents a lipid receptor itself because SMNP1^{KD} are able to taste dietary fats.

We conclude that neuronal SMNP1 is one regulating element in the perception of dietary lipids in adult *Drosophila*. Nevertheless, the fact that SMNP1 knock outs taste lipids points to other molecular machineries required to modulate lipid taste-dependent feeding behavior.

Flies discriminate yeast cultured at different temperatures

Genetically identical yeast cells can produce different lipidomes in response to changing temperatures. To validate if and to what extent the tested lipid species are present in *Saccharomyces cerevisiae*, we decided to analyze the lipidome of the wild type strain BY4741 cultured in lipid-free SD media at 10 (yeast^{10°C}) or 30°C (yeast^{30°C}) by mass spectrometry. Although we were technically not able to measure ergosterol levels directly, we did obtain data for ergosterol-esters and in addition, used thin layer chromatography to show yeast sterols. Like reported for yeast cultured at different temperatures¹⁶, yeast^{10°C} produce more sterols, sterol-esters and TAGs if compared to yeast^{30°C} (Figure S1C, D, E). In addition, we found that yeast produce mainly TAG^{C48} and TAG^{C50} (Figure S1F). TAG^{C42} or TAG^{C54} are detectable in yeast^{10°C} and yeast^{30°C} (Figure S1F). It is certainly interesting if flies are able to detect these lipids, however, respective yeast mutants are not available.

To assess if flies are able to discriminate between yeast cultured at 10 or 30°C, we decided to track their feeding behavior. We allowed adult flies to choose between both yeast qualities at 20°C (Figure 3A) and found that flies show a preference for the yeast^{30°C} (Figure 3B). To verify if volatile yeast^{30°C} cues attract flies, we repeated the experiment with smell defective *orb83b* mutants^{35,51}. In contrast to wild type flies, *orb83b* do not prefer yeast^{30°C} confirming that olfactory cues are instructive for food choice decisions of fruit flies (Figure 3C). Wild flies feed on rotting fruits that produce a multitude of different volatiles cues. To orient themselves, the taste of lipids could help flies to identify optimal food resources. To assess whether flies also taste dietary yeast lipids, we extracted hydrophobic compounds from our yeast cultures and supplemented defined lipid-free food with the respective lipid extracts (YAF^{10°C} or YAF^{30°C}). We found that wild type *Drosophila* do not discriminate between YAF^{10°C} and YAF^{30°C} (Figure 3D). To test if SMNP1^{KD} show a similar behavior, we repeated our food choice assay with YAF^{10°C} and YAF^{30°C}. Surprisingly, we found that SMNP1^{KD} flies show directed feeding behavior and prefer YAF^{10°C} (Figure 3E). Thus, SMNP1 is essential to maintain the sensitivity of flies to dietary lipids possibly preventing *Drosophila* from selecting unfavorable food.

We wondered, if wrong lipid perception could be of any consequence for flies. Adult females tend to position their eggs close to available food sources⁵² and hatched larvae are not able to discriminate food at different temperatures³⁵. Therefore, we decided to track individual larval development on YF prepared from our yeast cultures³⁹. Interestingly, larval development at comfortable temperatures was severely reduced on both food types (Figure S2A) contrasting results published earlier with YF prepared from undefined yeast purchased in local grocery stores³⁵ or YF made from BY4741 grown at 20°C^{17,39}. We have also shown earlier that larval pupation rate was compromised on undefined yeast food in cold environments³⁵. Thus, we speculated that larvae will not survive on YF^{10°C} or YF^{30°C} kept at 14°C. Interestingly, at 14°C a high percentage of larvae kept on YF^{30°C} reached pupariation faster compared to YF^{10°C} fed siblings (Figure S2B-D).

Taken together, our results confirm the dominant role of olfactory cues in food choice decisions at comfortable temperatures. However, we showed the taste-modulating role for neuronal SMNP1 in the perception of dietary lipids with complex lipid mixtures.

Systemic insulin signaling levels modulate lipid taste perception

Insulin signaling (IS) is implicated to regulate the functionality of CD36. It was shown, that high IS levels re-localize cellular CD36⁵³. To test if yeast^{10°C} or yeast^{30°C} food modulate IS levels, we probed

the phosphorylation state of Akt and MAPK indicating the activity of both enzymes. We found at 20°C that MAPK phosphorylation levels are higher in flies kept on yeast^{30°C} compared to siblings kept on yeast^{10°C} (Figure 4A). In addition, we found that phosphorylation specifically at the Akt^{SER505} but not at the Akt^{THR342} site were increased (Figure 4B, C and Figure S3A, B). Yeast^{10°C} do not differ significantly in their protein yields from yeast^{30°C}, but do contain more sugars (Figure S3C, D). To validate if proteins or sugars may elevate IS in flies, we fed adult with either glucose or soy peptone. We found that the protein component is more potent to activate cellular IS (Figure S3E-G). To assess if flies fed with glucose have a different lipid perception than flies kept on peptone, we measured the feeding behavior in response to glucose or peptone food supplemented with TAG^{C42:0}. On glucose we see no statistically validated feeding response of flies to TAG^{C42:0} (YAF vs 100µg/ml TAG^{C42:0} p-value = 0.23; test ANOVA) (Figure 4C). In contrast, flies are repelled to lipid supplemented peptone, albeit only at low lipid concentrations (Figure 4D).

Our results show that sugars and proteins activate insulin signaling at different rates in flies, and suggest a critical role of insulin signaling in the perception of lipids. We speculate that given protein/sugar ratios likely instruct insulin signaling levels and that way, modulate the lipid taste perception of flies.

Neuronal insulin signaling is essential for lipid taste

To confirm that IS is essential to taste dietary lipids, we decided to screen insulin mutants and measure their feeding preference for TAG^{C42:0}. In adult flies, insulin producing cells secrete the insulin-like peptides Dilp2,3 and 5. All three respective *Δdllp* mutants are viable and fertile²². Interestingly, we found that none of the *Δdllp* mutants is attracted by food supplemented with TAG^{C42:0} (Figure 5A, B, C). However, Dilps are systemic hormones and the loss of either could impair systemic IS. Thus, we decided to probe for the neuronal expression of active insulin receptors (InR) in the SEG region – one synaptic target region for IPC projections. We probed the CNS of adult females with antibodies directed against phosphorylated InRs. To control for the specificity of the probe we knocked down InRs in all neurons (*rab3>InR^{RNAi}*) or glia (*repo>InR^{RNAi}*) expressing dsRNAi (InR^{KD}). Neuronal InR^{KD} did not allow the detection of activated InR, but glial InR^{KD} did not affect InR localization in the SEG region with respect to controls (Figure S4). To assess if neuronal InR expression is required to taste TAG^{C42:0} we repeated our feeding experiments with flies with neuronal InR^{KD}. We show, InR^{KD} flies are unable to detect food enriched with TAG^{C42:0} (Figure 5D). Interestingly, CD36 family proteins and the InR are suggested to initiate cellular signaling by activating the PLC kinase⁵⁴. To test if neuronal PLC is involved in lipid taste detection, we knocked down neuronal PLC using dsRNAi (*rab3>PLC^{RNAi}*). Indeed, we found that neuronal PLC depletion resulted in the loss of sensitivity to TAG^{C42:0} (Figure 5E).

Taken together, neuronal *dllp*/InR interaction is required to facilitate TAG^{C42:0} sensation. Thus, we deem it likely that neuronal SMNP1 function is regulated by the cellular IS cascade.

Discussion

To thrive successfully in given environments many animals rely on specific essential dietary lipids. In addition, absorbed lipids are metabolically no less important than sugars or proteins providing energy and building blocks to consumers. Nevertheless, although it is shown that many animals can discriminate lipid qualities in experimental foods, a selective lipid taste comparable to sweet or umami is not identified. We decided to use adult *Drosophila melanogaster* and investigate if these insects are capable to taste different lipid species in their sugar and protein rich food, and to reveal potential molecular circuits responsible to convey lipid taste to define feeding behavior.

We found that wild type flies are repelled by low ergosterol concentrations but do not respond to higher sterol yields typical for yeast cells¹⁶. In our assay all specimen were able to choose between different ergosterol concentrations and we show that experiments with phytosterols do not produce similar behaviors. Given that our experimental results reflect the behavior of wild flies, we speculate that the sensation of dietary ergosterol levels could represent a way to evaluate food quality – especially since yeast are important to accelerate the proliferation rate and aging^{21,55,56}.

TAGs are another major lipid class present in most diets. We found that flies are able to taste some particular TAGs. Experiments with free fatty acids pointed already to some of our candidates. For instance, the fatty acid C8:0 (TAG^{C24:0}) modulates the feeding behavior of adults³ and the fatty acid C18:0 (TAG^{C54:0}) is toxic at low concentrations^{57,58}. We show food supplemented with TAG^{C42:0} is attractive to flies and the high number of successfully identified tasty lipid in our candidate screen indicates that more lipid species are tasty to *Drosophila*. We used synthetic TAGs formed by three identical fatty acids (chain length, saturation). In real food such lipids are rare or non-existent. TAGs produced by organisms are mostly formed by different fatty acid types. For instance, in contrast to synthetic TAGs formed by identical FAs endogenous TAGs can be formed by fatty acids with different chain lengths and saturation grades. It remains open if and which lipids produced by organisms are tasty to consumers.

In vertebrates, different lipid receptors are suggested to detect fatty acids. For instance, in mouse GPR120 and GPR40 are candidates for binding poly-unsaturated fatty acids. Fruit flies do not encode respective orthologous. However, like most vertebrates *Drosophila* express a member of the CD36 protein family which is known to bind and traffic fatty acids⁹. We speculated that the CD36 receptor in flies possibly aids the detection of dietary lipids. The neuronal elimination of CD36 changed the response of flies to ergosterol and TAG^{C54:0}, and suppressed the feeding response to food supplemented with TAG^{C42:0}. Our results show that CD36 is not a direct neuronal target of ergosterol and TAGs but modulates the lipid perception.

In vertebrates, evidence was presented that CD36 localization is dependent on insulin signaling⁵⁹. We found that the presence of neuronal InR or insulin peptides is essential for flies to taste dietary lipids. In addition, we show that protein or sugar diets activate the cellular insulin signaling cascade at different rates and that on such diets the perception of dietary lipids is changed. Thus, in addition to SNMP1 we have found another dietary variable capable to modulate the feeding response to defined dietary lipids. We confirmed the neuronal activity of the kinase PLC essential for the lipid-taste³. PLC is targeted by CD36 protein family members and is downstream of the InR signal cascade and therefore, links both pathways directly. Although it remains to be shown that SMNP1 and InR are expressed by the same neuronal subsets, our data strongly support the established vertebrate model pointing to a conserved mechanism (Figure 6).

We show that yeast cultured at 10 and 30°C in lipid-free media produce different lipidomes¹⁶ and flies are selective in their food preferences. However, olfactory cues guide wild type flies in their eating behavior^{35,51}. Further, lipid extracts of neither yeast culture instruct the feeding behavior of wild type flies. Lipid mass spectrometry reveals that *Saccharomyces* hardly produce TAGs we have identified in our candidate screens. Yeast produce mainly fatty acids with 16 carbons, and we have shown that TAGs composed from such lipids are not tasty to flies. We did not yet establish if two different TAGs intermixed in one food type do not compete for the same receptor and therefore, potentially change each other's activity. At least, we like to point out that wild *Drosophila* feed on very different yeast species^{12,17} and many known yeast lipidomes divert strongly from lipid configurations produced by *Saccharomyces*⁶⁰. Astonishingly, our experiments still prove the importance of the neuronal CD36

expression in feeding decisions. In contrast to wild type flies, neuronal SMNP1 knock out mutants are attracted to food supplemented with lipids from yeast cultured at 10°C. It remains open, if SMNP1 shows temperature-dependent expression however, it is shown that insulin producing cells are regulated by cold responsive cells⁵³. We provide evidence that the activity of the insulin signaling cascade determines the quality of lipid perception and propose a model that links the cellular insulin signaling with the fatty acid transport machinery. Thus, our results provide a possibility on how wild fruit flies select optimal food in rotting plant materials to endure environmental stress.

To its end, our findings show the strict requirement of defined food to study lipid taste. Here, we present *Drosophila* as a suitable pioneering tool to investigate the molecular base for the perception of dietary lipids systematically.

Material/Methods

Fly culture

If not indicated otherwise, fly stocks were kept on corn-based food (<https://bdsc.indiana.edu>) at 25°C maintaining a 12h light/dark cycle. Fly strain used are *cantonS* (REF), $\Delta dilp2$ (REF), $\Delta dilp3$ (REF) and $\Delta dilp5$ (REF), $\Delta or83b$ (Bloomington Stock Center, #23129), *repoGal4* (REF), *rab3Gal4* (REF), UAS:*inr*^{RNAi} (REF), UAS:*smnp1*^{RNAi} (Vienna *Drosophila* Resource Center #45883) and UAS:*plc*^{RNAi} (REF). Unless specified, animals used in experiments were aged for 5-15 days at 20°C.

Fly food recipes

If not indicated otherwise, each food type supplemented with 0.2% Methylparaben (Roth). YAF food: 100 g/l yeast autolysate (Sigma Aldrich), 100 g/l glucose (Roth). Synthetic lipids including ergosterol (Cayman), stigmaterol (Avanti), phosphatidylethanolamin (Avanti), Tricaprylin (Larodan), Trilaurin (Larodan), Tricaprin (Larodan), Trimyrustin (Larodan), Tripalmitin (Larodan), Tripalmitolein (Larodan) and Tristearin (Larodan) resolved in Methanol/Chloroform (2:1) and added to YAF as indicated.

Yeast culture

Saccharomyces cerevisiae (BY4741) were cultured in minimal medium (1.9 g/L yeast nitrogen base, 5 g/L ammonium sulfate, 20 g/L glucose, 20 g/L peptone) at specified temperatures. Growth control by optical density measurements at OD600. If not specified, yeast cultures harvested in stationary growth phase (Trautenberg et al., 2019).

Biochemistry

Lipid extraction: BY4741 pellets lipid-extracted after modified Folch protocol. Initial lipid extraction with 2:1 Methanol:Chloroform and subsequent re-extraction of the resin with 100% Acetone. Lipids were air-dried at RT, stored at -80 °C for no longer than one night and subsequently pellets resolved in 2:1 Methanol:Chloroform.

Westernblotting: adult female flies were snap-frozen with liquid nitrogen, 3 fly heads per sample pooled, heads were homogenized on ice, heat treated at 95°C for 10min and subsequently loaded onto Tris-SDS-PAGE. Membranes blocked in 5% BSA in 0,1% Triton X-100/PBS and subsequently probed with antibodies including anti-Akt (Invitrogen), anti-Akt^{pSer505} (Cell Signaling), anti-Akt^{pThr308} (Invitrogen), anti-MAPK (Cell Signaling), anti-pMAPK (Cell Signaling) and HRP conjugated

secondary antibodies (Thermo Fisher). Detected signals analyzed with Fiji software. Data was corrected for outliers using the IQR (interquartile range) method.

Thin-Layer Chromatography (TLC): Yeast lipid extracts in Methanol:Chloroform loaded onto a silica plate (VWR) and separated with: Heptane:Diethylether:Acetic acid (70:30:1, v/v/v) or Chloroform:Methanol:water (75:25:2.5, v/v/v). Lipids detected using primulin dye and plates were scanned with ImageQuant software.

Immunohistochemistry

Adult brain dissected and fixed in Graces medium with 4% formaldehyde at RT. Samples probed with antibodies directed against phosphorylated insulin receptor (Cell Signaling). Images take with confocal microscope (ZEISS-LSM 700) and analyzed using FIJI software.

Mass spectrometry

Yeast were cultured as specified in section 'Yeast culture' and samples were collected according to protocols published by Lipotype (<https://www.lipotype.com>). Lipid extraction and measurements performed by services offered by Lipotype.

Developmental tracking

Larval development was monitored on YF^{10°C} and YF^{30°C} at specified temperatures. Experimental details published in Trautenberg et al., 2020 (REF).

Behavior

Capillary feeder assay (CAFE assay): Aged experimental flies transferred into YAF vials for 24h at 25°C. Later, flies were placed in empty vials, containing wetted filter paper. The opening was secured with a sponge, holding four capillaries (Vitrex medical A/S, Blaubrand, Drummond Scientific Company) each filled with food as specified. In parallel, capillar-tubes without flies placed to controls for evaporation. After 18h at 20°C into the experiment the capillaries were removed and photographed. Images analyzed using FIJI software (Figure S5A).

Food-choice assay: Experimental flies were transferred onto agar plate with food baits 12-14h before recording at 20°C. Feeding was recorded for 8 h, taking 1 frame/sec. Videos analyzed using FIJI software (Figure S5B).

Statistical analyses

Hypothesis testing was performed using ANOVA with the aov function of the stats package in R. To account for the complexity of the comparison and the dependence between samples, food consumption was modelled over concentration with date as interactive factor. To account for the paired design of the experiment, different replicates were included as a blocking variable. The final design formula was thus `food_consumption ~ concentration * date + replicate_group`.

The associated code will be made available at https://github.com/arpoe/Trautenberg_journal_2022.

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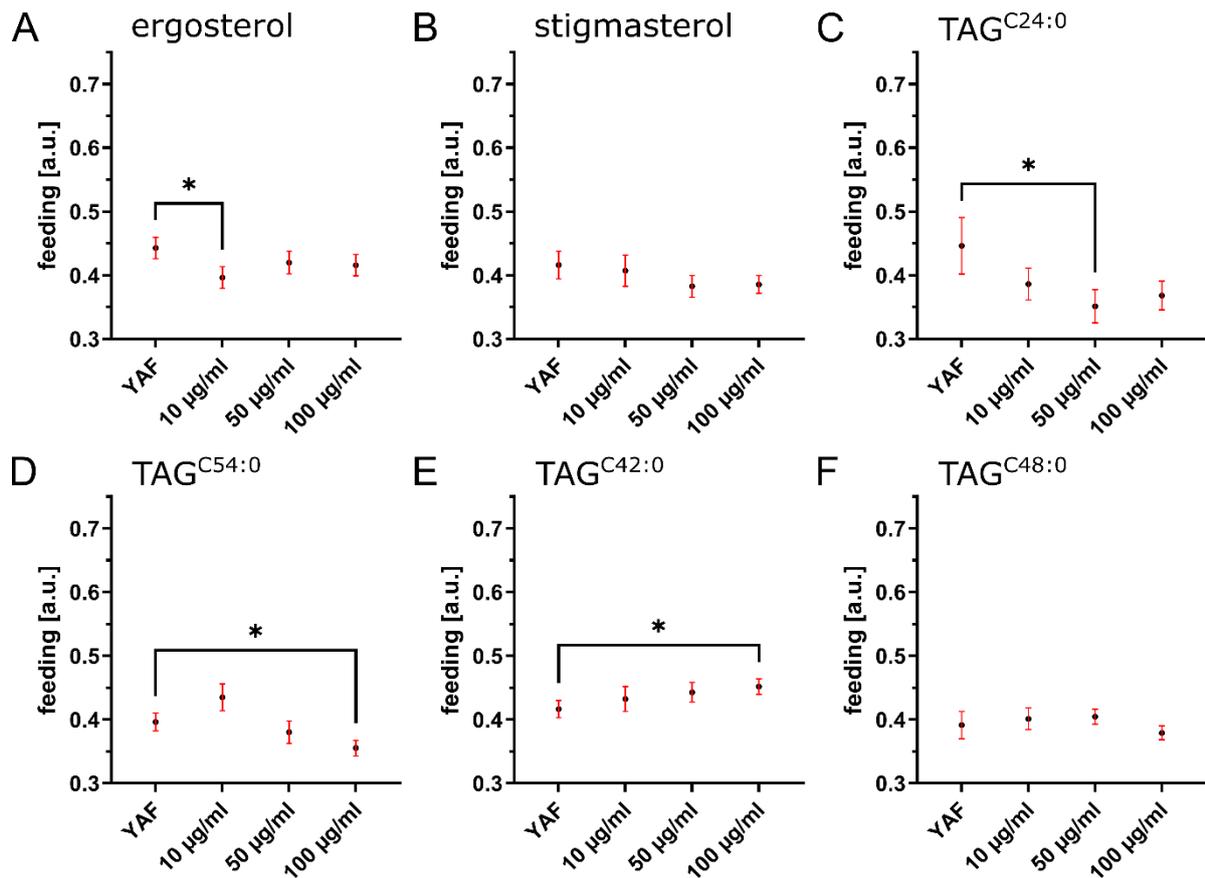


Figure 1: Adult *Drosophila* taste distinct dietary yeast lipid species

A-F Plotted are measured food consumptions of adult wild type flies feeding on YAF supplemented with different concentration (10, 50 and 100µg/ml) of ergosterol (A), stigmasterol (B), TAG^{C24:0} (C), TAG^{C54:0} (D), TAG^{C42:0} (E) or TAG^{C48:0} (F). Shown is the mean (dot) and the standard error of the mean calculated from n=14 or more independent experiments. Significance calculated using ANOVA, * indicates p<0.05.

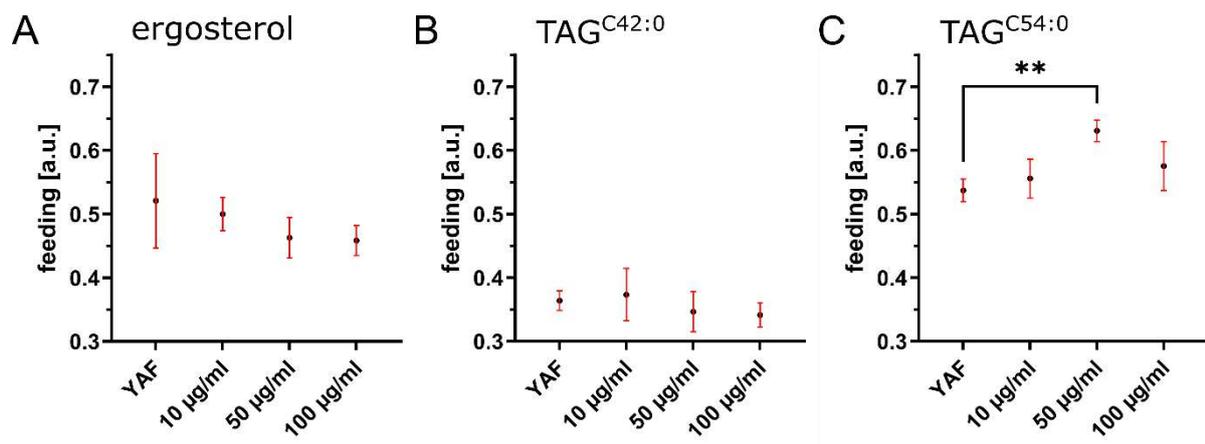


Figure 2: Neuronal loss of SMNP1 modulates the feeding response to specific lipids

A-C Plotted are measured food consumptions of adult *rab3>SMNP1^{RNAi}* flies feeding on YAF supplemented with different concentration (10, 50 and 100µg/ml) of ergosterol (A), TAG^{C42:0} (B), or TAG^{C54:0} (C). Shown is the mean (dot) and the standard error of the mean calculated from n=11 or more independent experiments. Significance calculated using ANOVA, ** indicates p<0.01.

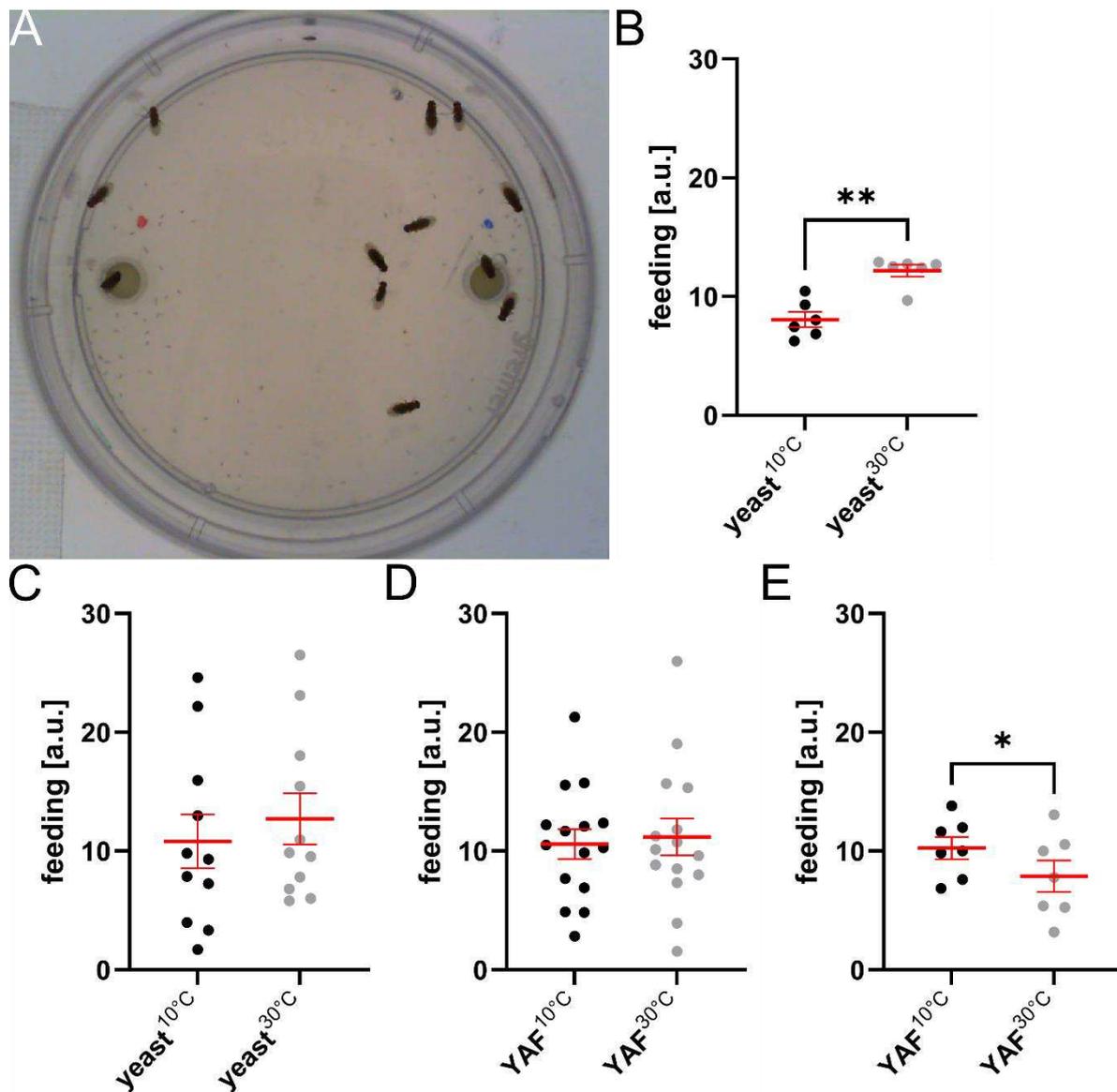


Figure 3: Neuronal SMNP1 regulates feeding behavior of flies

A-E Plotted is the feeding frequency of adult flies in binary food choice assays. Representative photograph from video-recorded movies depicts the experimental buildup used to assess the feeding frequency of *Drosophila*. Shown is the assay plate with flies and food baits on opposing each other (A). Plotted is the food preference of wild type flies on yeast (B) and on lipid-free food (YAF) supplemented with yeast lipid extracts (D), olfactory mutants $\Delta or83b$ on yeast (C) and $rab3 > SMNP1^{RNAi}$ flies on lipid-free food (YAF) supplemented with yeast lipid extracts (E). Shown is the mean (red line) and the standard error of the mean calculated from n=6 or more independent experiments. Significance calculated using Student's paired t-test, * indicates $p < 0.05$ ** indicates $p < 0.01$.

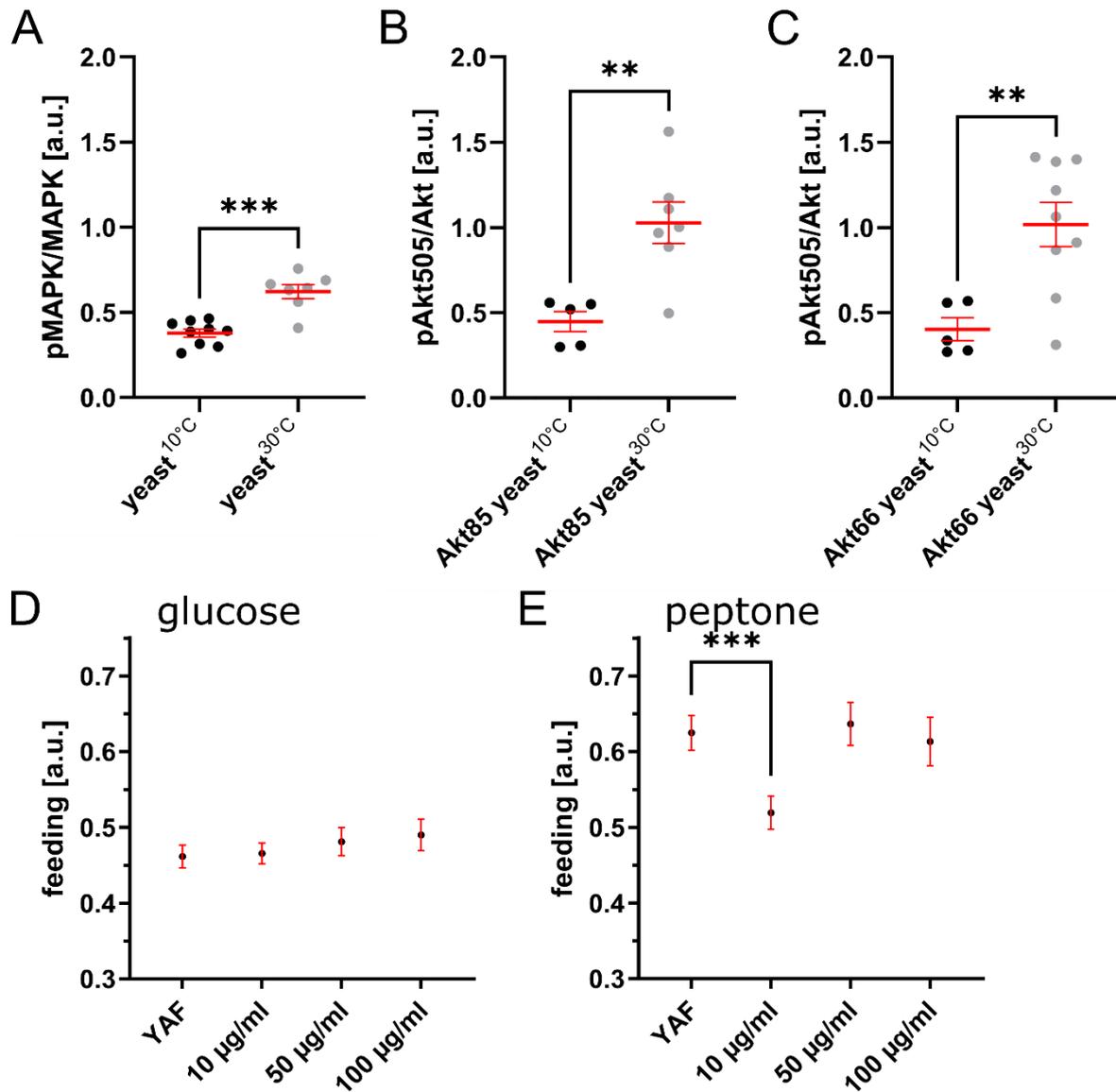


Figure 4: Systemic Insulin signaling modulates lipid-taste perception

A-C Systemic Insulin levels of adult flies were assessed by probing the phosphorylation state of the enzymes pMAPK (A) and Akt (B, D) with dedicated antibodies. Shown are quantified ratios calculated from recorded signals of the phosphorylated proportion of the enzyme in relation to the total. Flies were feeding on yeast^{10°C} or yeast^{30°C} diets. Shown are data of individual replica experiment (dots), the mean (red line) and the standard error of the mean calculated from n=5 or more independent experiments. Significance calculated using Student's unpaired t-test, ** indicates p<0.01, *** indicates p<0.001.

D,E Plotted are measured food consumptions of adult wild type flies feeding on glucose (D) or peptone (E) supplemented with different concentration (10, 50 and 100µg/ml) of TAG^{C42:0}. Shown is the mean (dot) and the standard error of the mean calculated from n=14 or more independent experiments. Significance calculated using ANOVA, *** indicates p<0.001.

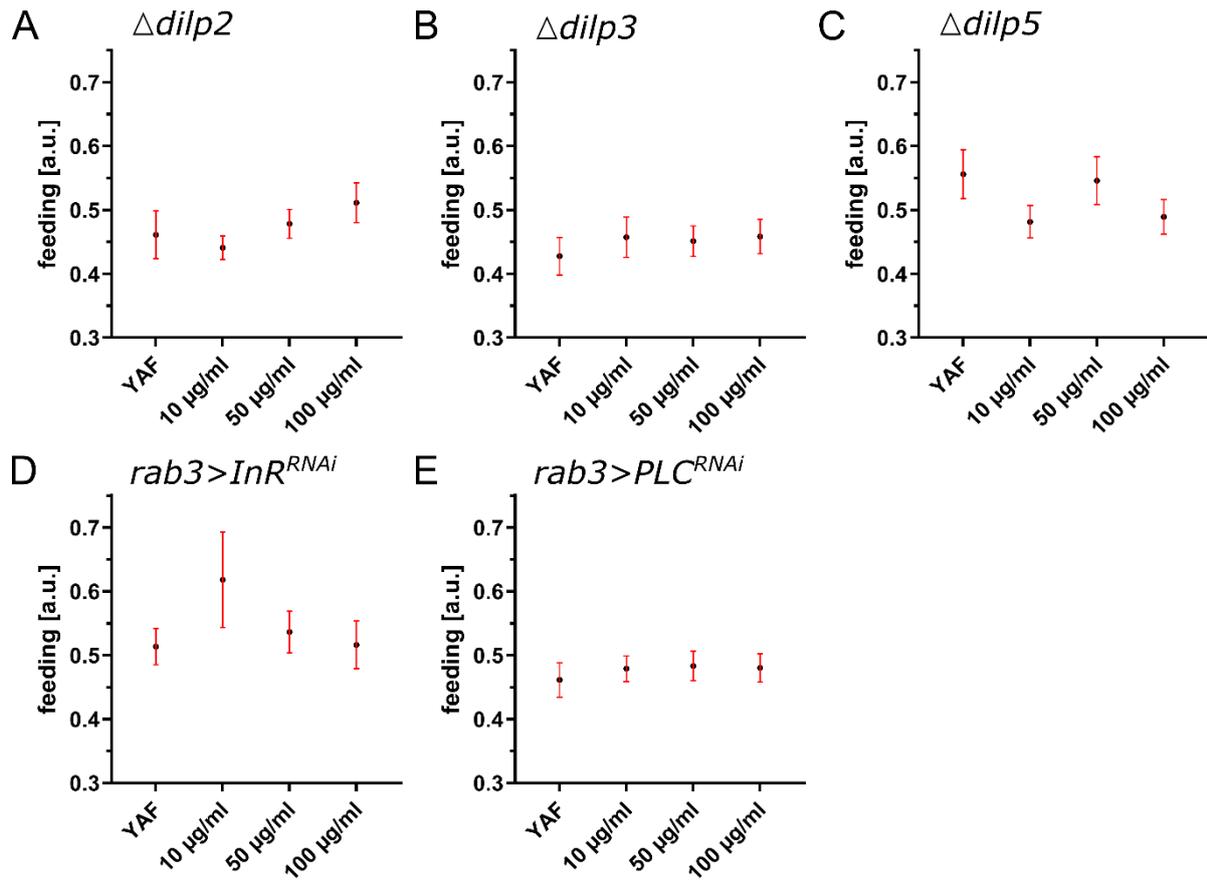


Figure 5: Neuronal insulin signaling regulates lipid-taste perception

A-E Plotted are measured food consumptions of adult $\Delta dilp2$ (A), $\Delta dilp3$ (B), $\Delta dilp5$ (C), $rab3>InR^{RNAi}$ (D) and $rab3>PLC^{RNAi}$ (E) flies feeding on YAF supplemented with different concentration (10, 50 and 100 μ g/ml) TAG^{C42:0}. Shown is the mean (dot) and the standard error of the mean calculated from n=13 or more independent experiments. Significance calculated using ANOVA, all calculated p > 0.05. In addition, please refer to Figure 1A to compare with wild type feeding behavior.

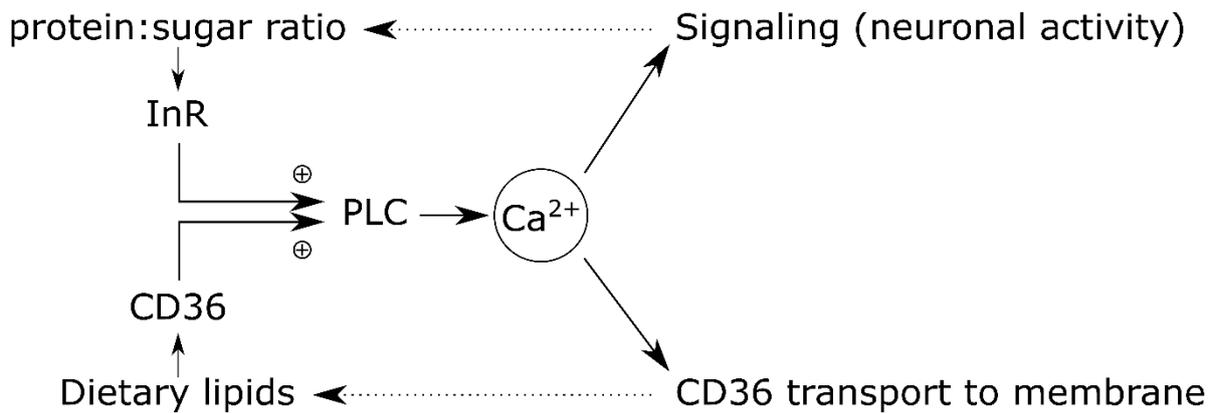


Figure 6: Model integrating neuronal InR signaling with FA transport

Depicted is the proposed regulatory loop responsible for the dietary lipid perception of *Drosophila*. We argue that the dietary protein:sugar ratio is modulating the activation level of the InR by ranged expression of different DILPs. On the other hand, transport of fatty acids originating from the diet

induces CD36 signaling. If both signaling pathways reach a threshold PLC activity elevates neuronal Ca^{2+} levels and hence, induces neuronal activity.

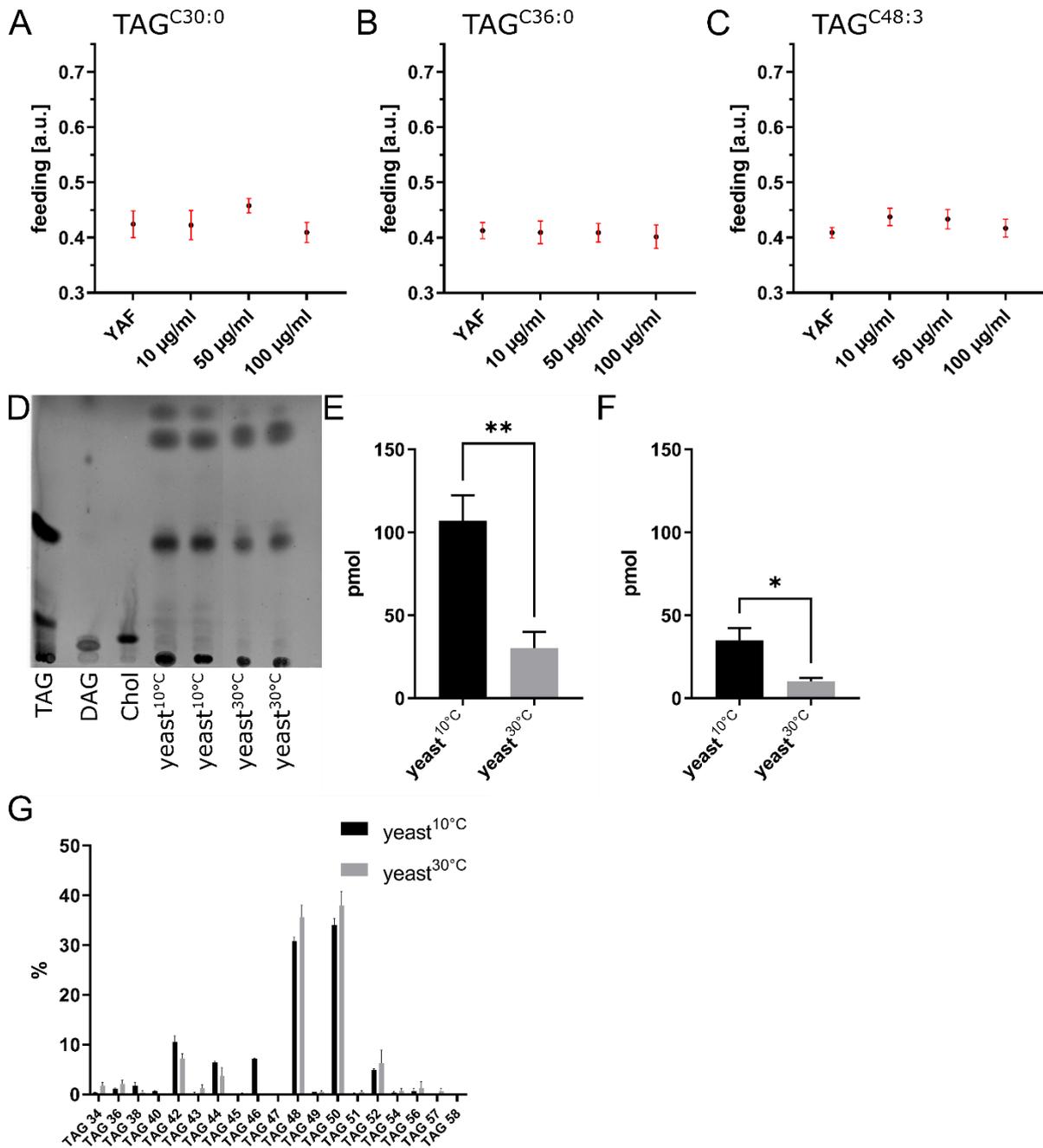


Figure S1: Temperature dependent yeast lipidomes

A-C Plotted are measured food consumptions of adult wild type flies feeding on YAF supplemented with different concentration (10, 50 and 100µg/ml) of TAG^{C30:0} (A) TAG^{C36:0} (B) and TAG^{C48:3} (B). Shown is the mean (dot) and the standard error of the mean calculated from n=14 or more independent experiments. Significance calculated using ANOVA, * indicates p<0.05.

D-G Yeast cultured at either 10 or 30°C vary in their lipid compositions. Yeast grown at 10°C have more sterols (D, representative thin layer chromatography plate stained with primulin). Mass spectrometry measurements reveal that yeast grown at 10°C have also more TAGs (E) and sterol esters (F). The TAG species profile (G) shows that both yeast cultures are indifferent in TAG qualities, albeit quantities between TAG species may vary.

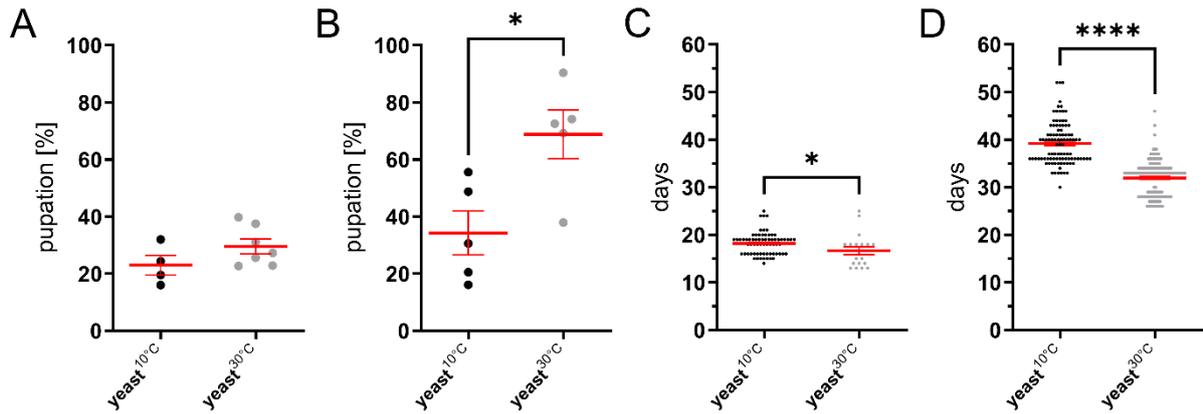


Figure S2: Larval survival on different YF types

A-D Plotted are survival (A, B) and pupariation (C, D) rates of larvae kept on YF made from yeast^{10°C} or yeast^{30°C} at 20 (A, C) or 14°C (B, D). Shown is the percentage of successfully pupariated larvae represented by one experimental cohort of minimum 20 individuals (A, B) in replica experiments (dot) or individual larvae tracked until pupation (C, D) in replica experiments (dot), the mean (red bar) and the calculated standard error of the mean. Significance calculated using Student's unpaired t-test, * indicates $p < 0.05$, **** indicates $p > 0.0001$.

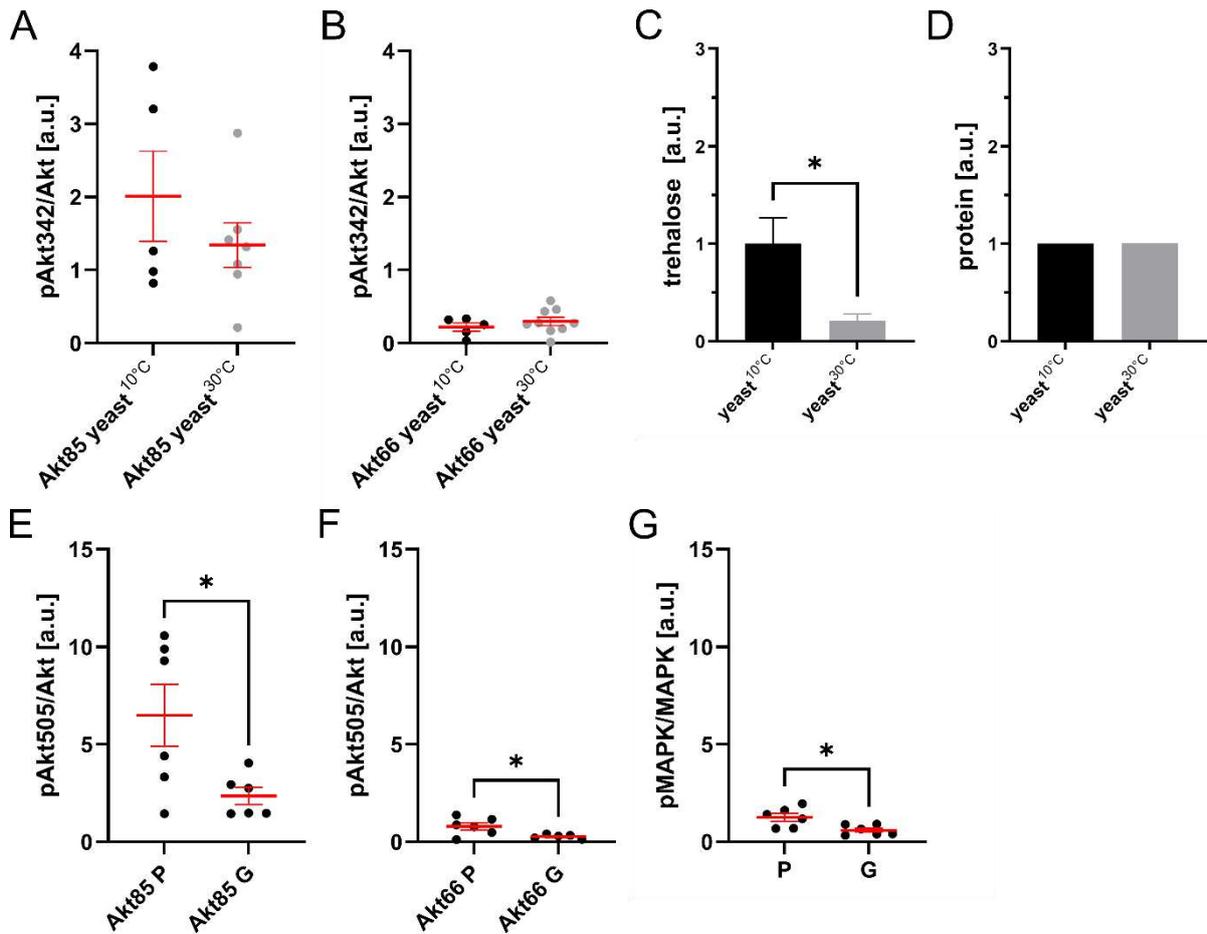


Figure S3: Insulin signaling levels differ in flies kept on glucose or protein diets

A, B Systemic Insulin levels of adult flies were assessed by probing the phosphorylation state of the enzymes Akt at the Thr342 site with dedicated antibodies. Shown are quantified ratios calculated from recorded signals of the phosphorylated proportion of the enzyme in relation to the total. Flies were feeding on yeast^{10°C} or yeast^{30°C} diets. Shown are data of individual replica experiment (dots), the mean (red line) and the standard error of the mean calculated from n=5 or more independent experiments. Significance calculated using Student's unpaired t-test.

C, D Plotted is the relative amount of total trehalose (C) or protein (D) in yeast grown at 10 or 30°C. Shown are the mean and the standard error of the mean calculated from n=3 or more independent experiments. Significance calculated using Student's unpaired t-test, * indicates p<0.05.

E-G Systemic Insulin levels of adult flies were assessed by probing the phosphorylation state of the enzymes Akt (E, F) and MAPK (E) with dedicated antibodies. Shown are quantified ratios calculated from recorded signals of the phosphorylated proportion of the enzyme in relation to the total. Flies were feeding on glucose (G) or protein (P) diets. Shown are data of individual replica experiment (dots), the mean (red line) and the standard error of the mean calculated from n=5 or more independent experiments. Significance calculated using Student's unpaired t-test, * indicates p<0.05.

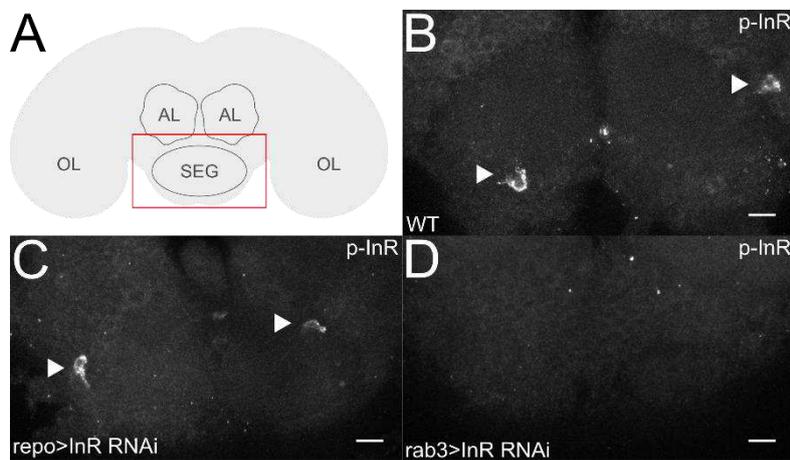


Figure S4: Neurons in the SEG region express phosphorylated InR

A-D Depicted is a sketch of the adult fly brain and boxed (red) is the region of interest (SEG = subesophageal ganglion, AL = antennal lobe, OL = optic lobe) (A). Photographs of maximum intensity projection of an example stack show neurons expressing the phosphorylated InR (white arrow heads). Shown are samples from wild type (B), *repo>InR^{RNAi}* (glial knock down) and *rab3>InR^{RNAi}* (neuronal knock down).

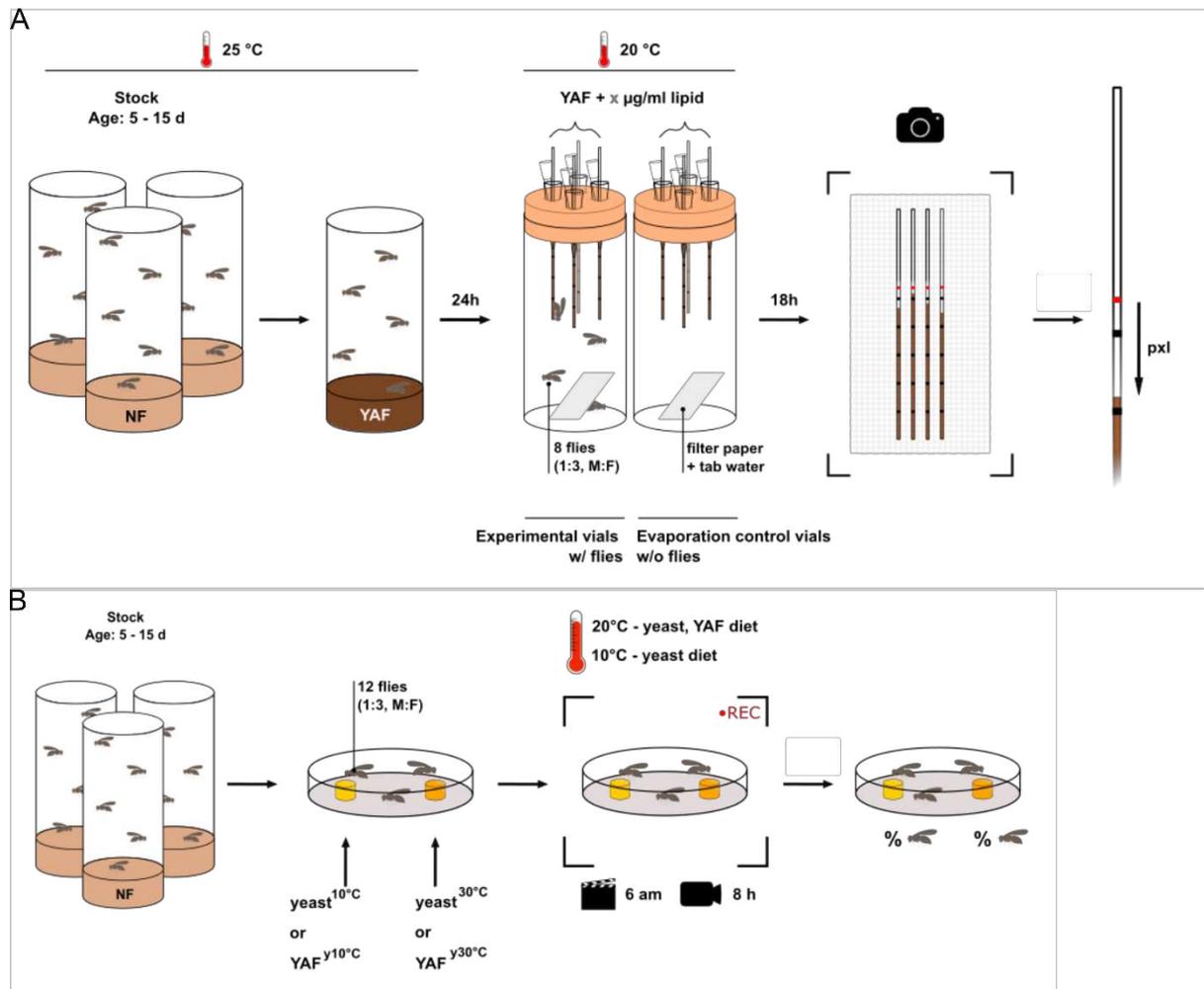


Figure S5: Graphic depiction of feeding behavior assay

A,B Shown are graphical abstracts detailing the food choice assay (A) and the feeding assay (B) protocols as described in the 'Method' section.