Effect of mating and isolation on preferential administration of methamphetamine in D. melanogaster

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SVEUČILIŠTE U RIJECI

ODJEL ZA BIOTEHNOLOGIJU

Diplomski sveučilišni studij

"Medicinska kemija"

Valentina Dukić

Utjecaj parenja i izolacije na preferencijalnu konzumaciju metamfetamina kod *D. melanogaster*

Diplomski rad

Rijeka, 2022.

Mentor: Dr. Rozi Andretić-Waldowski

Ko – mentor: Dr. Ana Filošević

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The thesis has 41 pages, 7 figures, 0 tables and 29 references.

Abstract:

Many molecular mechanisms are involved in the reward system of the brain. Natural rewards include food, mating and socialization. Artificial rewards include psychostimulative substances, such as methamphetamine (METH). Dopaminergic reward system of fruit fly in *Drosophila melanogaster* can be stimulated by both natural and artificial rewards. Reward system in *D. melanogaster* is mainly regulated by signal molecules dopamine (DA) and neuropeptide F (NPF) signalling. Positive social experiences are rewarding and increase the levels of DA and NPF in *D. melanogaster*. Sexual deprivation in *D. melanogaster* has led to an increased preference for ethanol and is explained through the mechanism of NPF signalling. Negative social experiences lead to increased aggression, lack of sleep, disturbed food intake and mating. It causes many epigenetic changes with the emphasis on DNA methylation. Social isolation increases the preference for psychostimulative substances in many species and in flies an increase in preference to ethanol post social isolation was discovered so far.

The aim of this thesis is to analyse the effect of different social experiences on the preferential consumption of METH in *D. melanogaster*. I analysed the effect of isolation/grouping and its duration, as well as, sexual deprivation, which has not yet been done in *D. melanogaster*.

I included four different groups of flies – isolated mated and virgin and grouped mated and virgin. The FlyCafe experiment allowed the self – administration of METH over the three days of the experiment. Isolated flies show higher METH preference and the preference decreases as the duration of isolation increases. Group housed flies show aversion to METH and this aversion increases with the duration of grouping. Social isolation shows the dominant effect on preference compared to sexual deprivation. The highest change in preference, with regards to duration of isolation/grouping, is present in group housed and mated flies. It indicates the importance of the mating duration in regards to the peak mating capacity in flies.

Future experiments should analyse the long-lasting effects of social isolation and sexual deprivation on preferential consumption of METH, to more precisely define environmental effect on the motivation for METH consumption.

Key words: *Drosophila melanogaster*, sexual deprivation, social isolation, METH preference, dopaminergic system

Sažetak:

Mnogi molekularni mehanizmi su uključeni u nagrađujući sustav u mozgu. Prirodne nagrade uključuju hranu, parenje i socijalizaciju. Umjetne nagrade uključuju psihostimulativna sredstva, poput metamfetamina (METH). Dopaminergični nagrađujući sustav vinske mušice u *Drosophila melanogaster* može se poticati prirodnim i umjetnim nagradama. Nagrađujući sustav u *D. melanogaster* većinski je reguliran signalizirajućim molekulama dopamina (DA) i neuropeptid F (NPF) signalizacijom. Pozitivna socijalna iskustva su nagrađujuća te povećavaju razinu DA i NPF u mušica. Deprivacija parenja je u *D. melanogaster* dovela do povećane preference za etanol te je objašnjena mehanizmom NPF signalizacije. Negativna socijalna iskustva dovode do povećane agresije, manjka sna, poremećaja hranjenja te parenja. Dolazi do mnoštva epigenetičkih promjena s naglaskom na promjene u metilaciji DNA. Socijalna izolacija povećava preferencu ka psihostimulativnim sredstivma u raznih vrsta, a u mušica je do sada otkriveno povećanje u preferenci ka etanolu kao posljedica socijalne izolacije.

Cilj ovog rada je ispitati utjecaj različitih socijalnih iskustava na preferencijalnu konzumaciju METH-a u *D. melanogaster*. Ispitan je efekt trajanja izolacije/grupiranja, kao i utjecaj parenja, što do sada nije bilo ispitano u *D. melanogaster*.

U eksperimenut su uključene četiri skupine *D. melanogaster* – izolirane parene i neparene te grupirane parene i neparene. FlyCafe eksperimentom omogućena im je samoadministracija METH-a kroz tri dana eksperimenta. Izolirane mušice pokazuju veću preferencu ka METH-u te je preferenca niža sa dužim periodom izolacije. Grupirane mušice pokazuju averziju ka METH-u te se averzija povećava s duljinom grupiranja. Socijalna izolacija pokazuje dominantni utjecaj na preferencu u usporedbi s deprivacijom parenja. Najveća promjena u preferenci s obzirom na duljinu izolacije/grupiranja je prisutna kod grupiranih i parenih mušica. To ukazuje na značaj duljine parenja s obzirom na vrhunac sposobnosti parenja u mušica.

Budući eksperimenti trebali bi ispitati dugotrajniji utjecaj socijalne izolacije i deprivacije parenja na preferencijalnu konzumaciju METH-a, kako bi se preciznije definirao okolinski utjecaj na motivaciju za konzumiranjem METH-a.

Ključne riječi: *Drosophila melanogaster*, deprivacija parenja, socijalna izolacija, METH preference, dopaminergični sustav

Contents

1. Introduction

1.1. Dopaminergic reward system

There are various biological mechanisms that mediate behaviour as a consequence of pleasurable experiences [1]. Those molecular mechanisms are called "reward". The brain responds to natural rewards like sex, socialization and food through the dopaminergic reward system [1]. The response to natural rewards is evolutionary important for fitness, survival and reproduction of species. Rewards are hedonic incentives which cause neural representations that elicit goal pursuit and motivation. On the other hand, aversive motivation is related to avoiding unpleasant conditions that could have bad consequences on the individual [2].

Neurochemical systems, plasticity mechanisms, neuronal organisations and intracellular information cascades are evolutionary conserved in across species [3]. Some components of the reward system and its mechanisms differ depending on the level of brain complexity. The reward system in insects has been mostly investigated in the model of fruit fly *Drosophila melanogaster*. Flies have a well-developed brain and mushroom body (MB) which have an important role in associative learning studied using appetitive or aversive conditioning.

The reward system is mostly examined in the context of olfactory learning [4]. Taste is detected through gustatory receptors while odours are detected through olfactory receptor neurons (ORNs). Signal is then sent to glomeruli in antennal lobes (ALs). The output is transmitted to projection neurons (PNs) and then to MB and the lateral horn (LH). MB have a potential role in sensory integration and are located between sensory processing centres and premotor centres [4]. MB only receives olfactory input that are crucial for memory formation. Outputs from MB are needed for reward learning. A small subset of MB neurons are also involved in the visual reward learning.

A subset of LH neurons express gustatory receptor Gr43a which may be involved in gustatory reward learning [4]. Also, the spatial arrangement of neurons that project from AL is indicative of whether they drive avoidance or approach. LH is perfectly situated to activate motor system depending on information it receives.

The Gnathal ganglion (GNG) is also involved in the reward processing [4]. It receives input from chemosensory receptor neurons. *Drosophila* has 26 octopaminergic neurons. One large neuron, OA–VUMa2, expresses tyramine decarboxylase and has octopamine (OA)–like immunoreactivity. It has a large cell body in the GNG and projects to the MB, LH and AL. It is proposed to be involved in the reward system, by mediating the primary gustatory and conditioned olfactory rewarding experience. This function is in parallel with properties of dopaminergic neurons in the mammalian brain, which respond to the same stimuli. OA signals are especially important for the rewarding experience due to due to sweetness of food.

In addition to OA, dopamine (DA) is also involved in the reward learning process [4]. Dopaminergic signals via the DopR receptor in MB and have a role in appetitive learning. In the protocerebral anterior medial neuronal cluster is a bundle of 100 dopaminergic neurons that project to the medial lobes of MB. Those neurons signal nutritive value and are involved in signalling food reward.

1.1.2. Natural and artificial rewards

In mammals, the mesocorticolimbic DA system is activated by natural rewards, as well as drug rewards [1]. The DA system mediates the intense pleasure of addictive drugs and anhedonia at the withdrawal state. The sensitized and altered cellular mechanisms of reward predictions and associative learning may cause the ingrained drug taking habits.

In flies, behavioural response to drugs of abuse can be measured through the changes in phenotypes such as: drug sensitivity, aversion or attraction, locomotor effects, development of drug tolerance, preference and sensitization [5]. Flies show higher locomotion at lower doses of ethanol and incoordination and sedation at higher doses, as well as dose–dependent aversion or attraction. Flies also develop a preference due to the rewarding effect.

DA has an important role in the drug mediated reward system [5]. For example, cocaine and METH block DA reuptake after the release at synapses. This results in higher extracellular DA. DA is also critical for the more complex behaviours, such as development of ethanol preference [5]. Flies are more likely to lay eggs on ethanol-containing food than on the regular food. Ethanol is rewarding and a neutral odour becomes attractive to flies when it is presented together with ethanol. Dopaminergic neurons in MB promote ethanol–induced preference, reward and hyperactivity. OA also has a role in the rewarding effect of ethanol as flies relate ethanol to the sweetness of fruit [5].

Neuropeptide F (NPF) is a molecule that regulates ethanol preference, reward and sensitivity [5]. Ethanol sensitivity is reduced when cells that express NPF are ablated or synaptically silenced during ethanol exposure. The raised levels of NPF increase ethanol sensitivity. When an intoxicating amount of ethanol is administrated, NPF production increases. NPF levels vary depending on the mating experience of male fruit flies and, therefore, regulate their preference for ethanol. Rejected males have lower levels on NPF and a higher preference for ethanol, while mated males have higher levels of NPF and lower ethanol preference [5]. Both high levels of NPF and ethanol consumption are rewarding to flies. Lower levels of NPF lead to a higher preference for ethanol. This indicates the role of NPF in responding to rewarding and threatening stimuli, including drug reward. Downstream targets of NPF are dopaminergic neurons which further confirms the role of DA in the reward system.

1.2. Impact of social isolation

Positive social interactions in most species, including humans, are associated with improved health [6]. Social interactions are a source of pleasurable sensations and feelings [2]. The pleasure and reward may come from attention, closeness, touch and safety. Sexual experience and communication can improve cognitive, emotional and motivational functions and reduce stress.

Social isolation, as a form of negative social interaction, adversely impacts behaviour and health [6]. Social isolation may cause myocardial infarction, negatively affect stroke recurrence, stroke survival and amount and efficiency of sleep. It also affects general morbidity and mortality as a consequence of alcoholism, obesity, high blood pressure and smoking [7]. The negative effects of social isolation are conserved in all social species [6,8]. The changes in behaviour occur due to differences in phosphorylation, physiology, epigenetics, gene expression, neurogenesis and neuronal morphology [7].

D. melanogaster are a social species and show complex social networks and collective behaviours [9]. Those contribute to many essential processes such as circadian rhythms, mating, fighting, sensing, feeding and foraging. Differences in social behaviour, like changes in population density, have an impact on mating, male courtship and aggression [10]. As social isolation has a large impact on the behaviour of fruit flies, they can be used as a good model to study how negative social interactions affect humans and what impact it has on the brain.

1.2.1. Epigenetic and transcriptional changes following social isolation

Epigenetic mechanisms that are engaged by stressors have a crucial role influencing gene expression in the brain [11,12]. Social isolation led to epigenetic changes in the brain of mice and increase of DNA methylation of dopaminergic neurons. In *D. melanogaster*, social isolation led to the decrease in DA levels [11].

The *Dopa decarboxylase* (*Ddc*) gene encodes an enzyme involved in the process of DA and serotonin synthesis [11]. *Ddc* mRNA is upregulated in the collectively housed flies compared to isolated flies. That is consistent with previous studies which have shown that levels of DA are lower in the heads of isolated flies. Levels of the activating histone mark H3K4me3 are significantly higher in group housed flies, around *Ddc* gene, compared to isolated flies. Activating mark H3K27ac was similarly increased, following the pattern of mRNA expression. Repressive marks, which are very low on *Ddc* gene, showed no significant changes.

All activating mark levels correlated positively with mRNA levels, and repressive marks correlated negatively with mRNA levels [11]. H3K9me2 and HrK9me3 changes are connected to Heterochromatin Protein 1 – mediated formation of heterochromatin and repression of transcription. There are small but significant changes in histone marks over the entire gene, but when examining islands that cover parts of genes, there are much larger changes. Those changes in activating or repressive marks are usually restricted to specific regions of genes.

There were several differently regulated genes when comparing male flies that have been socially isolated for four days to collectively housed males [11]. These are mainly genes involved in epigenetic regulation of gene expression (for example, histone modifications, DNA methylation) and transcription factors. These genes include several histone acetyltransferase genes and peptide with n–acetyltransferase activity.

Daytime activity is significantly lower in group housed flies compared to isolated flies [11]. This suggests higher metabolic activity in socially isolated flies. Out of 15 known mitochondrially encoded genes, which are upregulated in waking flies, 14 were upregulated in socially isolated flies. Altogether, transcript levels of many genes that are expressed in dopaminergic neurons were changed in isolated flies, including epigenetic reader and writer genes. Several house–keeping genes such as mitochondrial, ribosomal and proteosome genes are also differently expressed in group housed and isolated flies [11]. Some genes with epigenetic functions, such as histone acetyltransferase, are upregulated in group housed flies while other are upregulated in isolated flies.

Genes that regulate neural function, such as transcription factors and glycolysis genes, are also differently expressed [11]. Heterochromatin Protein 1–associated H3K9me3 is higher in group housed flies than isolated flies, while Polycomb repressive complex 2–associated H3K27me3 mark is upregulated in socially isolated flies.

Lastly, neural function genes that regulate male mating behaviour, memory, learning, synaptic, serotonin and neuropeptide signalling, transcription and ion channels regulation genes are differently expressed [11]. These genes are generally expressed more in isolated flies.

Five transcriptional factors have shown higher expression in group housed flies – Hr38 (Hormone receptor – like in 38), Sr (Stripe), CrebA, Cbt (Cabut) and Pho (Pleiohomeotic) [11]. Genes encoding for first four are orthologs of immediate early genes in vertebrates. Group housing provides more stimuli for male flies and dopaminergic neurons. These five genes are part of Activity Related Genes (ARG). The epigenetic effects of social housing on different marks were more highly correlated between ARG genes than among all genes.

Genes in several groups were repressed differently by ARG transcriptional repressors, depending on the housing conditions [11]. Nine functional groups of such genes are: sleep, neuropeptide, male mating, ligand–gated ion channels, MAPK signalling, catecholamine metabolism, G–protein signalling and some epigenetic genes. ARGs that were found to be differently expressed may have repressive effects on transcription in different brain regions, such as MB.

Hr38 and *Stripe* affect DA pathway genes [11]. *Hr38* regulates dopaminergic neuron development and transcription. Its overexpression increased *Ddc* transcription. *Hr38* and *Ddc* are significantly increased in group housed flies. *Cbt* is upregulated in 94% dopaminergic neurons of group housed males compared to isolated males [11]. *Cbt* primary acts as a transcriptional repressor in dopaminergic neurons following social stimulation.

1.2.2. Impact of social isolation on different behaviours

Socially isolated flies have significantly reduced total number of sleeping hours compared to the group housed flies. Fruit flies usually sleep for 8 to 10 hours in total [13]. Sleep is particularly reduced during the day while night – time sleep is fragmented [6,14]. Sleep state is needed to form synaptic connections made during the learning processes in the wake state [15]. Flies that were kept in groups were shown to have more synapses compared to those that were isolated. Isolated flies need less sleep due to the sensory deprived environment. ER stress and reduction in protein synthesis contribute to the reduction of synapses [6].

Aggression is important for survival and reproduction of various species [16]. Aggressive behaviours increase in males and females of *D. melanogaster* following social isolation [17]. Isolation has been correlated with food scarcity, causing increased aggression and competitiveness. Isolated male fruit flies needed less time to establish territory than group housed males, due to their higher aggressiveness [16]. Higher aggressiveness also leads to higher mating success. Aggressiveness can be reversed by grouping isolated flies, suggesting that socialising suppresses aggressiveness.

In species where courtship is learned, social isolation has a large negative influence on mating. Several sexual traits in *D. melanogaster* show neural plasticity dependant on social experiences [18]. Examples are mating success, ejaculate characteristics, mate choice and response to courtship song. Mating success is based on previous experience. If males are isolated from females, they have lower mating success [19]. Males also adjust their courtship behaviour to be more competitive if in the presence of other males. Group housing leads to a higher copulation rate. The social experience in the younger age has the highest effect on the courtship behaviour. Aspects like sperm resource and accessory gland secretion are also under the influence of social experience and density [18].

Social experience may have an effect on the feeding behaviours in *D. melanogaster* [9]. Many genes that differ in expression between isolated and group housed flies are involved in metabolic processes such as one – carbon metabolic, oxidation – reduction and carbohydrate metabolic processes. Many differentially expressed genes are involved in metabolism of pyruvate, amino acids, glucose and fatty acids. In chronically isolated flies, there was an increase in the feeding and total food consumption compared to the group housed flies [9]. This study shows that chronic social isolation induces starvation, on the behavioural and gene expression level, therefore isolated flies ate more to compensate for the starvation that occurred.

1.2.3. Impact of social isolation on addiction

In mammals, stressful events during adolescence increase the likelihood of addiction and substance abuse, depression and anxiety in adulthood [20,21]. Social stressors are one of the strongest and most prevalent stressful stimuli that one can experience [20]. Socially isolated rodents often show increased cocaine and ethanol intake, cocaine – evoked DA release and increased locomotor response to amphetamine. Isolated rodents have increased reward–related stimuli sensitivity [22]. On the other hand, social isolation during adulthood does not affect the self – administration of addictive drugs. Social isolation during adolescence could cause the modifications in neurogenesis.

Stress during adolescence induces neurotransmission and neurogenesis modifications in various brain regions such as the hippocampus, amygdala and PFC in rats [21]. Social isolation has been shown to lead to reduced long – term potentiation which is a neural model of memory and learning. Similar patterns and neurogenesis malfunctions may also be present in fruit flies. As young age is the time when brain is in development, the stress during that period has a high chance of altering the development of the reward system [20]. Therefore, it has been shown to lead to the dysfunction in reward processing, such as increased DA response to stimuli.

1.3. Impact of sexual deprivation on addiction

Positive sexual experience is also a behaviour reinforced by the brain's reward system [23]. Sexual activity is one of the fundamental physiological conditions present throughout many species [24]. The pleasure experience following sexual activity depends on the dopaminergic neurons of mesolimbic DA pathways. This pathway is very important for the study of drug addiction. Sexual deprivation may cause depression, low self–esteem, anxiety, loneliness and increase drug–seeking behaviours. Sexually experienced individuals have a preference for lower dosages of drugs, such as amphetamine, compared with higher doses [24]. This process may also be mediated by hormonal changes.

In one study, two cohorts of *D. melanogaster* males, with different sexual experiences, were used [23]. The group which was housed in isolation experienced sexual rejection by mated females. This supresses future courtship behaviour even to virgin females. The other cohort was mated and group housed with other males. The mated and group housed flies showed avoidance of ethanol during the first two days of the FlyCafe experiment and a slight positive preference on the third day of the experiment.

The rejected cohort had higher preference for the food with ethanol compared to the mated cohort [23]. Even when both rejected and mated groups were group housed and had positive social experience, rejected males had higher preference for ethanol enriched food. Males that experienced neither copulation nor rejection had higher preference for ethanol compared to the mated group but similar to the rejected group. The lack or presence of sexual experience showed higher effect for the ethanol preference compared to the social isolation. The rejected males that were subsequently mated with virgin females had reduced preference for ethanol food. Therefore, the effects of sexual deprivation can be reversed by the positive sexual experience.

NPF is a possible mediator of the effects of sexual experience on the ethanol preference [23]. The homologue of NPF in mammals, neuropeptide Y, regulates ethanol consumption. NPF – NPF receptor complex is important for the regulation of acute ethanol sensitivity in *Drosophila*. Neuropeptide Y levels are regulated by stressful experiences like restraint stress, post-traumatic stress disorder and early maternal separation [23].

The rejected group showed the lowest levels of NPF transcripts in fly brains. Virgin grouped flies showed higher levels and mated grouped flies the highest levels of NPF [23]. Sexual deprivation leads to the NPF deficit and increases the reward – seeking behaviour like consumption of ethanol. The positive sexual experience leads to the high levels of NPF and reduces the reward – seeking behaviours.

NPF had a role in the several fly behaviours like changes in feeding behaviour, response to the ethanol and physical stressor [23]. NPF neurons also modulate the effects of satiety and sugar reward memory. In this research it has been shown that levels of NPF are regulated by the sexual experience and that NPF neurons act as reward signals. Neuropeptide Y has many roles apart from the regulation of ethanol consumption, such as its role in feeding, stress, sexual motivation, sleep regulation and anxiety.

In this thesis I will focus on the effect of sex deprivation and social isolation on the preference for METH in *D. melanogaster*. If flies lack natural rewards, we hypothesize that they will turn to the artificial rewarding experiences. This thesis will focus on the possibility that METH can replace natural rewards by acting on the reward system.

2. Aims

The main aim of this thesis is to determine the influence of different social experiences on METH preference of *D. melanogaster*. The main hypothesis is that if social experiences have an effect on the METH preference of *D. melanogaster*, then there will be differences in METH self–administration between groups with different social experiences.

The first sub aim of this thesis is to determine the influence of social isolation on the METH preference of *D. melanogaster*. If social isolation, a negative social experience, has an effect on the METH preference, then there will be differences in METH self-administration between flies that were socially isolated and flies that were group housed.

The second sub aim is to determine the influence of duration of social isolation on METH preference of *D. melanogaster*. If the duration of social isolation has an effect on the METH preference, then there will be difference in METH self–administration between groups of flies that were isolated for different periods of time.

The third sub aim is to determine the influence of the mating experience on METH preference of *D. melanogaster*. If mating experience has an effect on METH preference, then there will be differences in METH self–administration between flies with different mating experiences.

3. Materials and methods

3.1. Chemicals and fly strain

METH-hydrochloride (≥97.5%) and mineral oil were purchased from Sigma-Aldrich, ethanol from VWR, and sucrose from a local store.

The experiments were done using males of the *wild type (wt) Canton S.* strain of *D. melanogaster*. Flies were maintained on standard cornmeal/agar medium at 25°C and 70% humidity on a 12-h light/dark cycle.

3.2. Experimental design

3.2.1. *FlyCafe*

In this work I used the FlyCafe method (Fig. 1) developed in the Laboratory for Behavioural Genetics at the Department of Biotechnology of University of Rijeka. The head of laboratory is dr. sc. Rozi Andretić Waldowski. The FlyCafe assay was developed as a combination of standard two – choice CAFE assay and Drosophila Activity Monitoring System (DAMS) [25].

The method is based on the simultaneous collection of data on activity of individual flies, the location of flies and the amount of consumed METH) [25]. It is based on the commercially available DAMS. Every DAMS monitor holds 32 flies placed individually in glass tubes (65 x 5 mm). A 1.5 cm long rubber tube is attached to each end of the glass tubes. The rubber cap is covered with nylon mesh and secured with parafilm to enable the entrance of water vapour and prevent dehydration. At the top of the rubber cups there is a whole that fits a 200 µl pipette tip also secured with parafilm. The tip is modified to hold a 5 µl glass capillary with food (Fig. 1 A)).

Capillaries are filled by capillary action with a small amount of mineral oil to minimize the evaporation of liquid food [25]. The rest of the capillary is filled with liquid food which consists of 0.1M sucrose and 0.05 g/ml of yeast solutions. The METH food has an addition of 0.1 mg/ml dose of METH in distilled water. The height of liquid food in the capillaries is measured with a ruler in mm and the capillaries are inserted in the pipette tip to be easily accessible to the flies. The amount of food that flies self–administrated is measured every day at 9:30AM for 3 consecutive days, and replaced with fresh capillaries.

I measured the change in meniscus position of liquid food and mineral oil in glass capillaries in mm and calculated the amount of ingested food. That was done by subtracting the evaporation correction and multiplying the result by the cross–sectional area of the capillaries. That provided the volume of consumed food in µl. Preferential consumption is calculated as a difference in consumption of METH food against regular sucrose–yeast food.

In each experiment, ten flies are controls which have regular food mixture provided on both ends of the glass tubes [25]. The control group is needed to eliminate the possibility of side bias. 22 flies are provided with METH food on one side, and regular food on the other side. Side was alternated to avoid side bias.

The DAMS monitor was placed on a pedestal in the plastic tub filled with 1L of tap water [25]. The tub was covered with cling film to minimize the fluctuations of humidity and evaporation. The monitors are connected to a computer using the PSIU9 Power Supply Interface Unit (TriKinetics) (Fig. 1 B)). The tub is placed in the incubator kept at 24 °C and in constant darkness to prevent the side preference due to environmental cues. The additional controls for correction of evaporation were three tubes without flies and with regular food on one side and METH food on the other side in capillaries. Each day the amount of consumed food was corrected for average evaporation in the control tubes.

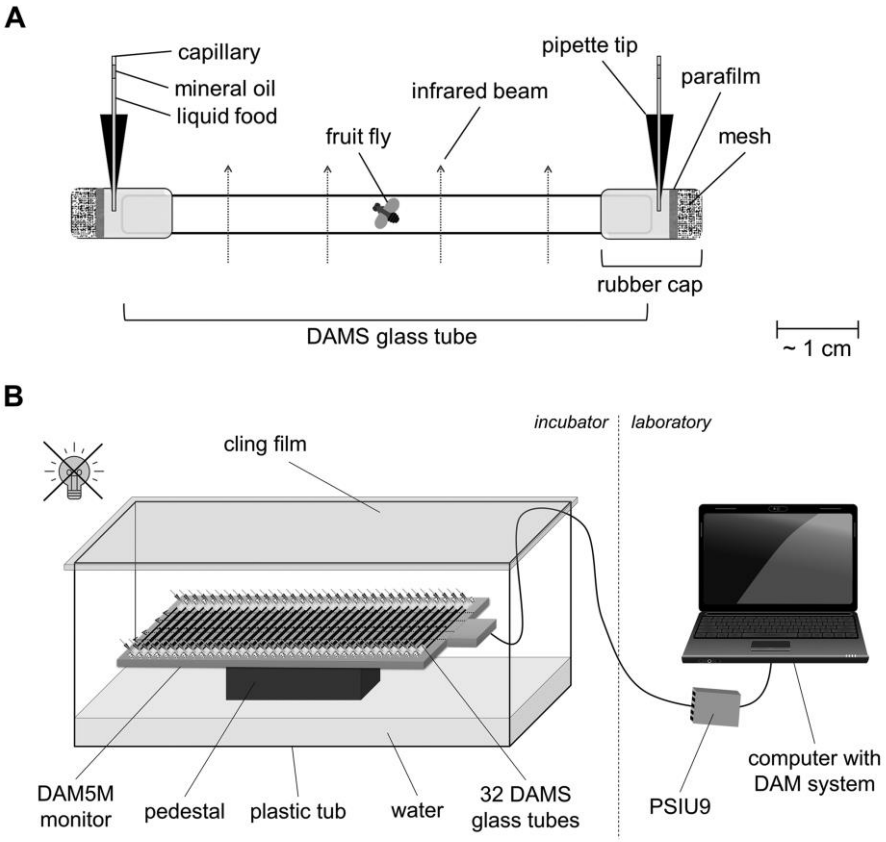

Fig. 1 FlyCafe-an assay for measuring preferential consumption of food of individual *D. melanogaster.* Adapted from [25]. A) Drosophila Activity Monitoring System (DAMS) glass tubes with rubber cups for capillary insertion on both ends. B) The setup of the FlyCafe installation consisting of a monitor which stores 32 glass tubes with individual flies in the container with water.

3.2.2. Experimental protocol

wt D. melanogaster were collected under the microscope and CO_2 anaesthesia. The flies were put in the vials with food. They were stored in groups, pairs or isolated depending on the four experimental groups. The flies were isolated or kept in groups for 1 or 5 days, in the same conditions as during the cultivation (section 1.1.).

I compared the differences in preference for METH in four experimental fly groups that were differentially treated before the FlyCafe experiment (Fig. 2). The four experimental groups are:

1) Grouped mated (**GM**) – 50 males and 50 virgin females were collected from the cultivation bottles. 25 virgin females and 25 males were put together in a vial with food and let to mate for one (GM–1) or five days (GM–5) in the incubator. On the first day of FlyCafe experiment, females were eliminated and 32 male flies were put in the DAMS monitors.
2) Grouped virgin (**GV**) – 50 virgin males were collected from the cultivation bottles. They were put in the vial with food and kept in the incubator for one (GV–1) or five days (GV–5). The FlyCafe experiment was conducted on 32 male flies.
3) Isolated mated (**IM**) – 50 males and 50 virgin females were collected from the cultivation bottles. In each of 50 vials with food one male and one virgin female were let to mate for 24 hours. The females were eliminated from the vials and males were isolated for one (IM–1) or five days (IM–5) in the incubator. The FlyCafe experiment was conducted on 32 male flies.
4) Isolated virgin (**IV**) – 50 virgin males were collected from the cultivation bottles. Each virgin male was put alone in vial and kept isolated for one (IV–1) or five days (IV–5). The FlyCafe experiment was conducted on 32 virgin male flies.

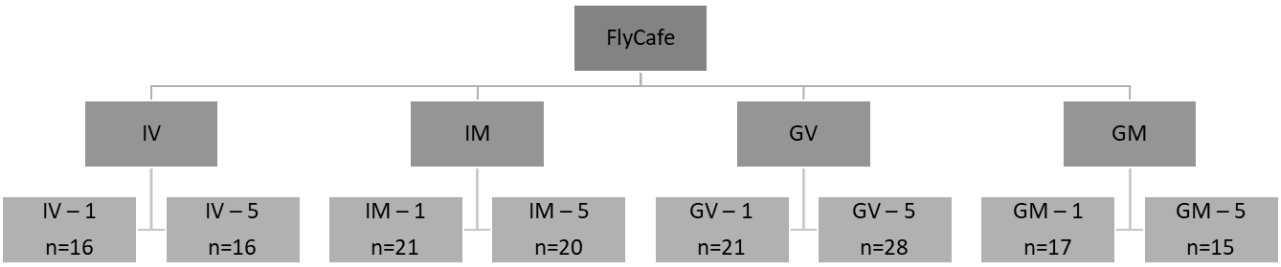

Fig. 2 The experimental protocol. Flies for all four groups were collected under the microscope and CO_2 anaesthesia. The males in IM (isolated mated) group were let to mate for 24 hours after which the females were removed. All four groups (IV (isolated virgin), IM, GV (grouped virgin) and GM (grouped mated)) were put in vials and in incubator for one (Group–1) or five (Group-5) day(s) (n = 50/vial). After five or one day(s) males were transferred in DAMS monitors (n = 32 * 4 groups) and the FlyCafe procedure was done. The n refers to the number of male flies alive after 3 days of FlyCafe.

3.3. Data processing and statistical analyses

Initial data processing was done using the MS Excel program. Further statistical analyses were done with GraphPad Prism. We used two–way ANOVA and Tukey's and Šidak's multiple comparison statistical tests.

4. Results

4.1. Social isolation decreases METH self–administration in male *D. melanogaster*

Our first aim was to determine the influence of social isolation in *D. melanogaster* on the self–administration of METH, based on the preliminary data which shows that social isolation has an effect on the METH preference. I tested the hypothesis that if social isolation has an effect on METH preference in *D. melanogaster*, then there will be a difference in METH self–administration in socially isolated flies compared to CTRL and group housed flies.

D. melanogaster males (virgin and not virgin) were isolated for one or five days preceding the FlyCafe experiment. The control group were males kept in the groups of 40 for one or five days. The additional control group were male flies introduced to experiment directly from the cultivation bottles (males and females together). In FlyCafe flies were offered the regular food on one side and food with addition of METH on the other side. Each group (CTRL, isolated and group housed flies) had an additional CTRL group which were offered food on both sides. Preference was calculated from the amount of food and METH food drank every day and expressed in µl/fly/day.

Fig. 3 shows results of the experiment done on *D. melanogaster* that were isolated for one day before the FlyCafe experiment. For comparison are flies that were group housed with 40 male individuals for one day (grouped) and flies that were put in the monitor directly from the cultivation bottle (CTRL). The preference for METH self–administration is statistically significantly lower in isolated flies compared to the CTRL group (Fig. 3). Isolated males showed aversion to METH (Fig. 3 A). Flies that were left in the group of 40 males for one day show preference for METH, but in comparison with isolated flies there is no statistically significant difference. The largest difference is present on the second day when the preference in isolated flies is significantly decreased compared to the other groups and rises again on the third day (Fig. 3 B). Grouped and CTRL group have constant preference throughout three days. Isolation shows significant influence on the preference for METH and the further experiment focused on the influence of different periods of isolation.

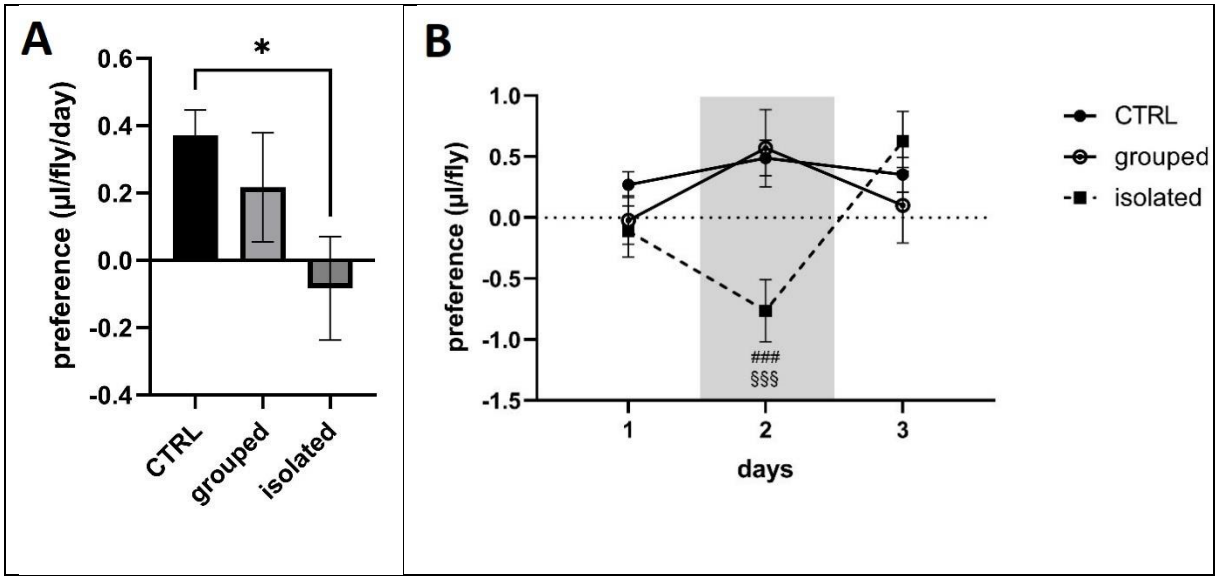

Fig. 3 The one-day isolation reduces preference for METH self-administration. *wild type Canton S.* males of *D. melanogaster* were isolated for one day (isolated), in the groups of 40 (grouped) or put in the monitor directly from the cultivation bottles (CTRL) (n = 32*3). On one side flies are offered food and on the other food + methamphetamine (experimental group) or food on the both sides (control) for three days. The amount of food drunk is measured in mm and the preference is calculated (µl/fly/day). The error bars represent standard deviation. **A) The average preference for all three days of experiment.** One–way ANOVA and Tukey's multiple comparison statistical test was done. * = p = 0.040. **B) Preference on individual days.** Two–way ANOVA and Tukey's multiple comparison statistical test was done. ### = p = 0.0001 (CTRL vs. isolated), §§§ = p = 0.0001 (grouped vs. isolated).

Fig. 4 shows the results of the experiment done on *D. melanogaster* isolated for one or five days before the FlyCafe experiment and for flies which were put in the monitor directly from cultivation bottles (CTRL). Preference for METH self–administration shows a statistically significant decrease (negative preference) after one day of isolation compared to the CTRL group (Fig. 4 A). After five days of isolation there is no preference, instead flies show aversion to METH. In the case of one day isolation, there is aversion on the second day, and preference on the third. (Fig. 4 B). In the case of five days of isolation, aversion increases over 3 days. The preference in the CTRL group is positive and constant during all three days. Based on these findings, I decided to further test the effect of different durations of isolation and grouping on the METH preference in *D. melanogaster*.

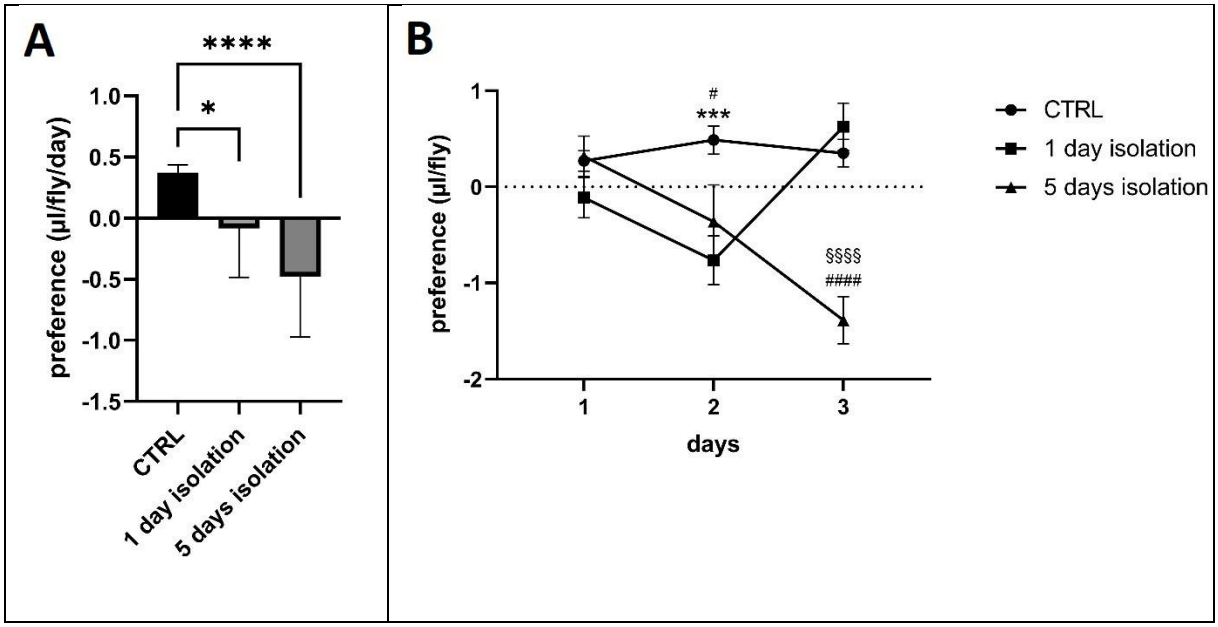

Fig. 4 Isolation for five days further decreases the preference for METH self–administration. *wild type Canton S.* males of *D. melanogaster* were isolated for one day (1 day isolation), five days (5-day isolation) or put in the monitor directly from the cultivation bottles (CTRL) (n = 32*3). On one side flies are offered food and on the other food + methamphetamine (experimental group) or food on the both sides (control) for three days. The amount of food drunk is measured in mm and the preference is calculated (µl/fly/day). The error bars represent standard deviation. **A) Average preference for all three days of experiment.** Two–way ANOVA and Tukey's multiple comparison statistical tests were done. * = p = 0.0255, *** = p = <0.0001 **B) Preference on individual days.** Two–way ANOVA and Tukey's multiple comparison statistical tests were done. *** = p = 0.0001 (CTRL vs. 1 day isolation), # = p = 0.0199 (CTRL vs. 5 days isolation), #### = p < 0.0001 (CTRL vs. 5 days isolation), §§§§ = p < 0.0001 (1 day isolation vs. 5 days isolation).

4.2. Duration of isolation/grouping has an effect on METH preference in *D. melanogaster*

The next experiments focused on the effect of duration of social isolation on METH preference, as well as differences between group housed and isolated and mated or not mated male *D. melanogaster*.

First, I analysed the effect of different duration of isolation/grouping on the METH preference. I tested the hypothesis that if the duration of social isolation and the duration of sexual deprivation has an effect on the METH preference there will be difference in METH self–administration between groups of flies that were isolated for different periods of time.

Flies were separated in four groups and treated accordingly – isolated virgin (IV), isolated mated (IM), grouped virgin (GV) and grouped mated (GM). Flies were in groups (with other males or females) or isolated for one or five days. After that period, they were put in the FlyCafe and the preference for METH self-administration was tested. Flies were offered regular food on one side and food with METH on the other side, while the CTRL group was offered regular food on both sides. The preference was obtained from the amount of food in capillaries that the flies drank and expressed in µl/fly/day.

Fig. 5 compares METH preference of IV and IM flies, isolated for one or five days (Fig. 5 A), and GV and GM flies, isolated for one or five days (Fig. 5 B), averaged for the first day of the FlyCafe experiment. There are no statistically significant differences between IV and IM or GV and GM flies that were isolated/group housed for the same amounts of time. There is also no statistically significant difference between IV and IM or GV and GM flies that were isolated/group housed for different amounts of time. However, IV and IM flies have positive METH preference after both one or five days of isolation, while GV and GM flies have negative METH preference.

Although not statistically significant, there is a trend for differences in preference depending on the duration of isolation/grouping. IV and IM flies isolated for one day have higher positive preference) for METH than those isolated for five days (Fig. 5 A). GV flies show no difference in negative METH preference depending on the duration of grouping, while GM flies that were group housed and let to mate for five days show increased aversion compared to GM flies group housed for one day (Fig. 5 B). Based on the findings, I further looked into the different effects of social experiences in different groups of flies (IV, IM, GV and GM).

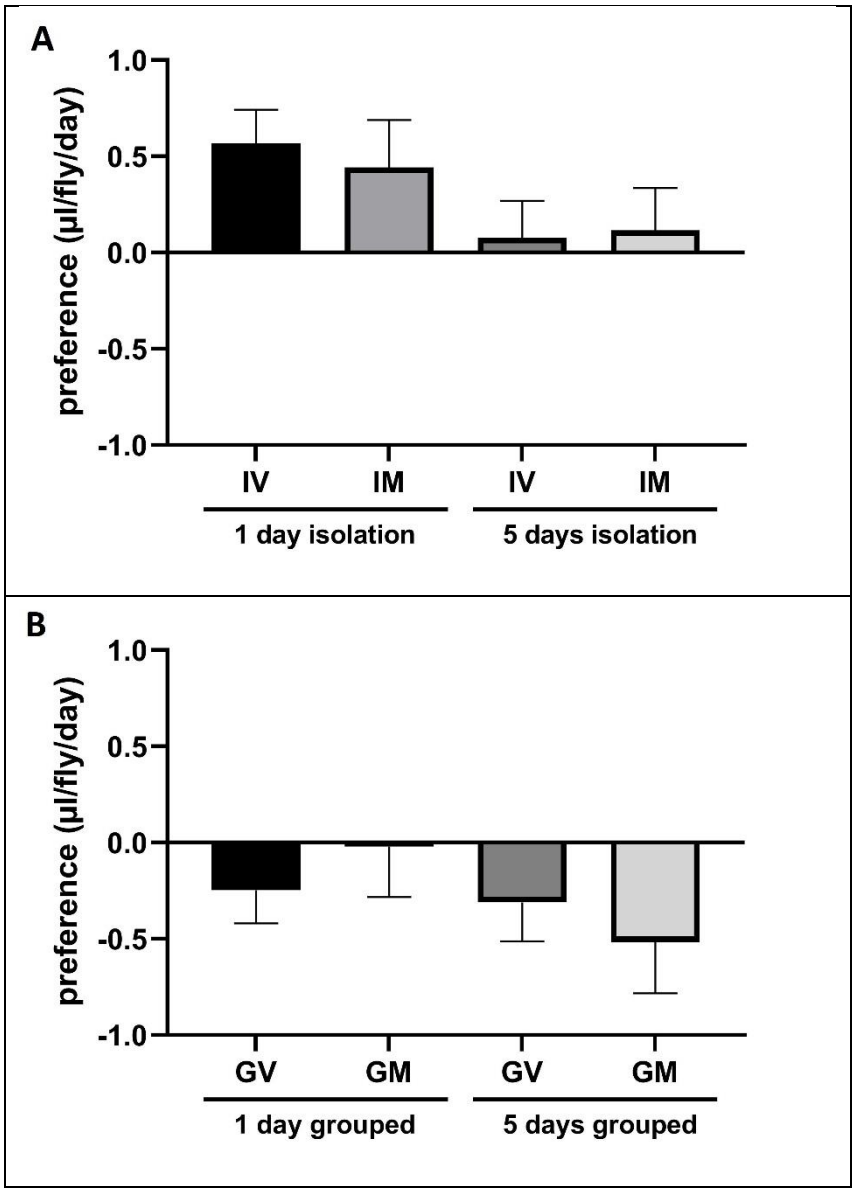

Fig. 5 METH preference in males of *D. melanogaster* depends on duration of isolation/grouping. 50 males of *D. melanogaster* were divided in four groups (isolated virgin (IV), isolated mated (IM), grouped virgin (GV) and grouped mated (GM)) and treated according to the protocol for each group. After 1 or 5 days, 32 males (n = 4*32 for each duration of isolation/grouping) were placed individually in glass tubes in Drosophila Activity Monitoring (DAM) monitor. The FlyCafe experiment was conducted. On one side flies are offered food and on the other food + methamphetamine (experimental group) or food on the both sides (control) for three days. The amount of food drunk is measured in mm and the preference is calculated (µl/fly/day) for four groups for the first day of FlyCafe experiment. One–way ANOVA statistical test was done. There are no statistically significant differences. The error bars represent standard deviation. **A) Isolated virgin and isolated mated flies. B) Grouped virgin and grouped mated flies.**

4.3. Group housed and mated flies show change in METH preference dependent on duration of isolation

Next, I compared the METH preference between four groups (IV, IM, GV, GM) that were isolated or group housed for the same amount of time (Fig. 6). The hypothesis is that if social experiences (grouping/isolation and mating) have an effect on METH preference, then there will be differences in METH self–administration between four groups. METH preference, expressed in µl/fly/day, was averaged for 1st and 3rd day of FlyCafe experiment for four groups.

GV flies after one day of grouping show negative METH preference while other groups show positive METH preference (Fig. 6 A). IV and IM flies show no statistically significant difference in METH preference compared to group housed flies after one day of isolation. GM flies show a statistically significant difference in METH preference compared to GV flies after one day of grouping (Fig. 6 A).

After five days of isolation, GV and GM flies show negative METH preference, while IV and IM flies show positive METH preference (Fig. 6 B). There is no statistically significant difference in METH preference between GV and GM flies, but GM flies show a higher negative METH preference than GV flies. There is also no statistically significant difference in METH preference between IV and IM flies. There is a statistically significant difference in METH preference when comparing GM flies to IV and IM flies (Fig. 6 B).

METH preference in GV flies is the same after one or five days of grouping. METH preference of IV and IM flies is slightly decreased after five days of isolation compared to one day of isolation. GM flies show the largest difference in METH preference. Preference is positive after one day of grouping and is negative after five days of grouping. Next, I decided to analyse the differences in preference on different days of the FlyCafe experiment.

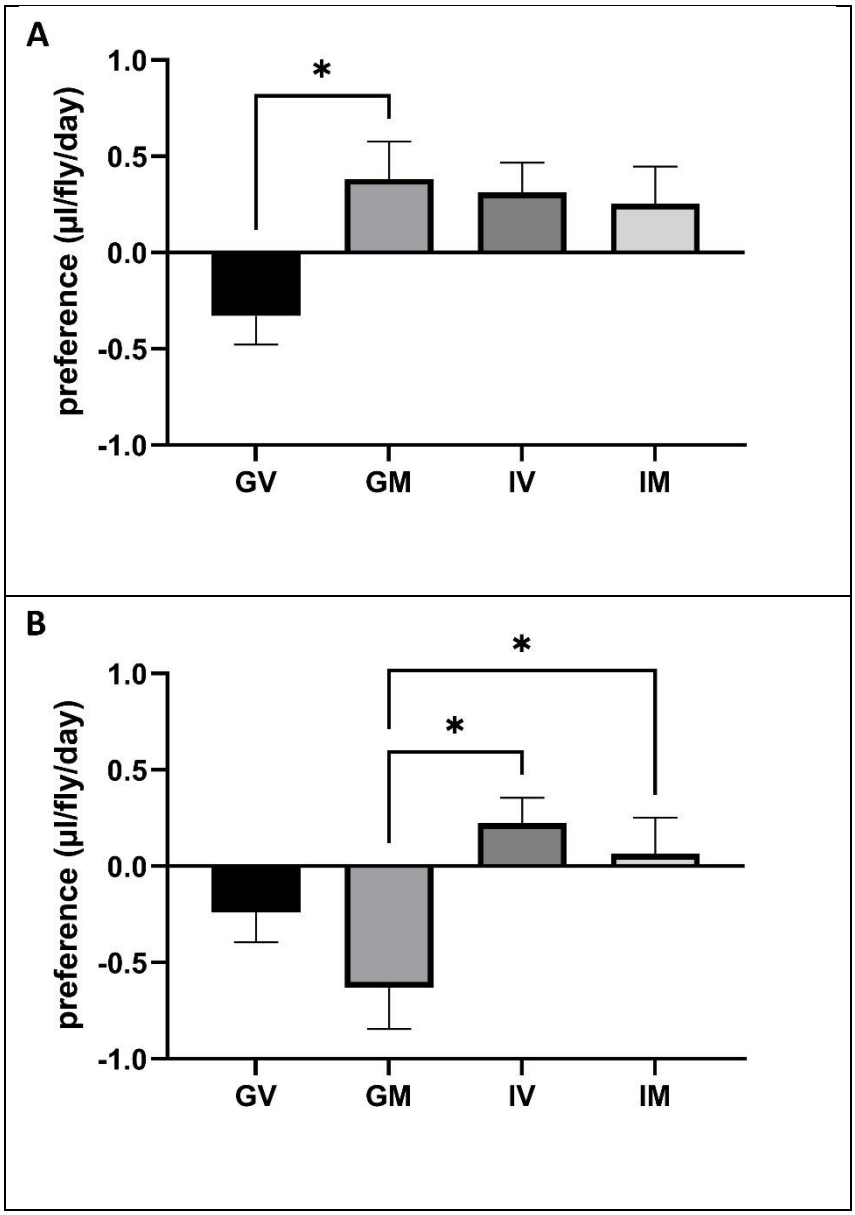

Fig. 6 Group housed and mated flies show the largest change in METH preference depending on the duration of isolation/grouping. 50 males of *D. melanogaster* were divided in four groups (isolated virgin (IV), isolated mated (IM), grouped virgin (GV) and grouped mated (GM)) and treated according to the protocol for each group. After 1 or 5 days, 32 males (n = 4*32 for each duration of isolation/grouping) were placed individually in glass tubes in Drosophila Activity Monitoring (DAM) monitor. The FlyCafe experiment was conducted. On one side flies are offered food and on the other food + methamphetamine (experimental group) or food on the both sides (control) for three days. The amount of food drunk is measured in mm and the preference is calculated (µl/fly/day) for four groups and averaged for the first and third day of the FlyCafe experiment. Errors bars represent standard deviation **A) METH preference in four groups after 1 day of isolation/grouping.** One–way ANOVA and Tukey's multiple comparison statistical test was done. * = p = 0.0231 **B) METH preference in four groups after 5 days of isolation/grouping.** One–way ANOVA and Tukey's multiple comparison statistical test was done. * = p = < 0.05

4.4. Direction of the change in METH preference depends on experimental conditions

Furthermore, I analysed how METH preference changes during the three days of FlyCafe experiments and how four groups differ on different days depending on the common factor (isolation, grouping, virgin, mated). Fig. 7 shows changes during the FlyCafe experiment for the groups that were isolated/group housed for one day preceding the FlyCafe experiment. The preference was observed based on the amount of food in capillaries that the flies drank and expressed separately for each day of FlyCafe in µl/fly/day.

IV and IM flies show no statistically significant difference in METH preference during the three days (Fig. 7 A). The preference is positive during all three days and has a slightly declining trend.

GV and GM flies have similar METH preference on the first day (Fig. 7 B). The preference increases for both groups on the second day. On the third day preference in GM flies increases even further while the preference in GV flies decreases. There is a statistically significant difference in METH preference between GV and GM flies on the third day of the FlyCafe experiment.

GV and IV flies show a statistically significant difference in METH preference on the first day of the FlyCafe experiment (Fig. 7 C). IV flies show positive METH preference while GV flies show negative METH preference. On the second day preference in GV flies increases slightly, while preference in IV flies decreases to a slightly positive value. On the third day preference in IV flies keeps decreasing, while preference in GV flies decreases, after the increase on the second day.

GM and IM flies show no statistically significant difference in METH preference during the three days (Fig. 7 D). IM flies show positive preference on the first day of the FlyCafe experiment, while GM flies show no preference. GM flies show a trend of increasing preference while IM flies show a trend of decreasing preference.

Based on these results, future experiments should focus on the prolongation of the FlyCafe experiments and see how the preference further changes as the days go and experiments are longer.

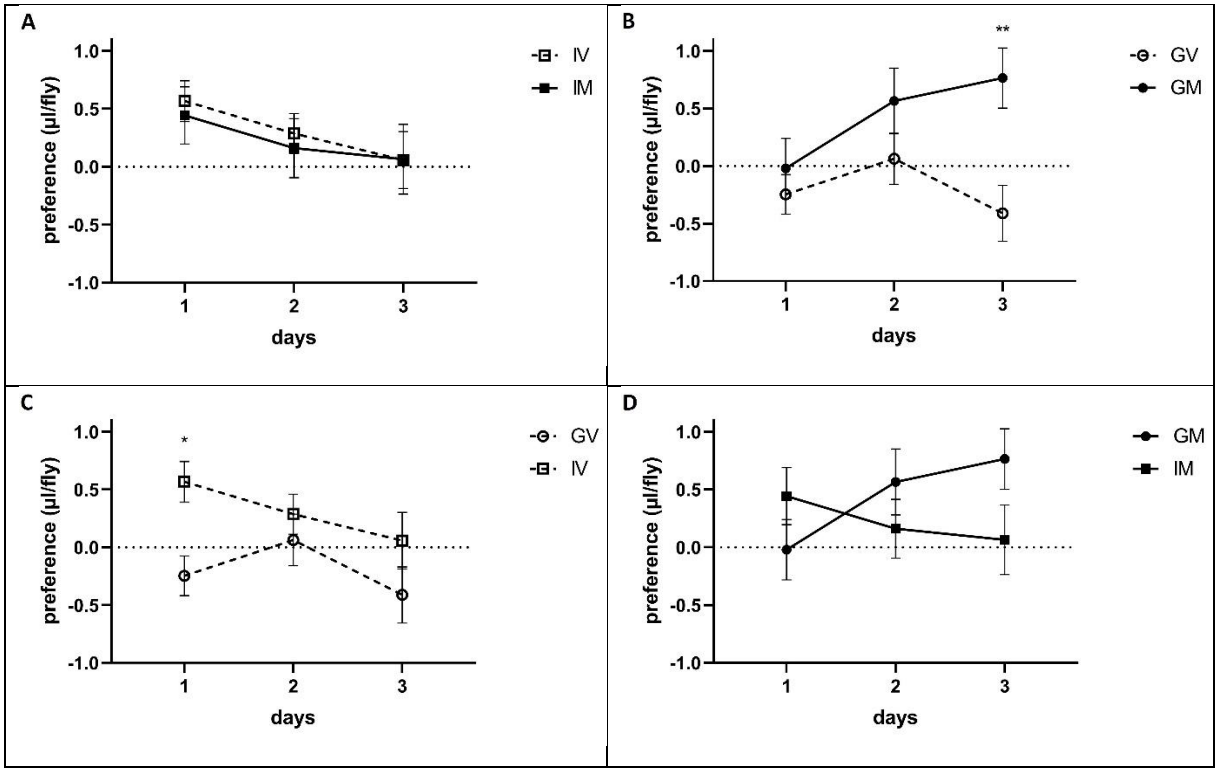

Fig. 7 Isolation has predominant effect on METH preference compared to mating experience. 50 males of *D. melanogaster* were put in four groups (isolated virgin (IV), isolated mated (IM), grouped virgin (GV) and grouped mated (GM)) and treated according to the protocol for each group. After isolation or grouping period (1 day) 32 males (n = 4*32) were placed individually in glass tubes in Drosophila Activity Monitoring (DAM) monitor. The FlyCafe experiment was conducted. On one side flies are offered food and on the other food + methamphetamine (experimental group) or food on the both sides (control) for three days. The amount of food drunk is measured in mm and the preference is calculated (µl/fly/day) for four groups. The results were compared in regards to four factors. Error bars represent standard deviation. **A) Isolated males.** Two–way ANOVA, Tukey's and Šidak's multiple comparison statistical tests were done. There are no statistically significant differences. **B) Group housed males.** Two–way ANOVA, Tukey's and Šidak's multiple comparison statistical tests were done. ** = p = 0.0022 **C) Virgin flies.** Two–way ANOVA, Tukey's and Šidak's multiple comparison statistical tests were done. * = p = 0.0241 **D) Mated flies.** Two–way ANOVA, Tukey's and Šidak's multiple comparison statistical tests were done. There are no statistically significant differences.

5. Discussion

Many previous studies have investigated the influence of social isolation and sexual deprivation on preference for psychostimulants in various model organisms, mainly mice and rats [20, 26]. It has been shown that rats and mice, which were exposed to the social isolation in adolescence, showed preference for drugs, including METH. Rodents also show hyperactivity, increased exploratory behaviour and disturbance in social behaviour [27]. In flies, social isolation had an effect on many behaviours like sleep, aggression, starvation and male courtship [10].

The influence of social isolation in *D. melanogaster* on METH addiction has not been studied. One study focused on the influence of sexual deprivation on ethanol preference [23]. This study showed that flies that were sexually deprived or rejected had higher preference for food with ethanol. It also focused on the NPF and its role in the rewarding effect of sexual experience and ethanol.

It has been shown previously that four-day social isolation has an effect on epigenetic and transcription in dopaminergic neurons within brain of *D. melanogaster* [11]. As the dopaminergic system and DA are important part of the reward pathway and the rewarding effect of natural (sex, socialisation) and artificial rewards (METH), this thesis tested how sexual deprivation and social isolation affects METH preference. This is the first time that the effect of the social isolation and sexual deprivation was analysed in the context of preference for METH, using the FlyCafe experiment.

In the first experiment, one – day isolated flies had negative preference for METH compared to the group housed flies and to the CTRL flies, which had the highest preference (Fig. 3 A). This is unexpected, as previous studies with rats and mice showed that isolated individuals have the highest preference for psychostimulants [20,26]. It is possible that one-day isolation is too short to have a strong effect on the METH preference. It is also unexpected that CTRL flies that were introduced to the experiment directly from cultivation bottles (females + males) had higher preference compared to flies group housed with other males. CTRL flies had a higher chance of mating and, therefore, more natural rewards.

CTRL and group housed flies showed consistent positive preference during three days of FlyCafe, while isolated flies had a drop in preference on the second day, which is statistically significant compared to other two groups, and again positive preference on the third day (Fig. 3 B). It is possible that there is adjustment time where on the first day flies learn on which side is which kind of food. On the second day the taste of METH may be aversive and predominant factor while on the third day it starts to be rewarding. It would be interesting to see if the pattern would continue on the fourth and fifth day of the experiment.

Alternatively, it is possible to analyse the preference on the first day of experiment, as on the second and third day all flies are individually in the tubes and isolated. So, the first day should give the best representation of the pre-treatment. The isolated and group housed flies have the same preference on the first day which would indicate that isolation for one day did not lead to significant difference.

Five-day isolation led to the even more negative preference to METH (Fig. 4 A). This is consistent with the previous finding that one–day isolation leads to the negative preference compared to CTRL and group housed flies. Some previous studies have shown a connection between negative social experiences with development of anhedonia [28]. Anhedonia is defined as inability to feel pleasure in normally pleasurable activities. It is possible that, following the stressful event, flies develop anhedonia and lose their interest for METH self–administration. That could partially explain the negative preference for METH after one–day isolation and even more negative preference after five – day isolation. As flies show a negative, and not neutral preference, there may also be another mechanism that would explain their aversion to METH.

The pattern of preference during the days of the FlyCafe experiment is different after five–day isolation compared to one–day isolation (Fig. 4 B). Preference drops on the second day, compared to CTRL, as well as in the case of one–day isolation but does not restore on the third day, preference drops even further. The dopaminergic system is highly adjustable. We would assume that flies isolated for five days had a longer period of negative social experience and, therefore, lower levels of baseline dopamine. Flies isolated for one day would have less negative social experience and higher levels of baseline dopamine than flies isolated for five days. CTRL flies should have the highest levels of baseline dopamine as they did not have the negative social experience.

CTRL flies did not show significant changes in METH preference, therefore, their DA levels stayed consistent. In the case of flies isolated for one day, preference drops on the second day after the possible initial rise in the DA production. On the third day preference for METH restores. In the case of flies isolated for five days there is an initial preference for METH and then preference keeps dropping. It is possible that the dopaminergic system adjusted to the negative social experiences. It has been shown that socially isolated rats have a heightened DA response to rewarding and aversive

stimuli [29]. The initial administration of METH on the first day of FlyCafe may have released higher amount of DA in flies isolated for five days, so they administrated less METH food on the following days. Also, there may have been some changes in the sensitivity to DA or in the number of DA receptors which would affect the changes in DA levels. In flies isolated for one day initial DA release could be smaller so they seek more METH on the third day. CTRL flies did not have a change in DA release so their administration of METH food was constant.

In the next experiments I further analysed the effect of different duration of grouping/isolation but also more strictly focused on defining the test groups. In the previous two experiments, males that were isolated or group housed were mixed virgin and non–virgin. In the further experiments, I used four groups with strictly defined virgin and non–virgin males. Therefore, the mating experience was controlled more closely. This was based on the previous studies which showed that mating experience has an effect on the ethanol preference [22] as well as on the results (Fig. 3 A) that show different preference of flies that were introduced to experiment directly from cultivation bottles. As cultivation bottles store male and female flies, compared to males only group, it is a reasonable assumption that males in the cultivation vials have mated.

Four groups that are analysed in further experiments are IV, IM (mated for 24 hours with one female before isolating), GV and GM (let to mate for the whole period of grouping). Fig. 5 compares the effect of different duration of isolation on different groups. The preference is averaged for the first day of the FlyCafe experiment as it is considered to be the most representative of the pre-treatment effects. IV and IM groups show positive preference (Fig. 5 A) which is expected and in accordance with previous studies that have shown the preference for psychostimulants following social isolation and sex deprivation [22,26]. The positive preference is higher for IV and IM flies isolated for one day compared to the five–day isolation. The IV and IM group shows almost the same level of preference

which shows that effect of social isolation is predominant. This is opposite to the finding that mating experience has higher influence on the ethanol preference [23].

GV and GM flies show avoidance regardless of grouping duration (Fig. 5 B). That is expected based on the previous studies that show lower preference for drugs and ethanol following the positive social experience [26]. GV flies have the same preference after one and five days of grouping. GM flies have increased aversion for METH after five days of grouping. This shows the positive effect of longer grouping and mating duration. Flies were exposed longer to the source of natural rewards and had lesser need for the rewarding effects from artificial sources.

GM males show the largest difference in METH preference depending on different duration of grouping/isolation (Fig. 6). IV, IM and GV flies show just a slight change in preference regarding grouping/isolation duration. GM flies show preference after one day of grouping with statistically significant difference compared to GV flies that have negative preference (Fig. 6 A). This is surprising as it would be expected that GM flies have the largest effect of natural rewards. There are studies that show that flies reach the highest mating efficacy after three days of age [30]. As GM flies are let to mate for only one day, and female flies are collected as virgins, they have not reached their optimal mating efficacy and we cannot be sure whether males put in the FlyCafe experiment had the opportunity to mate in that period. Flies that were group housed and let to mate for five days reach their optimal mating potential. After five days of grouping, GM flies show aversion for METH with statistically significant difference compared to IM and IV flies (Fig 6 B). This is the largest difference depending on the duration of isolation/grouping. In this case, mating has a significant effect on METH preference. This is consistent considering that GM and IM flies isolated/group housed for one day and IM flies isolated for five days were let to mate for only 24 hours, while GM flies group housed for five days had a mating period of five days. Considering that mating efficacy is highest on

the third day, it is expected that GM flies mated for five days show the highest effect of mating.

As was seen from the results on Fig. 3 and Fig. 4, preference changes during the days of the FlyCafe experiments. I further analysed how this preference changes in regard to four factors: isolation, grouping, mating and virgin flies. IV and IM flies show similar preference during all three days of the FlyCafe experiment (Fig. 7 A). It is expected that isolated flies would have similar preference. This is also consistent with the previous conclusion that isolation/grouping has larger effect on METH preference compared to the mating experience. The preference is also slightly declining which may be the result of flies getting the reward from METH and losing the need for reward over 3 days of the experiment.

GV and GM flies show differences in preference, especially on the third day of the FlyCafe experiment (Fig. 7 B). GM flies, surprisingly, show positive preference for METH while GV flies show aversion for METH. It is possible, as results are based on the flies group housed/isolated for one day, that GM flies experienced rejection. As previously stated, one day old female virgins do not reach their maximum mating efficiency, so it is possible that the males were rejected. As it has been previously shown, rejection may have an even stronger effect than lack of mating on the preference for psychostimulants [23].

IV flies show positive preference while GV flies show aversion (Fig. 7 C). This is consistent with the conclusion that isolation has a larger effect on METH preference. The statistically significant difference is on the first day of experiment, which is considered the most representative.

On the other hand, GM flies have higher and positive preference for METH compared to IM flies, except on the first day (Fig. 7 D). As first day is considered most representative, it is possible that it is the best reflection of the pre – treatment effect. The preference of IM flies drops after the first day while preference of GM flies rises. As both groups mated for only 24 hours with virgin females, the chances of being rejected are present in both groups. GM flies may have had higher chances of rejection as males were group housed with larger number of females which may have rejected numerous males. It is also possible that, after the first day, GM flies, which are not used to isolation, started having higher preference for METH because of the new state of isolation in the FlyCafe experiment. As IM flies have been isolated previously, this did not have such an effect on them.

The data in this thesis shows that, when averaged for all three days of the FlyCafe experiment, isolated flies show an aversion for METH while flies that were group housed with females show highest preference. The negative preference increases further with the longer duration of isolation. Further experiments showed the positive preference in IV and IM flies and aversion in GV and GM flies when averaged for the first, most representative day. GM flies vary the most when comparing the duration of grouping, probably due to the fact that flies reach the highest mating efficacy after three days of age. Therefore, testing the effect of mating should be done with older flies or for the longer time period. Data from Fig. 7 confirms that isolation has a larger effect than mating, but experiments with older flies, or with longer mating period should be considered to further investigate the effect of mating. Also, the FlyCafe experiment isolates flies individually in the tubes so the results for the second and third day may not be a good representation of the pre – experimental treatment of different groups, especially for the group housed flies. The experiment that would enable flies to stay in the same environment as the pre – treatment may help to provide more reliable results on the all days of experiment.

The transcriptional and epigenetic bases of this results should be further investigated. NPF levels seem to be mostly influenced by the mating experience [23] which further influences ethanol preference. It is possible that NPF does not have such an important role in METH preference, therefore, mating experience showed to be of the lesser influence. OA has also been shown to have a main role in the rewarding response to sweetness [4]. Considering that METH and other drugs have bitter taste, it is quite possible that OA has a smaller or no role in the METH preference. So far, the studies on other model organisms showed the highest effect of DA reward system in relation to psychostimulants [4,11], so that could probably be the base of development of METH preference post social isolation and sex deprivation.

6. Conclusion

The results from this thesis show that social isolation and sexual deprivation have an effect on the preference for METH of *D. melanogaster*. It seems that social isolation has larger effect on the preference to METH compared to the effect of sexual deprivation. On the other hand, when flies were allowed to mate for five days, that had a significant effect and flies developed aversion to METH. Further research of the effect of sexual deprivation on the preference to METH of *D. melanogaster* is needed to obtain stronger evidence. In most experiments, longer duration of grouping, but also isolation, seemed to have a positive effect by decreasing the preference to METH. This is surprising for isolation and the possible effects of anhedonia and modifications of mechanisms of reward system need to be better understood. Experiments presented in this thesis were also done on the young (virgin) flies. It has been previously shown that social isolation had lesser effect when older mice and rats were exposed to it. It would be interesting to investigate the influence of age in *D. melanogaster* as well.

Overall, this thesis shows that social isolation indeed has an important effect on the METH preference in *D. melanogaster*. Sexual deprivation also has an effect, but it is important to carefully design the study and take into the consideration the optimal mating potential of species to get reliable results. *D. melanogaster* is a good model organism because many genes and transcriptional factors that differ between isolated and group housed flies, as well as signalling molecules of reward system, are orthologue and homologue to those in mammals and humans. Investigating the effects of social isolation is especially important these days when, due to COVID–19 pandemic, we have been exposed to the effects of social isolation more often.

7. Literature

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