The Potential of LSD in Treating Binge Eating: Findings from Two Different Experimental Designs in Mice

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The advancements in modern food technology have made palatable foods more accessible resulting in somatic and psychological problems. Based on this, a new classification in eating disorders has been proposed: binge eating disorder (BED). Research on 5-HT2A agonist psychedelic compounds is a popular topic and preliminary clinical trials show promising results in the treatment of many psychiatric conditions and emotional problems. However, research specifically addressing psychedelics and BED is limited.

This master's thesis aimed to observe whether the classical psychedelic lysergic acid diethylamide (LSD) influences the consumption of normal and palatable foods in mice. Binge eating was operationalized in two different experimental designs. The first one was sucrose escalation where the mice received ordinary and sucrose tablets from a feeding device. The second experiment, the high-calorie fat bingeing, divided the mice into three groups that would get standard rodent chow and high-calorie fat pellets either continuously or intermittently for 24 hours. After testing the levels of eating, the effects of different doses of LSD on eating behaviors were investigated.

The results indicated that the mice in the sucrose escalation experiment did not show a preference for the hedonic sucrose pellets although the sucrose group consumed more food overall compared to the control. LSD did not affect their eating patterns. In the high-calorie fat bingeing experiment, the intermittent group showed significant fat bingeing at baseline, but the psychedelic effect did not manifest after administering three different doses of LSD.

The results raise questions about the suitability of LSD in treating BED and in general if animal models shed light on the emotional and psychological complexity of psychiatric diseases. This master's thesis and its findings pave the way for future applications for psychedelic research for binge eating disorder and food addiction.

Keywords: binge eating disorder (BED), psychedelics, serotonin (5-HT), LSD, mouse models

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Muutokset modernissa ruokateknologiassa ovat helpottaneet epäterveellisten valintojen tekemistä, mikä on johtanut somaattisiin ja psykologisiin ongelmiin. Tähän perustuen uudeksi psykiatriseksi häiriöksi on lisätty ahmintahäiriö. Tutkimus 5-HT2A agonististen psykedeelien tutkimus on nouseva trendi. Alustavien kliinisten kokeiden tulosten perusteella niistä voi olla apua monissa psykiatrisissa sekä tunne-elämän häiriöissä. Psykedeelien vaikutusta ahmintahäiriöön ei kuitenkaan ole juurikaan tutkittu.

Tämän pro gradu -tutkielman tavoitteena oli tarkastella vaikuttaako klassinen psykedeeli lysergihapon dietyyliamidi (LSD) hiirten normaaliin sekä herkkuruuan kulutukseen. Ahmiminen operationalisoitiin kahdella eri koeasetelmalla. Sukroosin eskalaatiokokeessa hiiret saivat sekä tavallista ruokaa että sukroositabletteja ruokinta-automaatista. Toisessa korkeakalorisessa rasva-ahmintamallissa hiiret jaettiin kolmeen ryhmään, jotka saivat tavallista jyrsijäpellettiä ja rasvaruokaa jatkuvasti tai 24 tunnin ajan. Kun syömistä oli testattu, tutkittiin eri LSD-annosten vaikutuksia syömiskäyttäytymiseen.

Sukroosieskalaation tuloksista ei ollut havaittavissa hiirten mieltymystä sukroosipelletteihin, vaan sukroosiryhmän hiiret söivät ylipäätään sekä tavallista ruokatyyppiä että sukroosia kontrolliryhmää enemmän. LSD ei vaikuttanut hiirten syömiskäyttäytymiseen. Runsaskalorisessa rasva-ahmintakokeessa rajoitetun ajan rasvaa saaneet hiiret ahmivat perustasolla rasvaa merkitsevästi muihin ryhmiin verrattuna, mutta psykedeelivaikutusta ei ilmennyt kolmella eri LSD-annoksella missään ryhmässä.

Tulokset herättävät kysymyksiä LSD:n soveltuvuudesta ahmintahäiriön hoidossa ja yleisesti siitä, mallintavatko eläinmallit tarpeeksi psykiatristen sairauksien psykologista ja emotionaalista kompleksisuutta. Tämä pro gradu -tutkielma ja sen tulokset luovat pohjaa tuleville tutkimuksille psykedeelien vaikutuksista ahmintahäiriöön ja ruokariippuvuuteen.

Avainsanat: ahmintahäiriö, psykedeelit, serotoniini (5-HT), LSD, hiirimallit

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1. INTRODUCTION

In modern society, the changes in food technology have facilitated access to palatable foods: we eat on the go, snack often, and consume more calories than necessary (Turton et al., 2017). This raises an inquiry of whether humans inherently prefer high-calorie diets over ordinary food and if so, why. Contrary to history, when famine and starvation were the main problems considering food, nowadays obesity and overeating cause several difficult health problems. These come with a serious economic impact: for example, a study conducted by World Obesity Atlas (2023) predicts that globally overweightness and obesity—meaning a BMI <25/m2—will reach 4 billion people by 2035, meaning a total cost of $4 trillion for countries all over the world. The economical cost itself is already motivational enough for the governments to tackle the issue. The question raised then is: Why does a modern person eat too much, and can there be some kind of aid for it?

When eating turns abnormal, a set of diseases called eating disorders come into the picture. To summarize, they are psychological and somatic conditions that refer to any kind of atypical eating that manifests in different ways: for example, restricted eating is typical for anorexia nervosa while in binge eating disorder, eating becomes uncontrollable which induces guilt in the patient (Calder et al., 2023). A way to understand the phenomena of overeating and the obesity epidemic is the research on binge eating disorder, BED, that contrary to anorexia or bulimia nervosa, is solely about overeating. Preliminary data suggests that drug abuse and binge eating may manifest similarly in the brain (Rodríquez et al., 2019). This intriguing finding prompts that overeating and addiction are related to each other: it is known that behaviorally and neurally, eating disorders resemble substance abuse (Calder et al., 2023; Fadahunsi et al., 2022). Given the wide economic, social, and individual effects of overweight and obesity, research focused on BED is needed.

Despite the known problems of overeating, an effective and well-tolerated pharmacotherapy for BED is still lacking (Czyzyk et al., 2010). One pharmacological tool, that has raised interest in the research field of eating disorders as well, could be psychedelics (Calder et al., 2023). Clinical interest in these mind-altering substances is resurging from the days when they were seen only as recreational drugs in need of strict controlling. The interest in these substances and their potential in mental health care is seen in the sharp rise of publications and studies in clinical research (Breeksema et al., 2020). Some tentative data on psychedelics and eating disorders exists, for example, Calder et al. (2023) found out in their review that psychedelic-assisted therapy, mainly using psilocybin and ketamine, showed improvement in patients with anorexia and bulimia nervosa.

However, the review did not present any data on the effectiveness of psychedelics in the treatment of BED.

Because of the lack of data in the important study field of BED, this master's thesis aims to find out whether one of the most well-known 5-HT2A agonists, LSD, effects eating behaviors, both in normal and palatable food consumption. The data was gathered via animal models in mice. Unfortunately, legislation and regulations on psychedelics have not reached a point yet where large clinical studies on humans would be easily conducted. Even though the suitability of mouse models in a complex human condition such as BED is always questioned, they can give tentative data on feeding behaviors and pave the way for more thorough clinical research on human BED patients in the future. As stated by Capitanio (2017) however how different humans and mice can be, they share similar functional and structural processes. These can be translated across different species and thus give us information on the processes of pharmacological effects on different substances on behavior.

In this master's thesis, binge eating is operationalized with two different experimental designs. In the first experiment, inspired by Feltmann et al.'s (2018) sucrose escalation on rats, the mice will receive standard rodent chow and sucrose tablets from a feeding device. The aim is to create a so-called negative anticipatory contrast where the mice would start preferring palatable sucrose tablets over ordinary chow tablets. In the second experiment, following the steps of Czyzyk et al.'s (2010) high-calorie fat bingeing experiment, another set of mice will receive standard rodent chow and high-calorie fat pellets. After establishing the desired eating patterns in both experiments, the effects of different doses of LSD on eating behaviors will be investigated.

Since the knowledge and research on the effects of psychedelic compounds on BED are still scarce, this master's thesis sheds some light on the issue and may inspire future research. Economical and psychological distress, caused by overeating and food addiction, are such a huge burden that tools to tackle the issues are worth investigating further.

1.1 Binge Eating Disorder

Even though nowadays BED is considered common, the exact prevalence is hard to estimate. Some data suggest that the prevalence is something between 1.9–3.5% in women and 0.3–2.0% in men, but because of the worldwide obesity epidemic, the numbers are constantly on the rise (Suokas & Rissanen, 2021). Considering the vague prevalence numbers, Scrandis and Arrow (2023) suggest that even though the research data on BED is still scarce, is has the highest lifetime prevalence of eating disorders in the US and worldwide. In overweight and obese populations, even a higher prevalence could occur, even something close to 30% (Kucharska et al., 2017). A typical person suffering from BED is an overweight young woman, but the disorder can affect anyone at any point in life. As it

often tends to be with eating disorders, BED is comorbid with other psychiatric conditions and is often paired with conditions such as anxiety, depression, and substance abuse (Calder et al., 2023). Thus, we cannot describe BED as excessive consumption of palatable foods—the underlying psychological and emotional factors play such a big role that they need to be addressed as well. The main symptom, overeating, is a simple way to operationalize the condition though, especially in pre-clinical animal studies.

In the research of eating disorders and clinical work, no clear consensus on how to define BED exists. This leads to the disorder being often underdiagnosed and treated. In the worst case, the patients in need of treatment are not receiving adequate help simply because the doctors do not have a certain protocol for treatment (Scrandis & Arrow, 2023). Some definitions perceive BED as a distinctive disease while for some it is more like an atypical and undefined form of eating disorder. Suokas and Rissanen (2021), define BED as a separate form of eating disorder and list some typical symptoms for only this condition that include recurring binge sessions with large quantities of food, lack of control, rapid eating until uncomfortably stuffed, and anxiety over continuous bingeing sessions. The bingeing sessions must occur at least once a week for three months and the criteria for bulimia nervosa must not be fulfilled. The DSM-5 manual specifies different severity classes ranging from mild, which refers to 1 to 3 bingeing sessions per week, to extreme which includes 14 bingeing episodes per week (Berkman et al., 2015).

According to Scrandis and Arrow (2023), endocannabinoid receptors in the brain increase appetite. Besides food, these receptors control the reward effects of anything pleasurable. Other neurotransmitters linked to food addiction and binge eating are opioid, serotonin, and dopamine receptors. Scrandis and Arrow (2023) carry on that in BED, alteration of homeostatic hormones, such as ghrelin and leptin, has been noticed. In everyday life, this could manifest in the lack of feeling full after a meal and can thus result in overeating until nauseous and ashamed.

Since its complex nature, there is no simple protocol for treating BED. The best strategy is to combine psychotherapy with some form of medication as well (Novelle & Diéquez, 2018). More in detail, two approaches for the treatment have been introduced: behavioral and cognitive therapy added to weight loss management with the support of weight-controlling pharmacotherapies (Czyzyk et al., 2010). According to Suokas and Rissanen (2021), despite the lack of certain treatment protocols, some commonly used methods are meal diaries, group therapies, and self-treatment guides. The treatment aims to support regular eating habits and stop the cycle of dieting and bingeing. Pharmacotherapies used for BED include SSRI medicines or topiramate (Suokas & Rissanen, 2021). In the United States, the only drug approved for the treatment of moderate or severe BED in adult patients is lisdexamphetamine (Novelle & Diéguez, 2018). The lack of a simple targeting drug to treat

the psychological and somatic symptoms of BED does not exist though (Czyzyk et al., 2010) which raises an interest in developing novel treatments for the disease.

1.2 Animal Models of Binge Eating

Since the rise of psychology, human behavior and psychiatric diseases have been tried to explain through not only human studies but using the aid of pre-clinical animal studies as well. Humans and animals share similar functional and structural processes that can be translated even across different species (Capitanio, 2017). For example, stress can be induced in mice and then measure how it affects eating behaviors (Jahng et al., 2011). The usage of animals to model psychiatric conditions has some ethical and moral discussions yet one of the assets of animal models is that in animal laboratories it is possible to create genetically and environmentally controlled settings (Capitanio, 2017; Van Gestel et al., 2014). For example, in the case of BED and other eating disorders, it would be impossible to calculate calorie intakes and control different food types in humans, while in mice, it is possible to use only certain foods whose ingredients and nutritional characteristics are known precisely. Even though in psychiatric conditions, observing behavior in investigating possible treatments is crucial, human diseases and behaviors modeled in animals vary in their resemblance to human conditions. Thus, simple animal models cannot paint the whole picture in complex phenomena like BED: for example, animal models are not capable of replicating the social context of human behavior or shame and a lack of control (Novelle & Diéguez, 2018). However, pre-clinical animal studies can pave the way for future studies and give novel guidelines on how to proceed to clinical trials.

When it comes to studying eating disorders, animal models are based on the clinical symptoms of humans (Kim, 2012). Based on this idea, animal models for BED are about creating an environment where animals would start consuming excessive amounts of palatable foods and even start restricting the intake of their standard food while waiting for the palatable food to come. This behavior is called the negative anticipatory contrast (Feltmann et al., 2018). According to Kim (2012), the classic study paradigm suggests that restriction of food can induce binge-like eating habits. However, considering the ethics in animal research, improving models that investigate creating as little stress and harm as possible is crucial (Travathan-Minnis & Saphiro, 2021). Paradigms where stress or food restriction is not in use, but the animals still start bingeing palatable foods, have been introduced. Examples of these models include the sucrose escalation model by Feltmann et al. (2018) and the high-calorie fat bingeing model by Czyzyk et al. (2010).

In Feltmann et al.'s (2018) study, rats were kept in food deprivation only for two hours on experiment days. In the 10-session training period, they were put into behavioral chambers with

access to standard rodent chow for 10 minutes from a first feeder of a feeding device, followed by a second feeder with the same chow for 10 minutes. In the actual experiment, the animals were divided into two groups, the test group now receiving high-palatable sucrose pellets from the second feeder while the control continued the same pattern as in the training period. The idea was to create an escalation eating pattern where the rats would inhibit the intake of standard rodent chow from the first feeder when they had learned that they would get palatable food soon from the second feeder. Interestingly, despite the short food deprivation and no stress, the rats started to prefer sucrose quickly and lowered the consumption of standard rodent chow from the first feeder. Besides it does not need long food deprivation or stress, the asset of this model is that the homeostatic hunger has been eliminated meaning that the animals are not bingeing just because they are starving. After the desired feeding behavior was established, the effects of monoamine stabilizer OSU6162 on food consumption were investigated and found that it reduced binge-like eating for sucrose pellets.

Similarly, in the hedonistic high-calorie fat bingeing model introduced by Czyzyk et al. (2010), their mice were not kept in stress nor hunger. At the beginning of the study, the animals were divided into three groups. Group 1, the chow group, received only standard rodent chow pellets while groups 2 and 3 received high-calorie fat pellets in addition to standard rodent chow either continuously or intermittently for 24 hours. The consumption of food was measured after 2.5 and 24 hours. It was noticed quickly that the intermittent group 3, similar to the sucrose escalation rats in Feltmann et al.'s (2018) study, started immediately bingeing the high-calorie fat pellets and intake of standard rodent chow. After the eating patterns were secured, the effects of fluoxetine, baclofen, and topiramate on eating behaviors were investigated since there was some prior research noting that these drugs had clinical efficiency in treating BED. From these drugs, SSRI drug fluoxetine was found to reduce the high-calorie fat pellet intake both at 2.5 and 24-hour timepoint.

As already noted, different neurotransmitters in the brain and the body are linked to binge-like eating behaviors. However, animal studies on the effects of serotonin agonists, such as LSD, on eating behaviors are still scarce. Thus, we do not have much data yet on how these compounds could affect eating.

1.3 The Neurotransmitter Serotonin

5-hydroxytryptamine (5-HT), more commonly serotonin, is one of the main neurotransmitters and hormones in the body (Kaneez, 2017; Nestler, 2020). It functions not only in the central nervous system but in the bowel and platelets as well (Kaneez, 2017; Ritter et al., 2024). The research on serotonergic pathways is relatively new: compared to the first psychedelic compounds that were

already studied in the 1800s, serotonin in the mammalian brain was found as late as 1953 (Nichols, 2018). Serotonin's several roles from homeostasis to cognition are acknowledged (Ligneul & Mainen, 2023) and nowadays, a popular topic in neuroscience is the role of mood, emotions, and mental health—for example, when typing the keyword "serotonin" in Pubmed database, it gives 158,638 results. Thus, there exists a great interest in serotonin's role in different functions as well as with psychiatric conditions.

The serotonergic pathway is located in the brain stem and raphe nuclei (Ligneul & Mainen, 2023; Woolsey et al., 2017). Serotonin is synthesized from the amino acid tryptophan by an enzyme called tryptophan hydroxylase (Gartside & Marsden, 2015). According to Ritter et al. (2024), some of the main actions of 5-HT are increased gastrointestinal motility, contraction of smooth muscle, and excitation or inhibition of neurons in the central nervous system. Psychology is the most interested in the functions of serotonin in the central nervous system. 5-HT affects many functions including appetite, mood, hallucinations, and vomiting (Ritter et al., 2024). 5-HT is associated with many clinical psychiatric conditions such as depression and anxiety that tend to be comorbid with BED.

Along with dopamine and norepinephrine, serotonin belongs to monoamine transmitters. The drugs prescribed for affective and psychotic disorders, such as SSRI, target these monoamines (Gartside & Marsden, 2015). The monoamine systems are essential since they influence mood, arousal, and cerebral flow (Nestler et al., 2020; Woolsey et al., 2017). Nestler et al. (2020) denote that even if the cell bodies of monoamines are restricted to a small number of nuclei, due to their wide axons, they affect the whole brain. This is one explanation for the problem of why drugs have difficulties targeting a single monoamine.

There is no clear consensus on the number of serotonin receptors in the human body, but the researchers have estimated something between 14–16 receptors that are grouped into 7 different families, 5-HT$_{1\text{-}7}$ (Berger et al., 2009; Ligneul & Mainen, 2023). Most of the 5-HT receptors are G-protein coupled except for the 5-HT$_3$ subtype which is a ligand-gated one (Gartside & Marsden, 2015). Considering psychedelic compounds, the main interest is targeted towards 5-HT$_{2A}$ receptors whose main effects include neuronal excitations and behavioral effects (Ritter et al., 2024). Most psychedelics, like LSD or psilocybin, are agonists or partial agonists for 5-HT$_{2A}$ receptors (Hanks & Gonzaléz-Maeso, 2013; Ritter et al., 2024).

Interestingly, different 5-HT receptors affect different eating patterns—for example, modifying the 5-HT$_{1B}$ receptors regulates food intake while 5-HT$_{2C}$ receptors affect the rate of eating (Kim, 2012). This is an intriguing finding considering that in BED, patients consume excessive amounts of food in a short period. Kim (2012) carries on, that genetically altered mice, who have

deletion of certain 5-HT receptors, can give researchers new possibilities for animal models in eating disorders. In general, according to Simansky (1996), 5-HT controls feeding behaviors in mammals, ranging from rodents to humans. More specifically, the 2A subtype may play a role in inhibiting the continuity of eating (Simansky, 1996).

1.4 Psychedelic Compounds

The widely known term psychedelics refers to the compounds that affect the serotonin receptors in the brain (Kelmendi et al., 2022). Originally, the researchers were invested in the possibilities of substances like mescaline or LSD mimicking mental disorders such as schizophrenia—thus back in the day, these substances were known as psychotomimetics (Nichols & Walter, 2020). Since it was later found out that the effects of psychedelics cannot be compared to psychoses a more fitting term, psychedelics, is now in wide use. The term originates from Greek, meaning mind-manifesting, which refers to the substances' ability to alter consciousness (Kelmendi et al., 2022).

Some of the known effects of psychedelics include ego loss, changes in the perception of time and space, hallucinations, acoustic alterations, and changes in the levels of alertness (Vollenweider & Preller, 2020). Because of their subjective manner, the effects differ from person to person. What is different compared to hallucinations induced by psychotic disorders is that the effects of psychedelics are only temporary: for example, the effects of LSD peak in 1–3 hours after administration and wear off in 6–8 hours with the dose of 1μg/kg (Hintzen & Passie, 2010).

Due to their chemical structures, psychedelics can be divided into three different classes: tryptamines, ergolines, and phenethylamines (Liechti, 2017; Kelmendi et al., 2022). Ergolines are non-selective which means that they are highly binding to many monoaminergic receptors in the brain while phenethylamines have their basis in the hallucinogenic effects of mescaline (Poulie et al., 2020). The most clinically studied and well-known psychedelics are psilocybin, a tryptamine, and LSD, an ergoline (Odland et al., 2022).

Classical psychedelics work by interacting with the serotonin receptors in the brain, especially the 5-HT2A receptors (Vollenweider & Preller, 2020). In addition to these serotonergic compounds, there exists a set of substances that induce similar effects, but their mechanism of action is different (Kelmendi et al., 2022). Kelmendi et al. (2022) list compounds such as phenethylamines, such as MDMA, or deliriants, such as scopolamine, or dissociates like PCP. Serotonergic psychedelics can also affect other receptors: for example, LSD and other ergolines are known to act upon dopamine receptors D1 and D2 as well as adrenergic receptors (Vollenweider & Preller, 2020).

According to Vollenweider and Preller (2020), the field of psychedelic-assisted therapy research was popular in the 1950s and the 60s. Even though the methodology was insufficient in these early studies, the tentative results point towards that psychedelic compounds can help alleviate symptoms of psychiatric conditions like depression, anxiety, and personality disorders. The research on psychedelic-assisted therapy on different eating disorders has also given preliminary evidence that these compounds could help in otherwise difficult treated conditions such as anorexia and bulimia nervosa (Calder et al., 2023).

1.5 Lysergic Acid Diethylamide

Lysergic acid diethylamide, LSD, is one of the most well-known psychedelic compounds. It is derived from rye's parasitic fungus called *Claviceps purpurea* (Hintzen & Passie, 2010). At the receptor level, LSD binds to 5-HT1A and 5-HT2C receptors in the brain (Liechti, 2017). However, the psychoactive effects of LSD are related to the brain's 5-HT2A receptors (Nichols, 2018). Compared to other psychedelic compounds, like psilocybin or mescaline, LSD binds more potently to 5-HT2A receptors (Liechti, 2017). Besides the interaction with post-synaptic 5-HT receptors, LSD also acts upon dopamine and adrenaline receptors (Hintzen & Passie, 2010).

As the founder of LSD, Albert Hoffmann reported in 1943 that despite unpleasant visuals and going insane during a trip, the next day, he woke up with a clear head as if a new door to the world had been opened (Carhart-Harris et al., 2016). It could be easy to assume that LSD would elicit frightening, psychosis-like states, while research has shown that LSD is non-toxic and safe in moderate doses is not associated with accidental deaths, suicides and does not have an abuse potential (Gukasyan et al., 2022; Liechti, 2017; Nichols & Grob, 2018). The effects induced by LSD include dream-like consciousness, increased capability for introspection, and alternation of mental association patterns (Hintzen & Passie, 2010).

LSD's known effects, for emotional processing, neuroplasticity, and improved self-perception (Hintzen & Passie, 2010; Ly et al., 2018) may underpin its therapeutic potential in BED. However, many studies conducted for eating disorders usually do not address BED. For example, in Gukasyan et al.'s (2022) review of psychedelic-assisted therapy's potential for eating disorders, the focus was on anorexia nervosa or binge-like eating with purging that is not a characteristic of BED. In a survey conducted by Spriggs et al. (2021) on depression and wellbeing in patients suffering from eating disorders, BED is mentioned along with anorexia and bulimia nervosa. However, because of its qualitative and survey-based nature, the positive results found can be interpreted only as tentative hints that psychedelics could alleviate the psychological symptoms of BED. In this survey, the

psychedelics used were not separated either, so the effects of solely LSD on BED cannot then be described.

The tentative data on LSD's therapeutic possibilities in human BED do not usually address the issue of overeating or consider whether LSD reduces eating in general and binge-like eating patterns. Some findings on LSD's effects on eating behavior have been found in animal studies: for example, an old study conducted by Hamilton and Wilpizeski (1961) showed that rats, who were administered different doses of LSD, suppressed their food intake. However, the researchers noted that this finding should be interpreted with caution since the eating behavior is dependent on food reinforcement. Also, no binge-like eating behavior was investigated in this research. More recently, Elsilä et al. (2022) investigated the effects of LSD in binge-like ethanol drinking in mice. Besides ethanol testing, the researchers also conducted a separate test for food and water intake. It was found that after administering 0.1mg/kg of LSD, the water and intake of standard rodent chow decreased. However, neither of these findings can answer the question of how LSD would affect the intake of palatable foods whether it influences binge-like behaviors, and if so, in what direction.

1.6 Research Questions and Hypotheses

This study aims to investigate eating behaviors in mice in both standard and high-palatable food types and whether LSD influences these feeding behaviors. From these aims, the study questions are:

A) Are the mice in two different experimental designs (sucrose escalation and high-calorie fat bingeing) showing a typical consumption of standard rodent chow tablets and pellets at the baseline?

B) Does LSD affect the consumption of standard rodent chow tablets and pellets in these two experimental designs?

The hypothesis is that LSD would not affect the consumption of the standard rodent chow tablets or pellets, so the homeostatic eating would remain the same throughout the experiments.

C) Are the mice in two different experimental designs (sucrose escalation and high-calorie fat bingeing) showing a preference for the consumption of palatable sucrose tablets and high-calorie fat pellets at the baseline?

D) Does LSD affect the consumption of high palatable foods in these two experimental designs?

The hypothesis is that the mice would start preferring the palatable foods over the standard rodent chow thus creating a negative anticipatory contrast where the anticipation of high palatable food would inhibit the intake of standard rodent chow. The hypothesized effect of LSD is that it would lower the consumption of palatable foods.

2. MATERIALS AND METHODS

2.1 Animals and Housing

All data was gathered at the University of Helsinki, in Biomedicum, in the Laboratory Animal Center. The study was a part of the Loss of Control research group's project in the department of pharmacology, faculty of medicine. This study aimed to investigate whether LSD influences the eating behaviors in mice. All experiments were authorized by the National Animal Experiment Board in Finland (Eläinkoelautakunta ELLA; license ESAVI/1218/2021) and were conducted in spring 2024 from January to June.

At the beginning of the sucrose escalation experiment, there were 21 4-month-old male C57BL/6JRCC mice—however, only 16 made it to the final study. Two mice were transferred to another study and three more mice were removed during the training period because they did not start eating properly from the feeding devices. The mice had ad libitum access to standard rodent chow Teklad (6.5 % fat, 19.1 % protein, 3.1 kcal/g) and water except on the experiment days when the standard rodent chow pellets were removed for two hours. The mice had a reversed dark-light cycle: the lights went off at 9 AM and were turned back on at 6 PM. The mice were either housed in pairs or three in standard plastic cages. One mouse had to be single-housed because its cage mate was removed from the study and a new one could not be introduced because of the risk of fighting.

The high-calorie fat bingeing experiment consisted of 18 4-month-old male C57BL/6JRCC mice. Because the food consumption had to be measured individually, all mice were housed in standard plastic cages during the experiment with ad libitum access to standard rodent chow Teklad (6.5 % fat, 19.1 % protein, 3.1 kcal/g) and water. The mice were on a 12-hour light-dark cycle: the lights turned on at 6 AM and off at 6 PM.

2.2 Experimental Design

The sucrose escalation experiment started with an acclimation period of seven days where the mice were introduced to the researchers and handling. This was followed by a ten-day training period where the animals were daily put into empty plastic cages with a feeding device that had two different feeders called feeder 1 and 2. Before the lights turned off at 9 AM, the racks in the homecage

containing standard rodent chow pellets were removed to secure the hunger in mice during the experiment. After two hours of the removal of the standard rodent chow racks, the animals were put into separate plastic test cages with a feeding device with the two separate feeders containing standard rodent chow tablets TestDiet 5TUM (4.7 % fat, 19.9 % protein, 3.09 % sucrose, 3.3 kcal/g). At first, feeder 1 opened for 10 minutes, followed by feeder 2 on the right which was also opened for 10 minutes. During this period, the animals could freely eat from the open feeder. The training period aimed to get the animals accustomed to the standard rodent chow tablets and to observe whether animals preferred either feeder. After the 20-minute testing period, the number of tablets eaten was read from the memory cards inside the devices. The tablets eaten and the calorie intake of each mouse were calculated.

After the training, the mice were divided into two groups (n = 8 per group) for the eleven-day sucrose escalation experiment. The division into two groups was balanced according to the baselines of each feeder from the training period to ensure that both groups had a similar intake from both feeders at the start. The control group, chow/chow, continued receiving standard rodent chow tablets from both feeders 1 and 2 while the test group, chow/sucrose, received standard rodent chow tablets from feeder 1 and palatable sucrose tablets TestDiet 5TUT (0.0 % fat, 0.0 % protein, 64.37 % sucrose, 3.4 kcal/g) from feeder 2. As in the training period, the standard rodent chow pellet racks were removed from the homecage in the morning for two hours before the animals were put into plastic test cages with feeding devices. Both feeders 1 and 2 were again open for 10 minutes and the food intake was measured after the test from each mouse.

At the end of the experiment, the mice were still in the same groups and the steps were similar to the eleven-day escalation period, but they received two injections on two different days, one day between them. The injections were saline and LSD and they were administered before the animals were put into the plastic test cage with the feeding device. Again, the food intake was measured from each mouse. All mice got a dose of vehicle saline and LSD 100 µg/kg. The injections were divided into two days, so one mouse got one injection per day. LSD was dissolved in isotonic saline. The injections were given intraperitoneally with 30G needles two hours after the racks with standard rodent chow were removed from the homecage in the morning, right before the animals were put into the plastic test cages with the feeding device. The doses were blinded from the researcher. The timeline of the experiment is visualized in Figure 1.

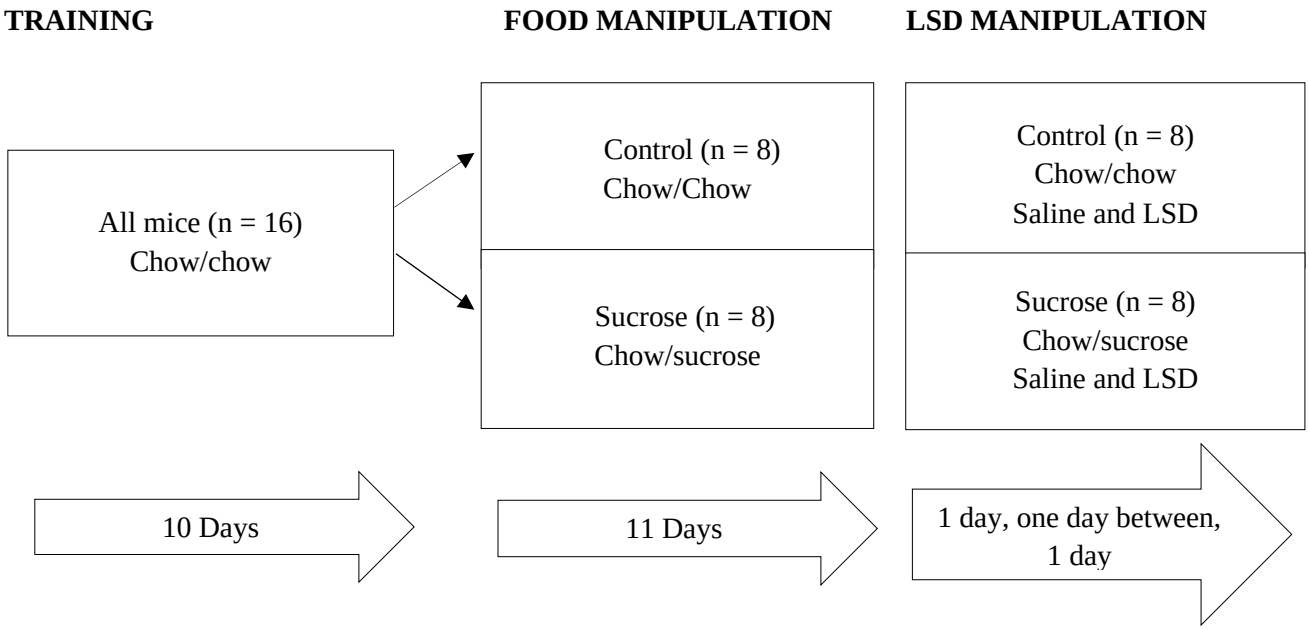
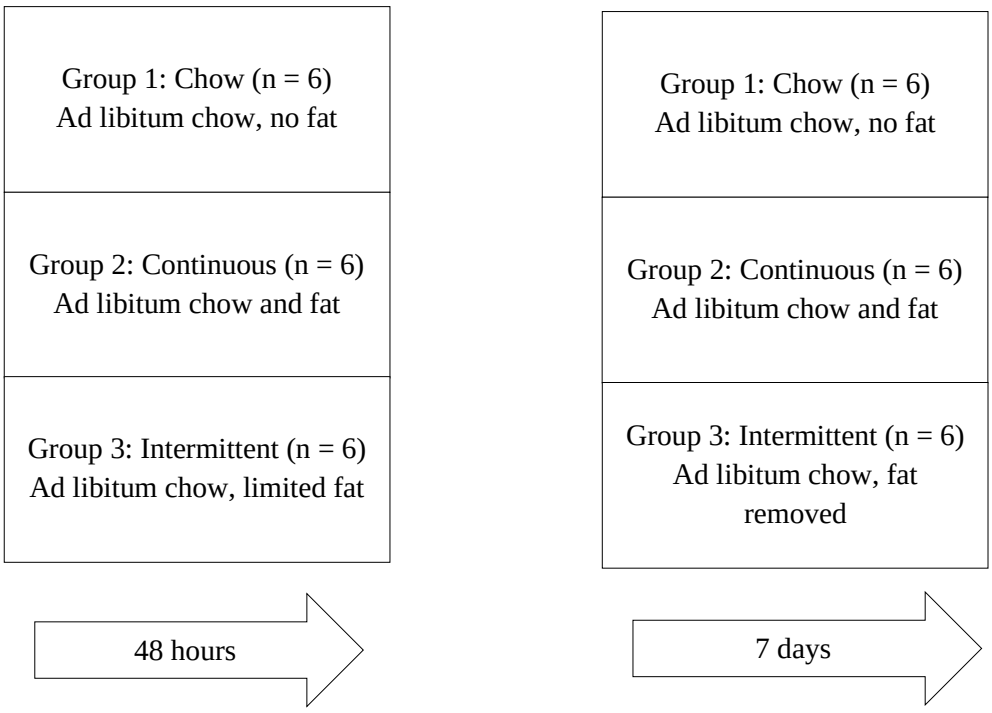


FIGURE 1. Timeline of the experimental procedures for the sucrose experiment. During the training period of ten days, all mice got access to standard rodent chow tablets in both feeders. After this, the mice were divided into a control group (n = 8) that continued getting chow from both feeders and a test group (n = 8) that received chow from the first feeder and sucrose tablets from the second feeder. This lasted for eleven days. In the end, the effects of saline and LSD were tested for two days on all mice. On day one, half of the animals received saline and half LSD, and on day two, the animals who first received saline now got LSD and vice versa. Between the injection days, there was one free day.

The high-calorie fat bingeing experiment aimed to introduce high-calorie fat pellets to mice and investigate whether they would create a binge-like eating pattern where they would start preferring the high-calorie fat over standard rodent chow. The experiment started with an induction period where the mice were divided into one of three groups: chow, continuous, or intermittent (n = 6 per group). The chow group, the control, had unlimited access to the standard rodent chow, but no high-calorie fat pellets were introduced. The continuous group had ad libitum access to both the standard rodent chow and high-calorie fat pellets Altromin C1090 (45 % fat, 18 % protein, 37 % carbohydrates, 4.5 kcal/kg). The intermittent group started with 48-hour access to both standard rodent chow and high-calorie fat pellets during the induction period. After 48 hours, the food consumption for all three groups was measured. The high-calorie fat pellets were then removed from the intermittent group, leaving them only the standard rodent chow. After 7 days, the high-calorie fat pellets were given back to the intermittent group. At this point, only the baselines were measured, so the mice did not get injections yet, but the food consumption of both standard rodent chow and high-calorie fat pellets was measured at different time points that were 0.5, 2.5, and 24 hours on the experimental days from

that point where the intermittent group got their high-calorie fat pellets. After the 24-hour measurements were done, the high-calorie fat pellets were then again removed from the intermittent group. The experiment continued after 9 days from the baseline measurements, and the mice started getting saline and LSD injections. The intermittent group got the high-calorie fat pellets back while the chow and continuous groups remained the same. After the mice got the injections, their food consumption was again measured at the three time points, and the high-calorie fat pellets were again removed from the intermittent group for seven days. This cycle was repeated four times in total. The drugs were vehicle saline and LSD, the dose being 50, 100, or 625 µg/kg. LSD was dissolved in isotonic saline. The injections were given once a week. The idea was that each mouse would get all the doses, so in total, they got four injections, one injection per week. The injections were given intraperitoneally with 30G needles in the morning before noon. The doses were blinded from the researcher. The steps of the experiment for each group are visualized in Figure 2 below.

A) INDUCTION



B) TEST PERIOD

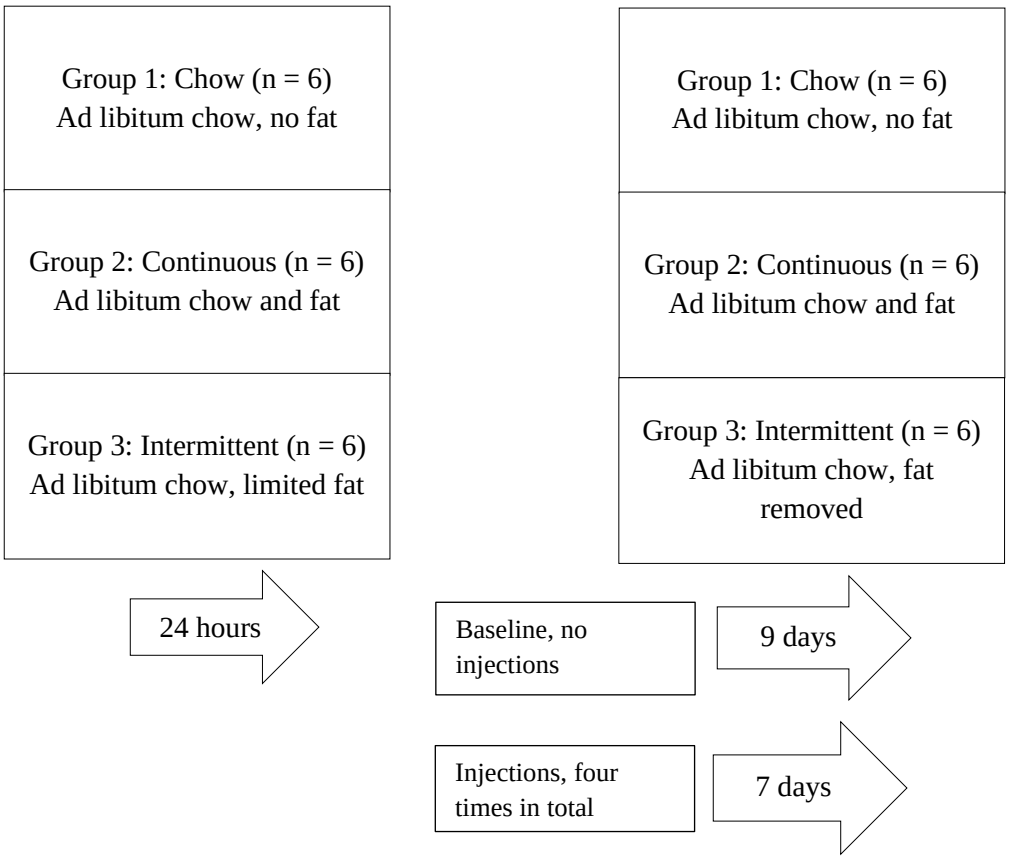


FIGURE 2. An illustration of the high-calorie fat experiment. A visualizes the induction period where the mice were divided into three groups and the intake of standard rodent chow and high-calorie fat pellets was measured after 48 hours. Illustrated in B, after 7 days, the test period started with measuring the baselines. From the baseline, after 9 days, the injections began where the effects of saline and LSD were investigated. This period with injections was repeated four times in total.

2.3 Statistical Analysis

The food intake in calories (kcal) for the ten-day training period for the sucrose escalation experiment was analyzed with two-way repeated measures ANOVA and Tukey's *post hoc* test. The main effects were tested on the day and feeder and the interactions of the day and feeder were investigated. The division into groups was conducted with repeated measures of two-way ANOVA and Uncorrected Fisher's LSD *post hoc* test to see the main effects of the feeder and group and their interactions. The eleven-day escalation period was analyzed with repeated measures of three-way ANOVA and Tukey's *post hoc* test to examine the main effects of day, group, and feeder, two-way interactions of day and group, day and feeder, and group and feeder, and three-way interactions of day, group and feeder. The effects of the LSD and saline doses on the intake of food were analyzed with repeated

measures of three-way ANOVA and Tukey's *post hoc* test to investigate the main effects of dose, feeder and group, two-way interactions between dose and feeder, dose and group, and feeder and group, and three-way interactions between dose, feeder, and group.

At the baseline of the high-calorie fat bingeing experiment, the food intake in grams (g) was analyzed using one-way ANOVA and Tukey's *post hoc* test. The three different groups (chow, continuous, and intermittent) were analyzed at three different time points (0.5, 2.5, and 24 hours). The effects of LSD (doses 50, 100, and 625 µg/kg) and saline were analyzed using one-way ANOVA and Tukey's *post hoc* test to compare food intake in the consumption of standard rodent chow and high-calorie fat pellets. Each time point (0.5, 2.5, and 24 hours) was analyzed separately.

Graphs and statistical analyses were conducted with Graphpad Prism (version 10.2.3 (403)).

3. RESULTS

3.1 Sucrose Escalation

Ten-day Testing Period

Significant main effects were found in the feeder (feeder 1 vs. feeder 2), $F(1, 30) = 8.277$, $p = .007$), day ($F(4, 119) = 7.246$, $p = < .001$) and in the two-way interaction of day and feeder ($F(9, 270 = 2.117$, $p = .028$). The *post hoc* analysis revealed that the differences between feeders became statistically significant on days 6, 7, 8, and 10—overall, the mice seemed to prefer feeder 1 and the preference became more pronounced, especially during days 6, 7, and 8. This finding is visualized in Figure 3A.

After division into different test groups after day ten, there was a significant main effect on the feeder ($F(1, 14) = 6.464$, $p = .023$), but not on the group ($F(1, 14) = 0.005$, $p = .947$) nor the two-way interaction between feeder and group ($F(1, 14) = 0.004$, $p = .949$). This division is visualized in Figure 3B.

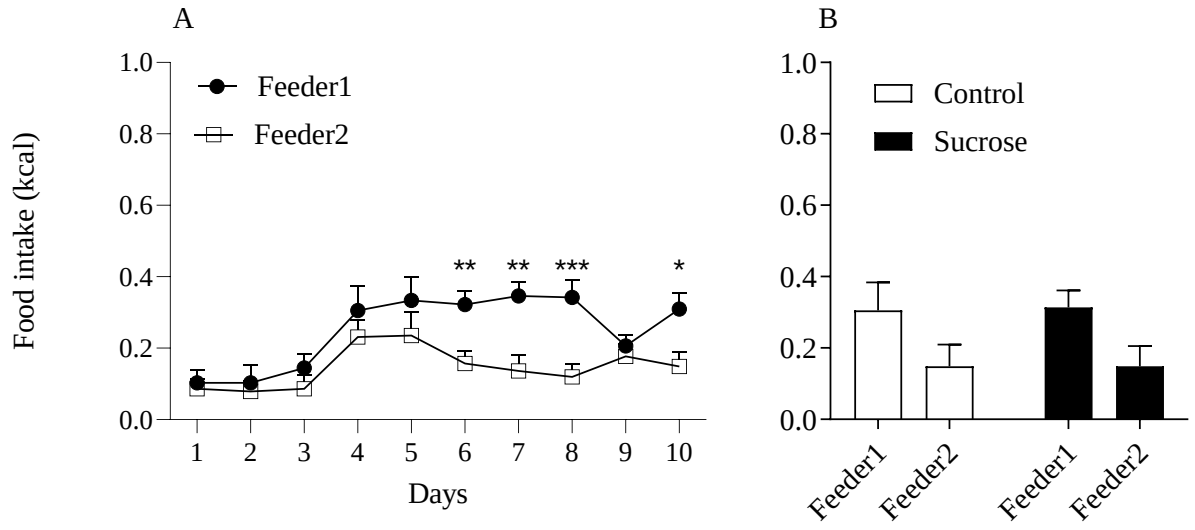


FIGURE 3. The trajectories of food intake for the ten-day training period are presented in Figure A. As shown by the *post hoc* analysis the differences between feeders 1 and 2 became significant on days 6, 7, 8, and 10 and the mice preferred feeder 1 over feeder 2. The preference became more pronounced, especially during days 6, 7 and 8. *p < .05, **p < .01, ***p < .001, difference between feeders 1 and 2. The division into two different groups for the sucrose escalation period is shown in Figure B. The groups were balanced according to food intake from both feeders. Even though there was not a significant main effect on the group, to illustrate the balancing to control and test groups, they are presented here. Average SEM +- is presented.

Eleven-day Sucrose Escalation Period

Significant main effects were found on feeder (feeder 1 vs. feeder 2) (F (1, 308) = 10.750, p = < .001), group (chow/chow vs. chow/sucrose) (F (1, 308), = 45.890, p = .001), and day (1-11) (F (10, 308) = 3.516, p = < .001). There were significant two-way interactions between group and feeder (F (1, 308) = 33.840, p = < .001) and day and group (10, 308) = 2.422, p = .009) but not on day and feeder (F (10, 308) = 0.296, p = .982). The *post hoc* analysis revealed that the sucrose group preferred feeder 1 compared to the control group which started to inhibit their food intake when feeder 2 was opened after feeder 1 had been at first open for 10 minutes. The analysis also showed that the sucrose group ate overall more compared to the control as the days passed. The three-way interaction between day, group, and feeder was not statistically significant (F (10, 308) = 0.855, p = .576). The findings are visualized in Figure 4.

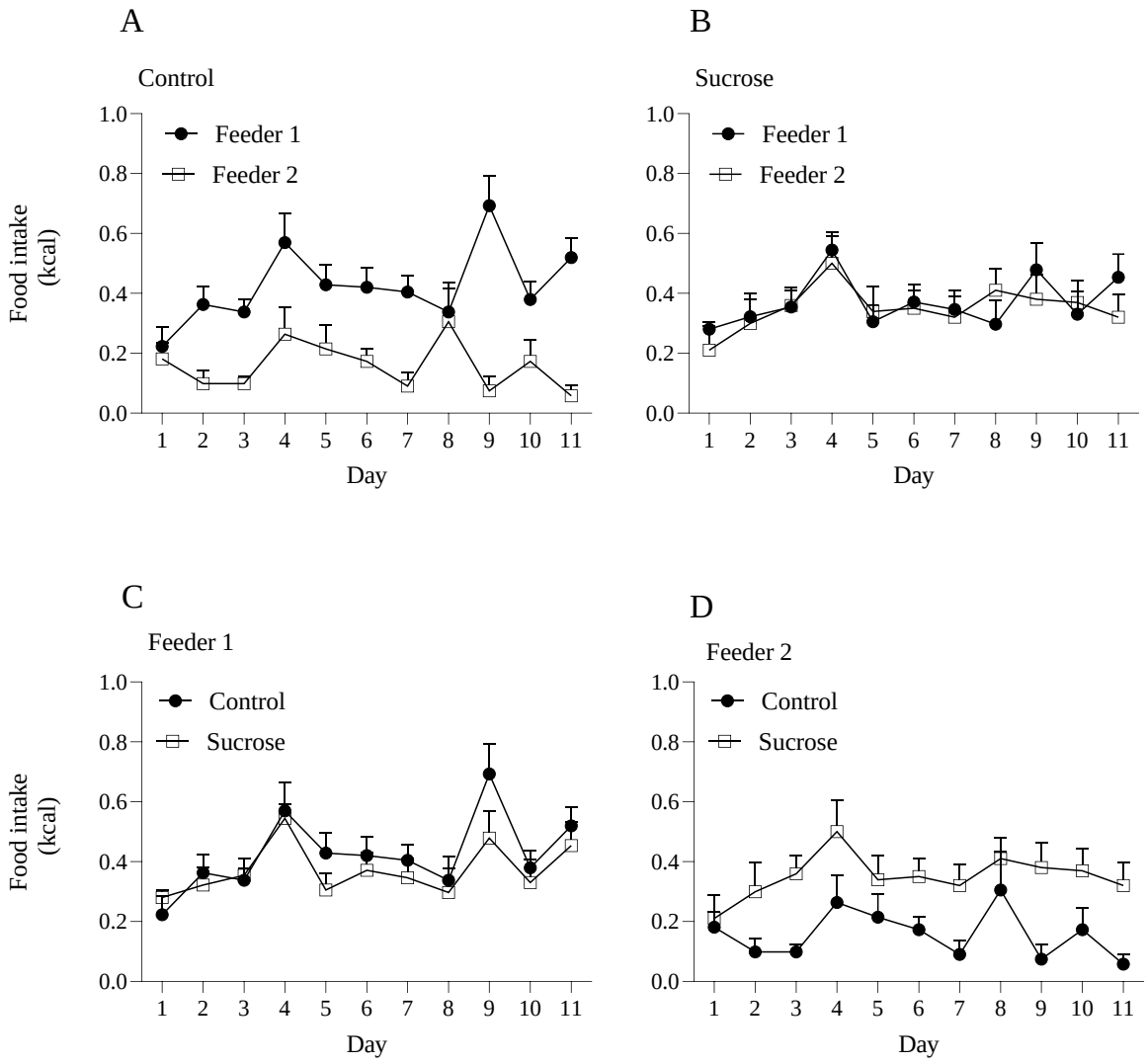


FIGURE 4. Trajectories from the eleven-day escalation period in the two groups. Figures A and B show the intake of food groups from both feeders while C and D show the intake of food feeders. The graphs and *post hoc* analysis showed that the animals in the sucrose group did not start inhibiting the intake of chow tablets from feeder 1 thus showing an escalation pattern. However, they still consumed more food from feeder 2 compared to the control. Even though three-way interaction was not found, for illustrative purposes, the days are also presented in the figure. Average SEM +- is presented.

Effects of LSD on Food Intake

Significant main effects were found on the feeder ($F(1, 28) = 6.667$, $p = .015$), but not on the group ($F(1, 28) = 2.782$, $p = .106$) nor dose ($F(1, 28) = 2.477$, $p = .127$). There were significant two-way interactions between feeder and group ($F(1, 28) = 16.790$, $p = .001$), but not on dose and group ($F(1, 28) = 0.846$, $p = .366$) nor dose and feeder ($F(1, 28) = 0.776$, $p = .386$). The *post hoc* analysis

revealed that the sucrose group ate more from feeder 2 compared to the control group as seen during the escalation period. Three-way interactions between dose, feeder, and group remained statistically insignificant (F (1, 28) = 0.115, p = .737). The findings are visualized in Figure 5.

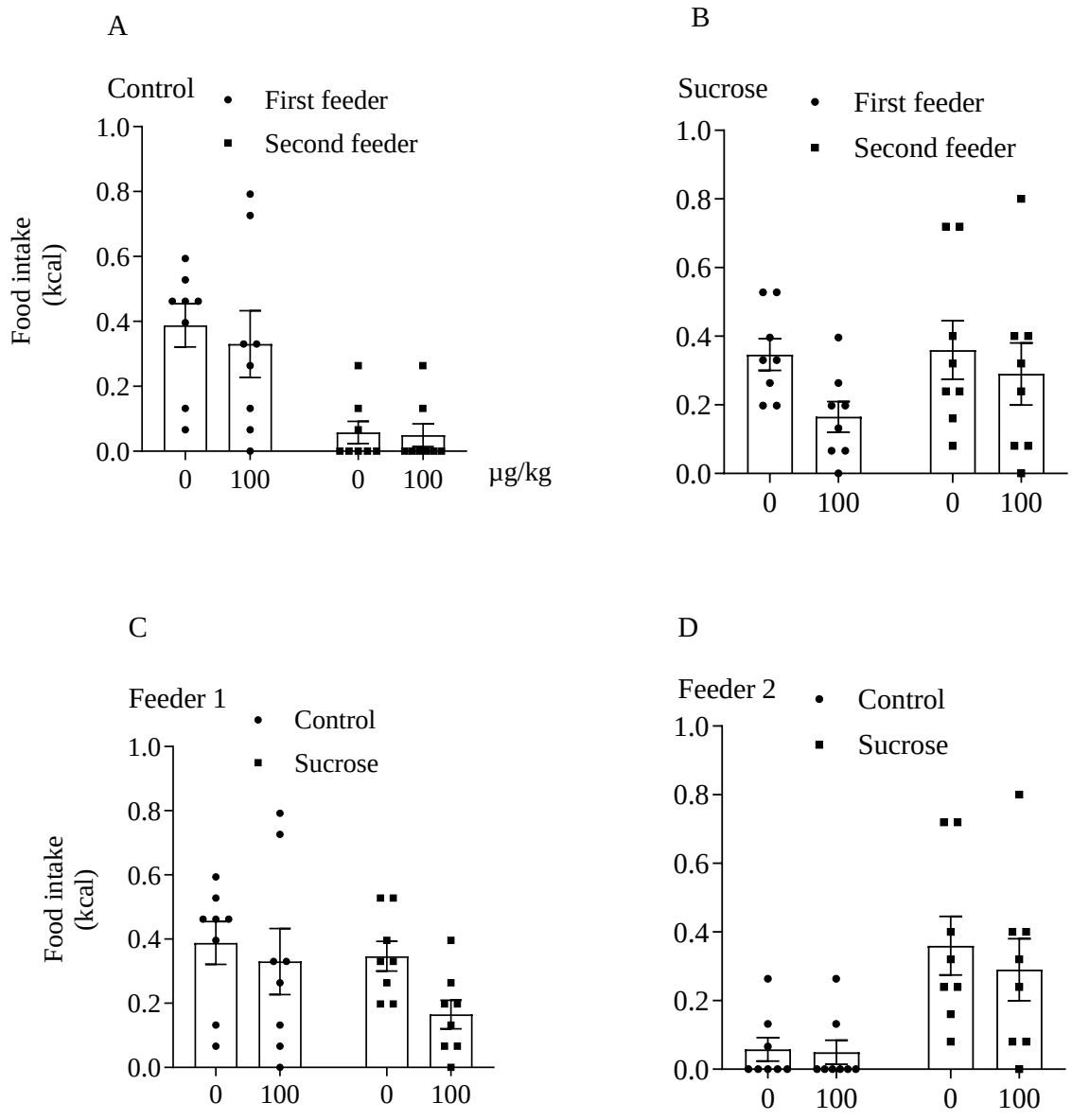


FIGURE 5. The effects of consumption of food after injections. A and B show the data groupwise while C and D show it feederwise. As revealed by the *post hoc* analysis and the graphs, the sucrose group continued eating from feeder 2 while the control dropped the intake of food after feeder 2 opened. However, LSD did not affect eating behaviors significantly in these mice. Average SEM +- is presented.

3.2 High-calorie Fat Bingeing

Baselines for Three Different Groups at Three Different Time Points

At the first 0.5-hour time point, no significant effects between the groups were observed on the standard rodent chow consumption ($F(2, 15) = 1.757$, $p = .206$). However, significant effects emerged already at the second time point of 2.5 hours: *post hoc* analysis showed the chow group consumed more standard rodent chow compared to continuous and intermittent groups ($F(2, 15) = 5.414$, $p = .017$). The significant effects between the groups started becoming more pronounced at the 24-hour time point where the chow group consumed way more standard rodent chow compared to continuous and intermittent groups ($F(2, 15) = 360.5$, $p = <.001$.

For high-calorie fat pellet consumption, significant effects were observed at every time point. The *post hoc* analysis revealed that at the 0.5-hour time point, the continuous and intermittent groups differed significantly: the intermittent group started bingeing high-calorie fat pellets already at this time point ($F(2, 15) = 31.28$, $p = < .001$). The significant effect increased at the 2.5-hour time point where the intermittent group continued consuming more high-calorie fat pellets compared to the continuous group ($F(2, 15) = 54.67$, $p < .001$). At the end of the 24-hour time point, the intermittent group increased their high-calorie fat pellet consumption compared to the continuous group who, as well, increased their high-calorie fat pellet intake ($F(2, 15) = 135.9$, $p = < .001$). The baselines are presented in Figure 6.

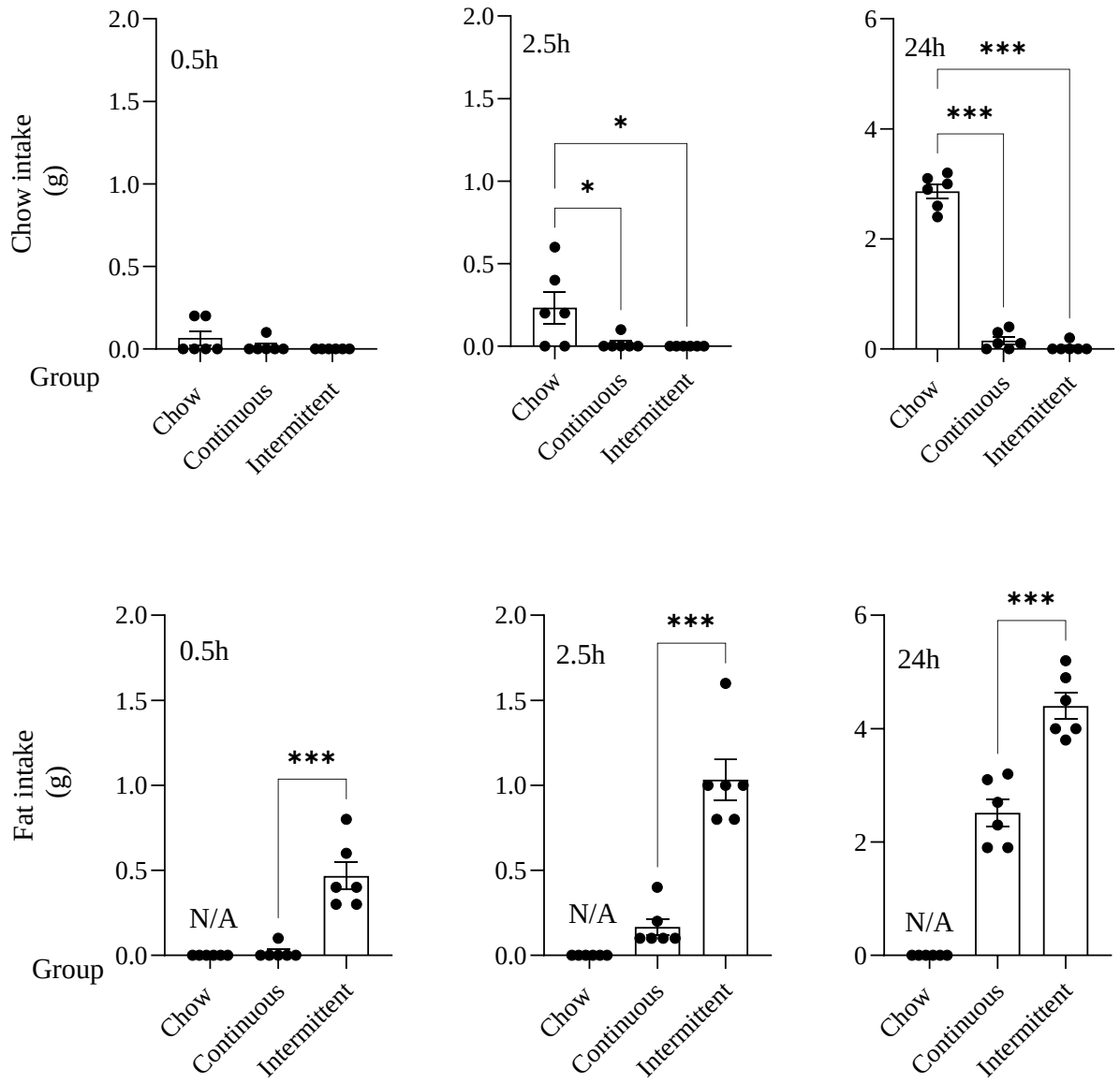


FIGURE 6. Baselines for standard rodent chow and high-calorie fat pellet consumption in each group at three different time points. As the *post hoc* analysis showed, the chow group consumed statistically significantly more compared to continuous and intermittent. Considering the consumption of high-calorie fat pellets intermittent group started bingeing the palatable high-calorie fat pellets already at the 0.5-hour time point and the bingeing continued for the 2.5 and 24-hour time points as well. *p < .05, **p < .001, ***p < .001, difference in the food intake between groups. Average +- SEM is presented.

Effects of Different Doses of LSD on Food Consumption

Administering a vehicle or different doses of LSD did not result in significant effects on food consumption for either standard rodent chow or high-calorie fat pellets. Overall, food consumption decreased slightly compared to the baseline.

In the chow group, no significant effect in the consumption of standard rodent chow was observed at the 0.5-hour ($F(3, 20) = 0.586$, $p = .631$), 2.5-hour ($F(3, 20) = 0.450$, $p = .720$), or 24-hour time point ($F(3, 20) = 2.980$, $p = .056$). The continuous group did not show significant effects at the 0.5-hour ($F(3, 20) = .787$, $p = .515$), 2.5-hour ($F(3, 20) = 0.707$, $p = .559$) or 24-hour time point ($F(3, 20) = 1.134$, $p = .359$). In the intermittent group, significant effects were not found at the 0.5-hour ($F(3, 20) = 3.240$, $p = .044$), 2.5-hour ($F(3, 20) = 2.321$, $p = .106$) or 24-hour time point ($F(3, 20) = 0.312$, $p = .817$).

Considering high-calorie fat pellet consumption, the continuous group did not show significant effects at the 0.5-hour ($F(3, 20) = 0.614$, $p = .614$), 2.5-hour ($F(3, 20) = 2.266$, $p = .112$ or 24-hour time point ($F(3, 20) = 1.251$, $p = .318$). No significant effects were found in the intermittent group either at 0.5-hour ($F(3, 20) = 0.709$, $p = .558$), 2.5-hour ($F(3, 20) = 1.900$, $p = .162$) or 24-hour time point ($F(3, 20) = 0.204$, $p = .892$).

The findings are presented in Figures 7 and 8.

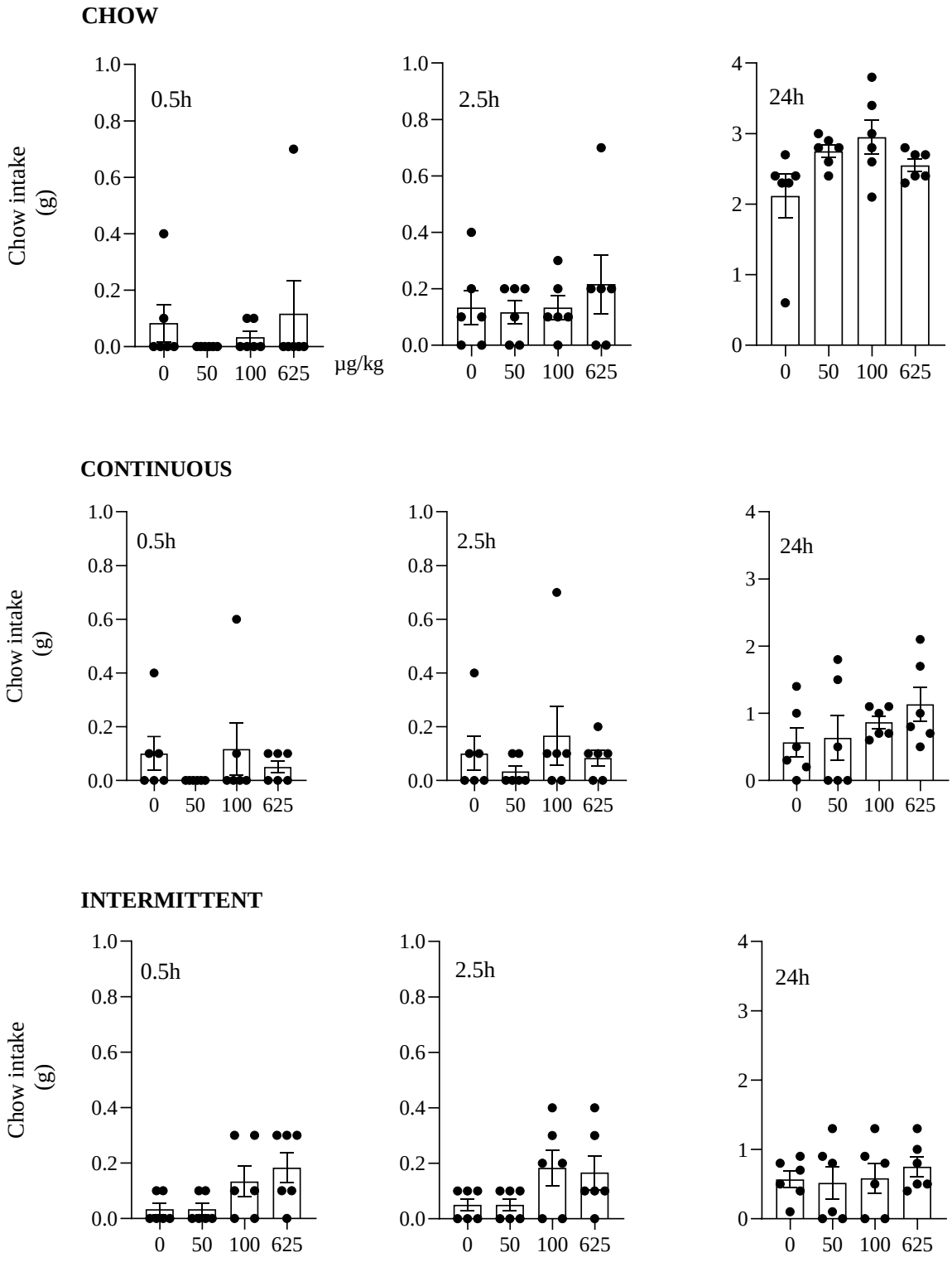


FIGURE 7. Intake of standard rodent chow in three different groups at three different time points after injections. Any dose of LSD did not have a significant effect on the consumption of this food type. Average SEM +- is presented.

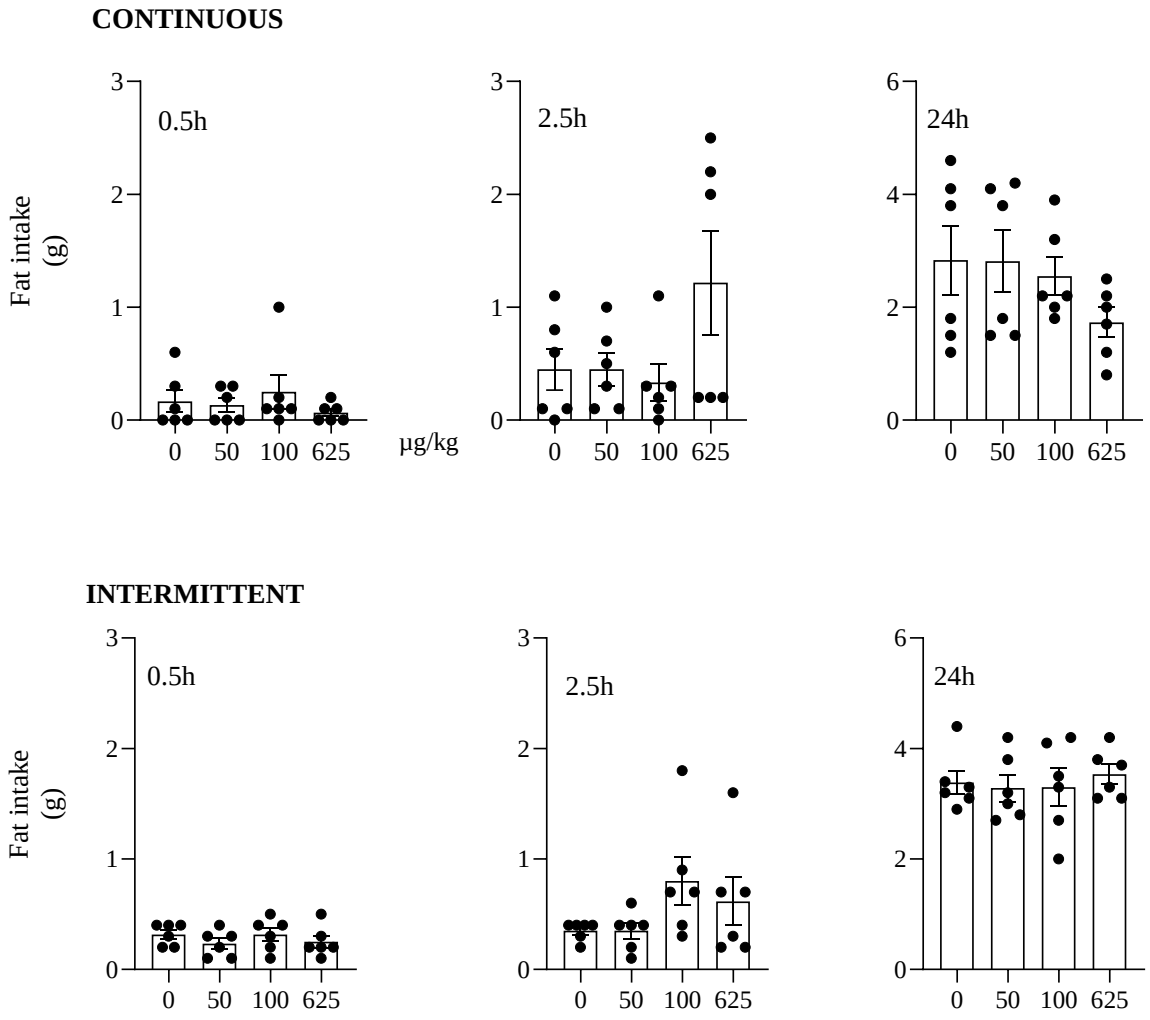


FIGURE 8. Intake of high-calorie fat pellets in two groups at three different time points. The chow group did not get high-calorie fat pellets, so it is not presented in this figure. Any dose of LSD did not have a significant effect on the consumption of this food type. Average SEM +- is presented.

4. DISCUSSION

This study investigated whether the mice in two experimental designs would show typical eating patterns considering standard rodent chow and palatable foods and if LSD would affect these eating behaviors. The eating and the effects of LSD were investigated via two different experimental designs.

4.1 The Sucrose Escalation Experiment

The mice in this experiment showed a typical eating pattern for standard rodent chow tablets during the ten-day testing period. LSD did not affect statistically significantly the consumption of standard rodent chow tablets, so thus the hypothesis was supported in this matter. However, the results

considering the consumption of palatable sucrose tablets pointed towards interesting findings. The mice in the test group did not seem to prefer the sucrose over standard rodent chow tablets: instead, as can be seen in the results, they ate almost the same amount of standard rodent chow and sucrose tablets. The hypothesis that the mice would start inhibiting the intake of standard rodent chow tablets in anticipation of the sucrose tablets was not supported though. LSD did not affect statistically significantly the intake of palatable sucrose tablets, so this hypothesis of reducing binge-like eating patterns was not supported.

In Feltmann et al.'s (2018) sucrose escalation model with rats, the animals quickly started inhibiting the intake of standard rodent chow compared to palatable sucrose pellets. In this research, a similar model was used, but with mice instead of rats, with the hypothesis that the animals would start showing a pattern of negative anticipatory contrast. However, the mice did not start escalating in the sucrose group but instead kept consuming both ordinary chow and high palatable sucrose tablets. The sucrose group overall ate more compared to the control group, so the assumed experimental design worked only partly. LSD did not lower the consumption of palatable sucrose tablets. Overall, the amount of food eaten decreased though, so some kind of effect of the pharmacotherapy might have manifested, but it is difficult to analyze whether it was caused by the drug itself or stress or some other reason.

4.2 High-calorie Fat Bingeing Experiment

At the baseline of the high-calorie fat bingeing experiment, the chow group showed a typical consumption of standard rodent chow pellets, while both continuous and intermittent groups started inhibiting the intake of the ordinary food type. Respectively, the continuous and intermittent groups showed high consumption of the high-energy fat pellets, especially the intermittent group. This points towards the support of the hypothesis that the animals would start inhibiting the intake of standard rodent chow in anticipation of palatable food. However, not any dose of LSD affected statistically significantly the consumption of standard rodent chow or high-calorie fat pellets. Thus, the hypothesis that LSD would not affect the consumption of standard rodent chow was supported while the hypothesis that LSD would decrease the intake of high-calorie fat pellets was not supported.

In this experiment, inspired by the model of Czyzyk et al. (2010), the baseline was in line with the previous research: especially the mice in the intermittent group started preferring the high-calorie fat pellets already at the 0.5-hour timepoint. Interestingly, there was no response for the LSD treatment though.

4.3 Interpretations of the Results

From the results, it can be concluded that LSD did not affect significantly the intake of standard rodent chow or palatable foods. It would then be tempting to assume that LSD would not be an effective pharmacotherapy for binge-like eating behaviors because at least in these kinds of pre-clinical animal studies, it did not affect the eating patterns. However, simple conclusions cannot be drawn from these results. In general, the mice in the two experiments did not eat that much, so a relevant question to ask is whether this kind of eating pattern could even be called binge eating—to our knowledge about human BED, a main symptom is excessive eating in short bingeing periods (Suokas & Rissanen, 2021), so to model human-like binge eating properly, excessive consumption of palatable foods should be induced. A point to consider then is how the results would differ if the mice were showing a clear binge eating pattern in the first place. In the upcoming research, it should be considered to find out how to create binge-like eating patterns—simply put, to make the animals eat more palatable foods in the first place.

One possible explanation for the animals not to start consuming excessive amounts of palatable foods could be anhedonia. According to Gorwood (2008), this condition stands for the reduced ability to enjoy normally pleasurable things, such as palatable foods. Especially in the sucrose escalation experiment, the main question is why—against the hypothesis—the animals did not manifest a preference for highly palatable sucrose tablets. In the experiments of this research, there were not physical stressors presented which usually are used in rodent models to induce anhedonia-like behavior. According to Primo et al. (2023), the reduction in sucrose intake in bottle-feeding mice sucrose water could point toward the direction that the animals are manifesting an anhedonia-like behavior that is not related to calorie intake or the reduction of overall consummatory behavior. However, in the results of this study, in the high-calorie fat pellet experiment, the mice in the intermittent group started showing a preference for palatable food, but the consumption decreased after administering LSD or saline, but the slight drop was not statistically significant in the end. It then should be considered that even though the intermittent group started showing a binge-like eating pattern at the baseline, was it enough for modeling human BED and for the psychedelic effects of LSD to manifest clearly?

A question to consider then is why the mice ate so little in the first place. Fadahunsi et al. (2022) reported a similar finding in their study where the effects of psilocybin on energy balance and feeding behavior in mice were investigated. The results indicated that psilocybin did not affect the feeding behaviors in mice—however, the consumption of food was low, so it should be considered if this behavior could be even called binge eating. Could it be that for the psychedelic effect to

manifest properly, the amount of food eaten needs to be on very high levels already at the baseline? To research this matter, a way to increase the appetite of animals needs to be investigated.

In their review of sucrose preference testing in animals, Primo et al. (2023) denoted that male and female mice have a difference in their preference for hedonistic foods. It seems that female mice prefer hedonistic foods compared to male ones. This pattern is seen in human BED as well since the condition has a higher prevalence in women than men (Suokas & Rissanen, 2021). This raises a question for future research: How would the results differ if we used the same experiments presented here, but tested the eating patterns in female mice instead of male ones?

A thing to take into account in the upcoming designing of animal models for BED is the species used. In this study, mice were investigated, but the original sucrose escalation model by Feltmann et al. (2018) tested the eating patterns in rats. In their study, the rats started showing a clear escalation pattern for the preference of sucrose very quickly. According to Primo et al. (2023), rats tend to favor sucrose better than mice. It would then be interesting to see how the results would differ if rats were used in similar experimental designs as in this research. Not only the animal species but the strain can influence the results as well—though it is known that the C57BL-6JRCC strain has been used in different addiction studies successfully, like in sucrose binge drinking studies by Rodríquez-Ortega et al., (2019), for example.

4.4 Limitations and Assets

The results of this study should be considered only tentative, so precise conclusions about the effects of LSD on eating behaviors should not be derived. The sample sizes used in the experimental designs can be questioned and talked about whether they are sufficient, especially when the three-way effect did not manifest in the sucrose escalation test. On the other hand, as in the ethics of animal studies, we as researchers should always aim to cause as little harm as possible and still aim for reliable results. Drawing the line where a sufficient sample size lies is not always simple in preclinical animal studies. For example, Charan and Kantharia (2013) suggest in their formula where E = total number of animals – total number of groups that the E should be something between 10 to 20. Considering this, the experiments here had adequate sample sizes, E in sucrose escalation being 14 and in high-calorie fat bingeing 15. Theoretically, these sample sizes look sufficient, but considering if they are enough in real life, is another matter to consider in future research.

As in all research conducted by humans, errors can occur. For example, in the sucrose escalation experiment, there are many things to consider like ensuring the functioning of the feeding devices and measuring the right number of tablets eaten from the right devices. One possibility would be to automatize the process as much as possible and to write down the results as soon as a session

has ended. Otherwise, there could be a possibility that the results are not written down clearly and can then bias the results. Experiments like this need precision and if many researchers are participating in the study, they need to be informed enough, so that everyone conducts and reports the results in the same way.

With injections, care should be implemented with the doses and administering routes. The intraperitoneal route was used in this study because it is quick, reliable, and does not cause a lot of stress on the animals (Al Shoyaib et al., 2019). However, even in this generally reliable administering route, errors can occur for example if the injection is misplaced (Steward et al., 1968). In laboratory settings, all stress in the animals cannot be eliminated—for example, simple procedures like handling and catching always cause a little stress in the animals that can affect the results (Akhtar, 2015). So, even though the experimental designs used in this research were chosen because the animals did not need to be kept in stress, a total elimination of stress cannot be secured because animal studies always include some form of stress in the form of handling and procedures.

Even though animal studies can offer us results of eating patterns and palatable food consumption, they cannot touch one important aspect: binge eating is not solely driven by physical hunger, but emotional factors play a big role as well (Kim, 2012). It is known that in BED patients, shame and stigma are typical, especially disgust toward oneself (Kim, 2012; Scrandis & Arrow, 2023). According to Dingemans et al. (2017), most of the people suffering from BED meet the criteria for some other psychiatric conditions as well, for example, anxiety and depression. Different moods are known to trigger binge attacks: not only sadness but frustration or anger as well. These are typical everyday emotions in humans, but how do you measure these in animals? If BED is more about emotions rather than the manifestation of the bad feeling in overeating, is it worth studying eating patterns only? These are relevant questions regarding the future direction of animal studies about any eating disorder.

As in almost every psychiatric condition, early emotional factors like attachment styles, childhood adversity, and stress can negatively impact normal eating habits and lead to excessive restrictions or bingeing (Turton et al., 2017). Stress can be induced in animal eating studies as well, but always weighing the ethical consequences. An interesting way to future animal experiments about BED would be not to include only the eating, but the emotional side as well. For example, it could be investigated how stressful conditions, like social isolation, or deficits in attachment to the mother would influence the eating patterns in animals and if LSD or other psychedelic compounds affect the eating patterns.

4.5 Future Implications for the Research on BED and Psychedelics

Obesity is highly connected to impairment in cognitive flexibility (Borgland & Neyens, 2022). This might be one of the reasons why weight-loss programs are not working for people with rigid thinking which is a characteristic of BED. As the treatment for BED needs lifestyle changes and commitment, the cognitive flexibility induced by psychedelics could be one tool to tackle the ever-growing issue. The motivation for remission for BED is high since approximately 80% of the weight lost via dieting is regained within 5 years (Borgland & Neyens, 2022). Since we know that psychedelic compounds increase cognitive flexibility, studying how they manifest in the everyday life of the patients could be one direction toward a better understanding of the effects of psychedelic compounds in BED.

Another possibility derived from the experimental designs of this research is the role of other psychedelic compounds and their effects on eating behaviors and BED symptoms. Here, only LSD from ergolines was investigated, but it would be worth investigating how the results would differ if psychedelic compounds from different chemical classes were used, such as psilocybin from the tryptamine class or relatively novel NBOHs from the phenethylamine class. Also, according to eating and the increasing of appetite mentioned earlier, one possibility would be investigating how cannabinoids would affect eating patterns in mice and humans. According to Borowska et al. (2018), endocannabinoids affect the endocrine system and thus appetite and eating behaviors. One interesting method to add to the experimental designs used in this research would be a way to increase the appetite of the animals with cannabinoids and see whether the psychedelic effects would manifest more profoundly.

The main goal of this master's thesis was to investigate the effects of eating patterns in mice in different experimental designs and the effects of LSD on eating behaviors, modeling the binge-like eating patterns in BED, but not the other aspects of the disease. The BED research is still scarce, so this master's thesis was poking a field that still of lacking data, but still has emotional, economic, and psychological impacts on society. Even though some animal models have been conducted for creating binge-like eating patterns, like the ones of Czyzyk et al. (2010), Fadahunsi et al. (2022), and Feltmann et al. (2018), no effects of LSD have been investigated except for older studies like Hamilton and Wilpizeski (1961) who used rats in their study. Due to the many possibilities of LSD, like cognitive flexibility and its high safety profile, the compound is worth investigating more. Even though in the results of this master's thesis, the psychedelic effects were not showing in these mice, it does not mean the subject should be abandoned for good. The unexpected results pave the way for the research of LSD as a possible aid for BED and food addiction. Of course, LSD or psychedelics cannot be seen as the final salvation for eating disorders and mental health

diseases, but rather as tools worth investigating. Instead of treating psychedelics as illicit drugs, they should be considered a new possibility for treating eating disorders, and their complex unique effects are worth researching both pre-clinically and clinically. According to Hintzen and Passie (2010), LSD is not merely used for getting high, but rather enhancing and expanding perceptions which can result in seeing the world in a new light. From this thought questions arise: What other possibilities lysergic acid diethylamide can give to the research besides only food intake? What psychological and spiritual aspects lie in its use? The findings and questions of this research shall act as an inspiration for improvements in designing novel experimental designs for BED.

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