The role of ghrelin and dopamine systems in the nucleus accumbens in the interaction of food restriction and amphetamine-induced locomotor sensitization

Lee, Seohyeon

Department of Medical Science
The Graduate School
Yonsei University

The role of ghrelin and dopamine systems in the nucleus accumbens in the interaction of food restriction and amphetamine-induced locomotor sensitization

Advisor Kim, Jeong-Hoon

A Dissertation Submitted
to the Department of Medical Science
and the Committee on Graduate School
of Yonsei University in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Philosophy in Medical Science

Lee, Seohyeon

July 2025

The role of ghrelin and dopamine systems in the nucleus accumbens in the interaction of food restriction and amphetamine-induced locomotor sensitization

This certifies that the Doctoral Dissertation of Lee, Seohyeon is approved

Thesis Supervisor Kim, Jeong-Hoon

Thesis Committee Member Ahn, Duck Sun

Thesis Committee Member Lee, Jong Eun

Thesis Committee Member Jung, Young-Chul

Thesis Committee Member Kim, Hee Young

The Graduate School
Yonsei University

January 2025

ACKNOWLEDGEMENTS

학위 과정은 저에게 있어서 학문적으로도, 개인적으로도 큰 배움을 얻고 성장하는 시간이었습니다. ‘중독’을 연구하는 연구실을 발견하고, 학사 전공과는 다른 분야에서 학위과정을 결정하던 그 순간부터 매순간 도전하며 수없이 울고 웃었던 시간들이 스쳐 지나갑니다. 이제 박사과정의 마무리를 앞두고 있습니다. 짧다면 짧고, 길다면 긴 학위기간 동안 많은 분들의 도움으로 한걸음, 한걸음 디딜 수 있었습니다. 이 지면을 빌려 감사의 말씀을 전합니다.

우선, 제 학위과정이 가능하도록 저에게 아낌없는 지원을 해 주신 김정훈 지도교수님께 감사드립니다. 학문적 호기심만 가지고 실험실에 들어온 저에게, 교수님께서는 참 많은 것을 알려주셨습니다. 학문을 대하는 올바른 태도와 열정을 몸소 보여주셨으며, 나아가 세상을 더 넓은 시각으로 바라보는 방법도 알려주셨습니다. 언제나 어려움 없이 학문적 질문을 할 수 있다는 스승이 있다는 것은 학위과정의 큰 자랑이자 버팀목이었습니다. 연구실을 떠나서도 교수님께 받은 가르침 마음에 품고, 자랑스러운 제자가 될 수 있도록 노력하겠습니다.

바쁘신 중에도 귀한 시간을 내어 심사를 맡아 주신 안덕선 교수님, 이종은 교수님, 정영철 교수님, 김희영 교수님께도 감사의 인사를 드립니다. 소중한 조언과 피드백을 통해 논문의 완성도를 높이고, 그 과정에서 많이 배울 수 있었습니다.

연구실에서 동고동락하며 서로 힘이 되어주던 선후배님들에게도 감사를 전합니다. 작은 고민에도 따뜻한 위로와 함께 앞으로 나아갈 방향으로 답을 주시던 김화영 연구교수님, 함께 걷던 교정은 저에게 오랜 추억으로 남을 것 같습니다. 제 학위과정 동안 거의 모든 연구를 함께한 윤형신 연구교수님, 부족한 모습에도 늘 진심으로 대해 주셔서 감사합니다. 연구에 대한 뜨거운 열정과 애정은 멈춰 있던 저를 여러 번 다시 일으켜 세워주셨습니다. 자바라방의 해결사, 열정적인 디스커션과 다정한 응원을 해주시던 채문정 박사님. 정말 많은 힘이 되었습니다. 우상향 그래프를 그리는 멋진 곽명지 박사님, 친절하게 도와주신 모든 순간들, 둘만 나눌 수 있던 모든 대화들 마음에

잘 간직하겠습니다. 탁월한 연구능력과 그 이상으로 멋있는 내면을 가진 구민정 박사님, 덕분에 힘들었던 순간들조차 따스한 기억으로 남았습니다. 불필요한 걱정보다는 해내는 것에 집중하던 한준엽 선배님, 서로의 연구를 보며 의견을 나누던 시간을 기억합니다. 함께 앞으로 나아가고자 진심 어린 고민을 나눈 동료인 박종우 오빠, 늘 밝고 맑을 나날들을 응원합니다. 전우애로 함께하며 이제는 마음을 나누는 친구가 된 임하은 석사님, 덕분에 많은 순간 힘을 낼 수 있었습니다. 묵묵히 자신의 일을 해내며 성장하고 있는 곽민석 후배님, 늘 밝은 인사를 건네어 주던 톡톡 튀는 장한별 후배님, 마지막으로 쉽지 않은 걸음이지만 그럼에도 나아가고 있는 유가영 후배님 모두 고맙습니다. 졸업 후 각자의 자리에서 멋지게 자리 잡고 계신, 짧지만 진솔한 대화를 나눴던 조보람 박사님과 영원한 나의 첫 사수 이정원 박사님, 그리고 연구 경험과 사회로 진출하신 경험을 바탕으로 유익한 이야기를 들려주시는 그 외 연구실 졸업생 선배님들 모두에게 감사 인사를 드립니다.

마지막으로 연구실 밖에서 저에게 무수한 응원을 보내주었던 분들께 감사드립니다. 세상에서 가장 사랑하는 부모님. 언제나 든든하게 괜찮다며, 당당하라며 응원해 주시던 아버지, 항상 뒤에 계시던 아버지 덕에 앞만 보고 달릴 수 있었습니다. 가장 쉽게 약한 마음을 털어놓았지만 언제나 강한 확신으로 답을 주신 어머니, 덕분에 지치지 않고 다시 일어설 수 있었습니다. 그리고 외로운 순간이면 언제나 함께 해준 가족들과 친구들. 모두 저에게 큰 용기와 원동력이 되었습니다. 늘 바쁘다는 핑계로 세심하게 살피지 못했던 저를 이해하고 믿어주어 고맙습니다.

학위 과정을 돌아보니, 고민하고, 부딪히고, 아파하면서도 치열하게 온 마음을 다했던 시간이었습니다. 앞으로 어떤 상황을 마주하더라도 지난 시간을 자양분 삼아 주저하지 않고 앞으로 나아가겠습니다. 언제나 사랑과 응원으로 품어 주셔서 감사합니다. 더욱 성장하는 모습으로 보답하겠습니다.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

The role of ghrelin and dopamine systems in the nucleus accumbens in the interaction of food restriction and amphetamine-induced locomotor sensitization

The mesolimbic dopamine system, which is involved in processing natural rewards such as food, as well as drugs of abuse, shares common neural pathways within the nucleus accumbens (NAcc) that influence both appetite and addictive behaviors. Recent research has increasingly focused on the bidirectional interaction between addictive drugs and hormones that regulate appetite. Ghrelin, widely recognized for its function in hunger signaling, also modulates addictive behaviors by working together with dopamine to regulate these processes. Food restriction (FR) elevates plasma ghrelin levels, and these ghrelin-induced effects resemble heightened drug-seeking behavior and psychostimulant sensitivity under food-restricted conditions. However, it remains unclear whether endogenous ghrelin, which is naturally elevated by FR rather than infusion, interacts with NAcc dopamine signaling to mediate amphetamine (AMPH)'s reinforcing effects. Additionally, the influence of the duration and timing of AMPH exposure relative to FR on these pathways is still uncertain. This study investigates how the duration and timing of FR, either before or after AMPH treatment, affect AMPH-induced behavioral responses, focusing on the roles of ghrelin signaling and the dopamine system within the NAcc core. Rats were divided into three groups: normal feeding (NF), acute FR (aFR; 1-2 days), and chronic FR (cFR; 2 weeks). After these conditions were established, AMPH was administered, and locomotor activity was measured. cFR significantly enhanced AMPH-induced locomotor activity compared to NF and aFR, which was associated with elevated plasma ghrelin and increased dopamine D1 receptor (D1R) expression in the NAcc. Systemic or NAcc core-specific administration of D1R and ghrelin receptor (GHSR) antagonists reduced this enhancement, confirming the role of ghrelin and dopamine signaling in modulating cFR-enhanced AMPH responses. Furthermore, it was found that, while locomotor activity was

increased with D1R agonist injections into the NAcc core only in cFR group in saline pre-exposed rats, rats pre-exposed to AMPH showed enhanced sensitivity to D1R agonists regardless of the duration of FR. Plasma ghrelin levels in the aFR group were significantly higher in AMPH pre-exposed rats compared to saline pre-exposed rats, suggesting that the AMPH pre-exposure condition may synergistically enhance the ghrelin system's response to D1R activation, even in aFR group. This study underscores the critical influence of FR on drug-induced behaviors, highlighting how the timing and duration of FR can significantly modulate the interaction between ghrelin and dopamine systems in the NAcc. These findings offer new insights into potential strategies for addiction treatment.

Key words: food restriction, ghrelin, dopamine D1 receptor, nucleus accumbens, amphetamine

1. Introduction

In contemporary society, the abundance of food contrasts dramatically with the prevalent culture of dieting for social, cosmetic, or health reasons, creating a paradoxical state of voluntary dietary restrictions.[1,2] This paradox unintentionally promotes vulnerability to binge eating,[3] excessive weight gain,[4] and an increased risk of substance abuse and relapse,[5,6] potentially driven by changes in neural pathways related to hunger signaling and reward. Indeed, there is significant overlap between the brain regions involved in eating disorders and substance use disorders,[7,8] which also exhibit high rates of comorbidity.[9] The prevalence of substance use has been shown to increase with the severity of food restriction,[5] and food restriction has been found to reinforce the central rewarding effects of substance abuse.[10-12] Additionally, individuals with psychostimulant use disorders, such as amphetamine (AMPH), often report decreased food intake, weight loss, and energy deficits.[13] This implies that food restriction might contribute significantly to both the initiation and relapse phases of substance abuse.

Based on these observations, ongoing research is investigating the interactions between psychostimulants and appetite-regulating hormones. Psychostimulants are known to interact with hormonal signals involved in appetite control, altering energy deficit monitoring and reward systems.[13] Among these hormones, ghrelin is a 28-amino-acid peptide primarily secreted by the stomach and gut that plays a critical role in hunger signaling and the regulation of appetite.[14-17] Its levels naturally fluctuate before and after meals,[18] reflecting its essential function in maintaining energy homeostasis.[19] While early research on ghrelin focused on its role in regulating feeding behavior, subsequent studies began to explore its association with psychostimulants.[20-22] Psychostimulants such as methamphetamine and cocaine have been shown to transiently alter plasma ghrelin levels.[23-25] Additionally, preclinical studies further demonstrate that systemic administration of ghrelin not only increases hyperactivity induced by stimulants,[26,27] but also enhances the rewarding effects, including conditioned place preference (CPP).[28] These findings suggest that ghrelin interacts with reward-related pathways, modulating responses to psychostimulants. Notably, ghrelin engages with the mesolimbic dopamine pathway,[29-31] where its activation heightens the reward system's sensitivity beyond basic feeding behaviors.[32] This interaction includes increased dopamine release in the NAcc,[33] a brain region crucial for mediating the rewarding effects of both natural stimuli and drugs, and playing an instrumental role in addiction

and motivation-related behaviors.[34,35] Furthermore, administration of ghrelin into the ventral tegmental area (VTA) or nucleus accumbens (NAcc), followed by psychostimulant treatment, significantly amplifies psychostimulant-related reward effects.[22,36,37] Conversely, these effects are diminished when ghrelin receptor, known as growth hormone secretagogue receptor (GHSR), is blocked using antagonists.[22,33] Together, these findings suggest that ghrelin is a crucial mediator in regulating psychostimulant sensitivity, through its interaction with the dopaminergic system.

The effects induced by ghrelin are similar to those observed under food restriction conditions, where drug-seeking behaviors and psychostimulant effects are significantly enhanced. Clinical studies have reported an increased sensitivity to psychostimulants in individuals subjected to food restriction,[5] while preclinical studies have also demonstrated increased locomotor activity, CPP and self-administration of psychostimulants following food restriction conditions.[38-41] These effects may result from food restriction enhancing dopaminergic signaling in a manner similar to ghrelin,[42-44] thereby increasing sensitivity to psychostimulants. Both ghrelin and food restriction enhance reward-related pathways by eliciting dopamine spikes and regulating dopamine release in the NAcc.[33,45,46] Additionally, food restriction increases the firing rate of dopamine neurons,[47] amplifying dopaminergic signaling and further heightening sensitivity to psychostimulants. During food restriction, ghrelin levels are markedly elevated,[48,49] facilitating its transport across the blood-brain barrier and subsequent action on the central nervous system.[50] These physiological changes suggest a close connection between food restriction and an alteration in the ghrelin system, which may work together to influence dopaminergic activity. However, it remains unclear whether the enhanced psychostimulant effects under food restriction are driven by increased plasma ghrelin levels directly interacting with dopaminergic signaling.

Interestingly, the enhancement of psychostimulant-induced locomotor activity by food restriction or ghrelin administration resembles the locomotor sensitization seen with repeated exposure to psychostimulants. Psychostimulants, including AMPH, are well known to induce behavioral sensitization, characterized by a progressive increase in locomotor activity.[51,52] Once this sensitization is established, it persists as a long-lasting memory that significantly contributes to drug-seeking and drug-taking behaviors in animals.[53] Due to these attributes, behavioral sensitization has been proposed as a theoretical model to explain the increase of drug use and the persistent cravings seen in human addiction.[51,52] The NAcc, composed of the core and shell, is central to behavioral

sensitization.[54,55] Evidence suggests that the core is more critical for mediating sensitization, while the shell is largely associated with drug reward.[56-58] Research further demonstrates that microinjection of ghrelin and dopamine D1 receptor (D1R) agonists within the NAcc core induce locomotor sensitization in rats pre-exposed to AMPH.[37] This finding supports the idea that ghrelin's interaction with dopaminergic pathways in the NAcc is essential for this enhanced response. The presence of the GHSR in the NAcc[59-61] provides further evidence that the increase in plasma ghrelin after food restriction may influence reward behavior through interactions with ghrelin and dopamine receptors in this brain region, indicating a possible synergistic interaction between ghrelin and dopamine in enhancing the effects of addictive drugs.

Despite evidence that food restriction enhances drug reward, the underlying mechanisms remain unclear. While food restriction has been shown to enhance drug reward, previous studies have demonstrated that one day of food deprivation does not significantly enhance this effect,[62] whereas longer periods of food restriction, such as protocols that result in significant weight loss,[63] consistently amplify drug reward. However, the fundamental mechanism differences according to the food restriction period have not yet been thoroughly elucidated. Additionally, while the effects of food restriction on psychostimulant responses have been studied, there is limited understanding of how prior psychostimulant exposure alters the pathways and outcomes associated with subsequent food restriction.

Building on these gaps, this study aims to determine whether the reinforcing effects of AMPH under food restriction are mediated by endogenous ghrelin, specifically through its interaction with dopaminergic signaling in the NAcc core. Given prior findings that ghrelin and dopamine interactions in this region induce behavioral sensitization, the study explores whether food restriction enhances AMPH effects through this mechanism. Furthermore, it examines how the duration of food restriction, whether classified as short-term or long-term, modifies AMPH-induced behavioral responses and investigates whether the timing of food restriction, occurring either before or after AMPH exposure, alters these outcomes. These investigations aim to elucidate the interplay between food restriction, ghrelin, and dopaminergic pathways in psychostimulant sensitivity.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200-230g) were obtained from Orient Bio Inc. (Seongnam-si, Korea) and housed two per cage under a 12-hour light/dark cycle. All experiments were conducted during the daytime. All animal procedures adhered to the Institutional Animal Care and Use Committee protocol of Yonsei University College of Medicine.

2.2. Perfusion, brain extraction and immunohistochemistry

After acclimatization, rats were anesthetized with intraperitoneal (IP) injections of ketamine (100 mg/kg) and xylazine (6 mg/kg). Perfusion was performed first with saline followed by 4% paraformaldehyde (PFA). Extracted brains were post-fixed overnight in 4% PFA at 4°C and submerged in 30% sucrose until they sank (3-4 days). Afterward, the brains were embedded in OCT compound with 30% sucrose, flash-frozen in isopentane on dry ice, and sectioned coronally at 50 μm thickness (0.7-2.2 mm from bregma) according to the Paxinos and Watson Rat Brain Atlas (5th ed.). Sections were blocked for 1 hour in PBS with 5% normal goat serum and 0.3% Triton X-100, then incubated at 4°C with primary antibodies in PBS with 2% normal goat serum and 0.1% Triton X-100. The primary antibodies used were rabbit polyclonal anti-GHSR (1:250, Phoenix Pharmaceuticals Inc., H-001-62), mouse monoclonal anti-NeuN (1:2000, Abcam, ab104224), and mouse monoclonal anti-DARPP-32 (1:1000, BD Science, 611520). After washing (3 x 10 min) with PBS containing 0.1% Triton X-100, sections were incubated with secondary antibodies for 2 hours at room temperature, washed, and mounted using Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) under a coverslip. The secondary antibodies used were Alexa 488 (1:2000, Abcam, ab150077) for GHSR, and Alexa 568 (1:2000, Abcam, ab175473) for NeuN and DARPP-32.

2.3. Confocal microscopy analysis

Immunostained sections of the brain, including the NAcc, were imaged using an LSM 700 confocal laser scanning microscope (Carl Zeiss) and analyzed with Zen 3.4 Software (Carl Zeiss, Jena, Germany).

2.4. Drugs and peptide

Dextroamphetamine sulfate (AMPH) (U.S. Pharmacopeia, Rockville, MD, USA) was dissolved in sterile 0.9% saline. The selective D1R antagonists R-(+)-SCH23390, GHSR antagonists JMV2959 (Molnova, Ann Arbor, MI, USA), and D1R agonist R-(+)-SKF-81297 (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in sterile 0.9% saline and stored at -20°C. Before use, frozen aliquots were thawed and diluted in sterile saline.

2.5. Food restriction

Food restriction groups received 60% of the daily intake for normal feeding (NF) group. Acute food restriction (aFR) lasted 1-2 days, and chronic food restriction (cFR) lasted 2 weeks. After 14 days, the aFR group weighed 95%, and the cFR group weighed 80% of the free-feeding group's weight. Water was available ad libitum.

2.6. Locomotor activity

Locomotor activity was evaluated using a set of nine activity chambers (35 x 25 x 40 cm), each constructed from translucent Plexiglas (IWOO Scientific Corporation, Seoul, Korea) and housed within sound-absorbing PVC enclosures. The floor of each chamber consisted of 21 stainless steel rods (5 mm in diameter) spaced 1.2 cm apart, center-to-center. Two infrared light beams (Med Associates, St. Albans, VT, USA) were positioned 4.5 cm above the floor and spaced evenly along the longitudinal axis to measure horizontal movement. Locomotor activity was recorded only when

a rat interrupted two consecutive beams, thereby minimizing the influence of confounding activities such as grooming that might only affect a single beam.

2.7. Surgery and intracranial microinjections

Rats were anesthetized with IP injections of ketamine (100 mg/kg) and xylazine (6 mg/kg) and then placed in a stereotaxic apparatus. Bilateral guide cannulas (22 gauge; Plastics One, Roanoke, VA, USA) were implanted into the NAcc core at coordinates A/P, +3.2; L, +2.8; D/V, -6.1 mm from bregma, angled at 10° to the vertical and positioned 1 mm above the intended injection site. Cannulas were secured with dental acrylic cement and stainless-steel skull screws. After surgery, obturators were inserted into the guide cannulas, and the rats were allowed 5–7 days of recovery period.

Bilateral intracranial microinjections were performed on freely moving rats. Injection cannulas (28 gauge) connected to 1-μl syringes (Hamilton, Reno, NV, USA) via PE-20 tubing were inserted 1 mm below the guide cannula tips. A volume of 0.5 μl per side were injected over 30 seconds. Cannulas were withdrawn after 1 minute, and obturators were replaced.

2.8. Blood sampling and plasma ghrelin analysis

Blood samples were collected from the jugular vein to quantify ghrelin levels, using a 26-gauge needle inserted in a caudocephalic direction. Approximately 400 μl of blood per rat was collected into Microtainer tubes with EDTA (BD Biosciences, USA) and centrifuged at 4000 rpm for 15 minutes at 4°C. Plasma ghrelin concentrations were measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals, EK-031-31) with the samples diluted 1:10 in assay buffer according to the manufacturer's protocol.

2.9. Brain tissue preparation and western blot assays

Animals were decapitated, and brains were rapidly excised on an ice-cold plate. Brains were

sliced and the NAcc core regions were punched out, immediately frozen on dry ice, and stored at -80°C. Tissue samples were homogenized in lysis buffer (0.32 M sucrose, 2 mM EDTA, 1% SDS) containing Pierce™ protease and phosphatase inhibitor mini tablets (EDTA-free) (Pierce, Rockford, IL, USA) and ultracentrifuged at 13,000 rpm for 15 minutes at 4°C. Protein concentrations were determined using the Bradford protein assay. Samples were boiled for 10 minutes after mixing with loading buffer and then subjected to 10% SDS-PAGE, followed by electrophoretic transfer to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Membranes were blocked with either 5% bovine serum albumin (BSA) or 5% skim milk in PBS-T buffer and incubated overnight at 4°C with primary antibodies: GHSR (1:1000 in PBS-T with 5% BSA, Abcam, ab95250), D1R (1:2000 in PBS-T with 5% skim milk, Abcam, ab40653), dopamine D2 receptor (D2R, 1:500 in PBS-T with 5% BSA, Santa Cruz, SC5303), and anti-actin (1:10,000 in PBS-T with 5% BSA, Abcam, ab6276) as a loading control. Peroxidase-conjugated secondary antibodies, anti-rabbit IgG (1:2000, LABISKOMA, k0211708) or anti-mouse IgG (1:5000; Cell Signaling, 7076S), were applied in PBS-T with 5% skim milk, followed by detection with enhanced chemiluminescence (ECL) reagents (AbFrontier, Seoul, South Korea) and X-ray film exposure. Band intensities were quantified using Fujifilm Science Lab 97 Image Gauge software (version 3.0) and Multi Gauge V3.0 for quantitative analysis.

2.10. Plasma membrane fraction

Plasma membrane fractions were isolated using the Plasma Membrane Protein Extraction Kit (Abcam, ab65400) according to the manufacturer's instructions. Brain tissue samples were lysed with protease inhibitors, initially centrifuged at 700g for 10 minutes at 4°C to separate the supernatant, and further centrifuged at 10,000g for 30 minutes at 4°C. The cytosolic proteins were isolated from total cellular membrane proteins, including plasma and organelle membranes in the pellet. A two-phase buffer system was then employed to further extract the plasma membrane, collected via subsequent centrifugation. The isolated plasma membrane fraction was solubilized in 0.5% Triton X-100 in PBS for Western blot analysis. SDS-PAGE on 10% gels and subsequent blotting with anti-cadherin (1:5,000, Cell Signaling, 4073S), α-tubulin (1:30,000, Santa Cruz, SC5303), and anti-D1R (1:2000 in PBS-T with 5% skim milk, Abcam, ab40653) antibodies were performed. Visualization was achieved using an ECL detection system (AbFrontier, Seoul, South

Korea) with quantitative analysis via Multi Gauge V3.0.

2.11. Design and procedures

Upon arrival, all rats underwent a week-long adaptation period to the new housing environment before any experiments were conducted.

2.11.1 Experiment 1

To investigate the presence and localization of GHSRs at the protein level, naïve rats were used to obtain brain samples for immunohistochemistry, which was followed by confocal microscopy.

2.11.2 Experiment 2

To examine the effects of food restriction on ghrelin levels and AMPH-induced locomotor activity, subjects were divided into three groups: NF, aFR, and cFR group. Plasma ghrelin levels were measured three times during the two-weeks of food restriction period: the day before beginning, on day 7, and on day 14. Each group was acclimatized to the test room in their home cages for 1 hour, followed by 1-hour baseline activity measurement in the locomotor activity boxes. For drug testing, animals received an IP injection of AMPH (1 mg/kg), and they were immediately returned to the locomotor activity boxes for another hour of activity measurement. Brain tissues (NAcc core) from all experimental rats were then collected for Western blot analysis.

2.11.3 Experiment 3

To assess systemic and NAcc core-specific effects of GHSR and dopamine D1R antagonists on AMPH induced locomotion in food-restricted rats, two experiments were conducted.

In experiment 3-1, rats were divided into two groups based on food restriction duration: acute and chronic, using the same methodology as in Experiment 2. Rats were placed in locomotor activity

boxes for 60 minutes for habituation. They then received an IP injection of either vehicle (VEH), JMV2959 (3.0 mg/kg or 6.0 mg/kg), or SCH23390 (0.01 mg/kg or 0.03 mg/kg). Fifteen minutes after treatment, AMPH (1.0 mg/kg, IP) was administered, and locomotor activity was measured for 60 minutes.

In experiment 3-2, only rats under cFR were studied. All rats underwent a 60-minute acclimation period in locomotor activity boxes before drug administration. A precise microinjection into the NAcc core was performed 10 minutes before administering AMPH (1.0 mg/kg, IP). The microinjection contained either VEH, JMV2959 (2 μg/0.5 μl/side or 10 μg/0.5 μl/side), or SCH23390 (0.1 μg/0.5 μl/side or 1 μg/0.5 μl/side). Locomotor activity was subsequently monitored for 60 minutes to assess the impact of treatments on behavior.

2.11.4 Experiment 4

To investigate the effects of D1R activation in the NAcc on the locomotor sensitization induced by AMPH pre-treatment and subsequent food restriction, rats were first pre-exposed to either saline or AMPH (1.0 mg/kg, IP) at intervals of 2 to 3 days, for a total of four administrations. To reduce any confounding conditioning effects, AMPH was administered in different locations (in activity boxes for the first and fourth injections, and in home cages for the intermediate injections). After pre-exposure was completed, rats were allowed for the 2-weeks of drug-free period, and they were divided into three sub-groups with distinct food restriction conditions: NF, aFR, and cFR. Then, sensitization testing was conducted after two weeks of drug-free period. Following a 60-minute acclimation in activity boxes, bilateral microinjection of either saline or the D1R agonist SKF81297 (0.5 μg/0.5 μl/side) was made for each group and their locomotor activity was measured for additional 60-minute.

To further confirm that the D1R system in the NAcc core interacts with a certain level of plasma ghrelin to affect AMPH-induced locomotor activity, plasma ghrelin levels were compared from rats subjected to either saline or AMPH pre-exposure with aFR. Blood samples were collected at five time points: D1+ (the next day after pre-exposure day 1), D4+ (the next day after pre-exposure day 4), WD 7 (the 7th day of withdrawal), WD 14 (the 14th day of withdrawal), and aFR+ (the next day of acute food restriction).

2.12. Histology

Rats that underwent surgery were anesthetized and perfused with saline followed by 10% formalin via intracardiac infusion. Brains were post-fixed in 10% formalin, sectioned coronally (40 μm), and cannula placement within the NAcc core was verified using cresyl violet staining. Only data from rats with accurately placed cannula tips were included in the analysis.

2.13. Data analysis

Data are presented as means + standard error of means (+ SEM). Statistical analyses were performed using SigmaPlot version 12.0 (Systat Software, San Jose, CA, USA). Unpaired t-test was used to compare the means between two groups. Depending on the experimental design, one-way ANOVA or two-way repeated measures ANOVA was employed. Significant differences were determined at $P < 0.05$. For analyses yielding significant main effects or interactions, post-hoc comparisons were conducted using the Bonferroni test to identify specific group differences.

3. Results

3.1. Visualization of the existence of GHSRs at the protein level in the NAcc

Before beginning to investigate the role of ghrelin signaling and the dopamine system within the NAcc core in response to food restriction and AMPH administration, it needs to be confirmed whether GHSRs exist at the protein level in the NAcc, as previous studies have only identified them at the mRNA level, and some have disputed about their expression in this site.

As shown in Figure 1, GHSR signals were detected at the protein level in the NAcc. Furthermore, double staining using antibodies against the neuronal marker NeuN and GHSR revealed that most GHSRs were expressed in neurons (Figure 1C-D). Given that more than 95% of NAcc neurons are medium spiny neurons (MSNs),[64] it was further investigated whether GHSRs are expressed in MSNs using the MSN marker dopamine- and cAMP-regulated neuronal phosphoprotein 32 kDa (DARPP-32). The results showed that GHSRs were highly expressed in MSNs, with some additional marginal expression observed in non-MSNs (Figure 1E-F).

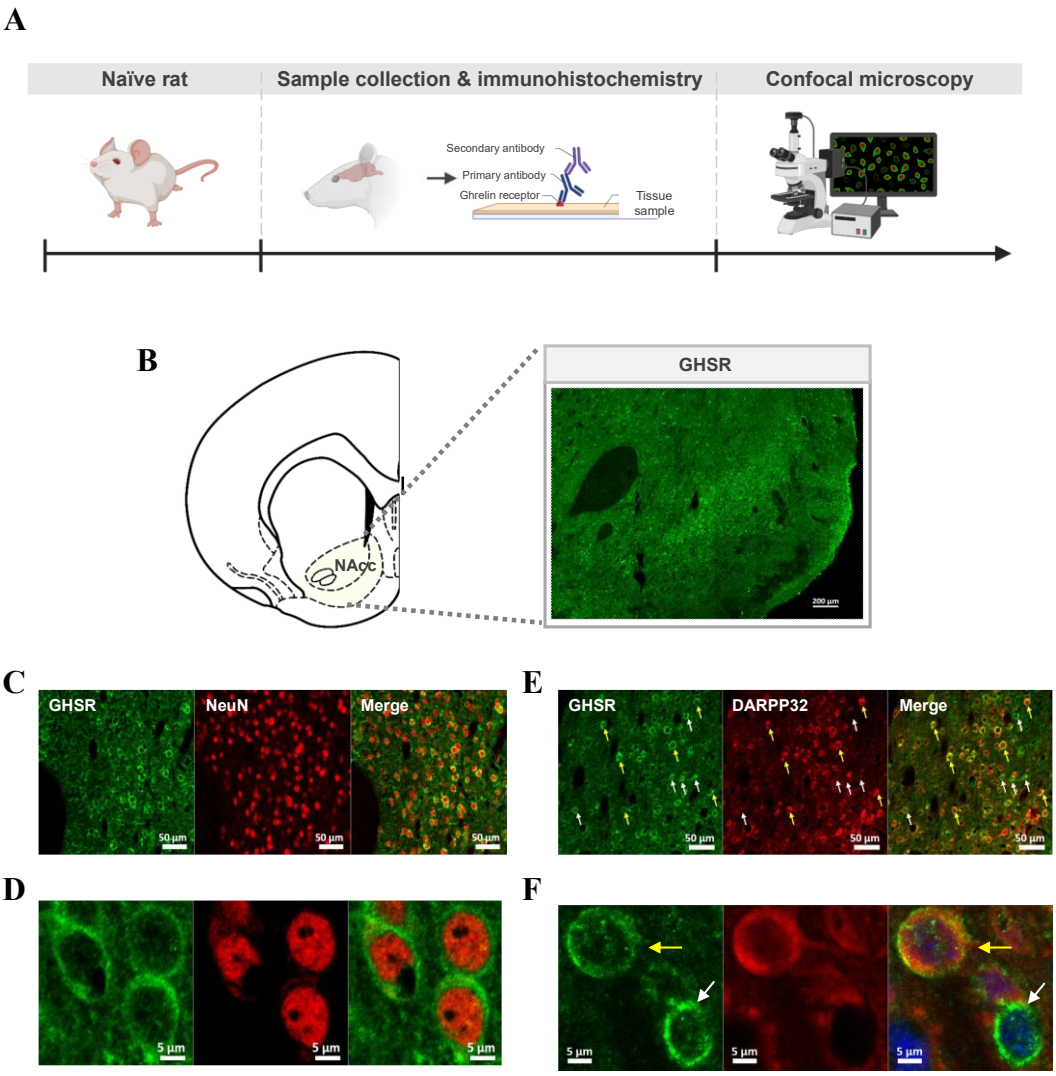

Figure 1. Visualization of GHSRs in the NAcc. A. Naïve rats were used to obtain brain samples for immunohistochemistry. These samples were subsequently processed for confocal microscopy to enable the visualization and localization of GHSR at the protein level within the NAcc. Created with Biorender.com. **B.** Fluorescence signals of GHSR in the NAcc confirm the protein-level presence of GHSR in this region (scale bar: 500 µm). Images were taken with 8x6 tiles at 20x magnification using confocal microscopy. **C-D.** Images at 20x magnification (C; scale bar: 50 µm) and 63x magnification with 3x zoom (D; scale bar: 5 µm) showing that nearly all GHSRs are expressed in

neurons in the NAcc. **E-F**. Images at 20x magnification (E; scale bar: 50 μm) and 63x magnification with 3x zoom (F; scale bar: 5 μm) showing that the majority of GHSR signals co-localize with DARPP-32, a marker for MSNs, in the NAcc. Yellow arrows point to GHSRs in MSNs, while white arrows indicate expression in non-MSNs.

3.2. cFR increases ghrelin levels and enhances acute AMPH-induced locomotor activity

The objective of this experiment was to investigate the effects of acute and chronic food restriction on plasma ghrelin levels and acute AMPH-induced locomotor activity. Rats were divided into three groups: NF, aFR and cFR, and subjected to their respective food restriction conditions. Our experimental design, as detailed in Figure 2A, tracked changes in body weight and ghrelin levels (Figures 2B and 2C). The body weight of rats in the aFR group was reduced to approximately 95% of the NF group, while the cFR group exhibited a more substantial reduction, maintaining only 80% of the weight of the NF group (Figure 2B). During the food restriction phase, plasma ghrelin levels were measured from jugular vein blood samples at three time points: baseline (before the food restriction, FR 0), 7^{th} day of food restriction (FR 7), and 14^{th} day of food restriction (FR 14) for all groups (Figure 2C). The two-way repeated measure ANOVA identified significant effects of the food restriction condition [$F_{2,37} = 5.730$, $P < 0.01$] and the interaction between the food restriction condition and the day of the food restriction period [$F_{4,74} = 4.005$, $P < 0.01$]. Specifically, in the cFR group, plasma ghrelin levels showed a notable increase on FR 7 and FR 14 compared to baseline levels within the same group ($P < 0.01$, by post hoc Bonferroni comparison). Furthermore, ghrelin concentrations in the cFR group were significantly elevated compared to the NF group at both FR 7 and FR 14 ($P < 0.01$ for both, as determined by post hoc Bonferroni comparisons), and to those in the aFR group on FR 7 and FR 14 ($P < 0.001$ and $P < 0.05$, respectively, by post hoc Bonferroni comparison), indicating that cFR provokes a more pronounced ghrelin response than aFR.

To investigate the effects of acute and chronic food restriction on the response to acute AMPH injection, rats were given AMPH (1 mg/kg, IP) after the food restriction period. Rats were first placed in a locomotor activity box for 1 hour to measure baseline locomotor activity (Figure 3A). The one-way ANOVA revealed significant differences in baseline locomotor activity between the groups [$F_{2,37} = 9.282$, $P < 0.001$]. Post hoc Bonferroni comparisons showed that both the aFR and cFR groups had significantly lower locomotor activity compared to the NF group ($P < 0.01$). After the baseline measurements, AMPH was administered to each group and locomotor activity was measured for an additional 60 min. The cFR group showed a significant increase in locomotor activity compared to the NF group ($P < 0.05$, post hoc Bonferroni comparison) (Figure 3B). The one-way ANOVA indicated significant variations in AMPH-induced locomotor activity between the

groups [$F_{2,37}$ = 4.351, P < 0.05]. Time-course data shown in Figure 3C illustrate significant differences in locomotor activity between the groups; the aFR and cFR exhibited a notable decrease in activity compared to the NF group before the AMPH injection, while the cFR group displayed a heightened response to AMPH post-injection. The two-way repeated measures ANOVA indicated significant differences in locomotor activity between the groups during the habituation phase [$F_{2,37}$ = 9.282, P < 0.001], with both aFR and cFR group showing significantly lower activity levels compared to the NF group at the -60 min time points (Bonferroni post hoc test, P < 0.001 and P < 0.05). The two-way repeated measures ANOVA after AMPH administration revealed a significant difference in locomotor activity between groups [$F_{2,37}$ = 4.351, P = 0.020]. The cFR group showed a significant increase in locomotor activity levels at 40 min compared to the NF and aFR groups, respectively (Bonferroni post hoc test, P < 0. 01 and P < 0.05).

To determine the relationship between AMPH-induced locomotor activity and plasma ghrelin levels under different food restriction conditions, a correlation analysis was conducted. A significant correlation was observed between plasma ghrelin levels after 14 days of FR and locomotor activity following AMPH administration, particularly within the cFR group (r = 0.557, P = 0.039, Figure 3F). No such correlation was evident in the NF and aFR groups (Figure 3D-E). This finding suggests that cFR uniquely affects the relationship between increased plasma ghrelin levels and AMPH-induced locomotor activity.

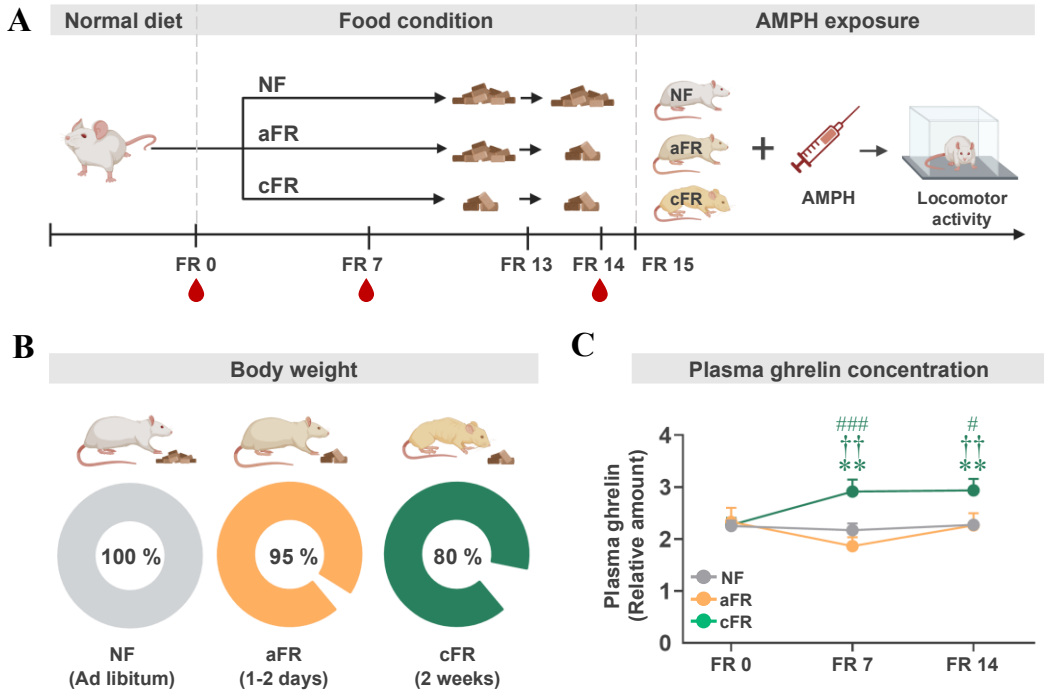

Figure 2. Effects of food restriction on body weight and plasma ghrelin levels in rats. A. A schematic representation of experimental design. Rats were subjected to different food conditions: NF, aFR or cFR. AMPH exposure occurred on day 15 of food restriction, and subsequent locomotor activity was measured. Created with Biorender.com. **B.** Pie charts display the relative body weight after the food restriction period, expressed as a percentage of the NF group's average body weight. **C.** The graph shows plasma ghrelin concentration levels at FR 0, FR 7, and FR 14 for each group. Data are presented as the average for each group mean (+SEM). Symbols denote statistically significant differences as determined by post hoc Bonferroni comparisons following two-way repeated measures ANOVA. **P<0.01 indicates significantly higher ghrelin levels on FR 7 and FR 14 in the cFR group compared to their own baseline. ††P<0.01 indicates significantly higher ghrelin levels in the cFR group compared to the NF group on FR 7 and FR 14. ###P<0.001, #P<0.05 denote significantly higher ghrelin levels in the cFR group compared to the aFR group on FR 7 and FR 14, respectively. Group sizes are as follows: NF (n=18), aFR (n=8), cFR (n=14).

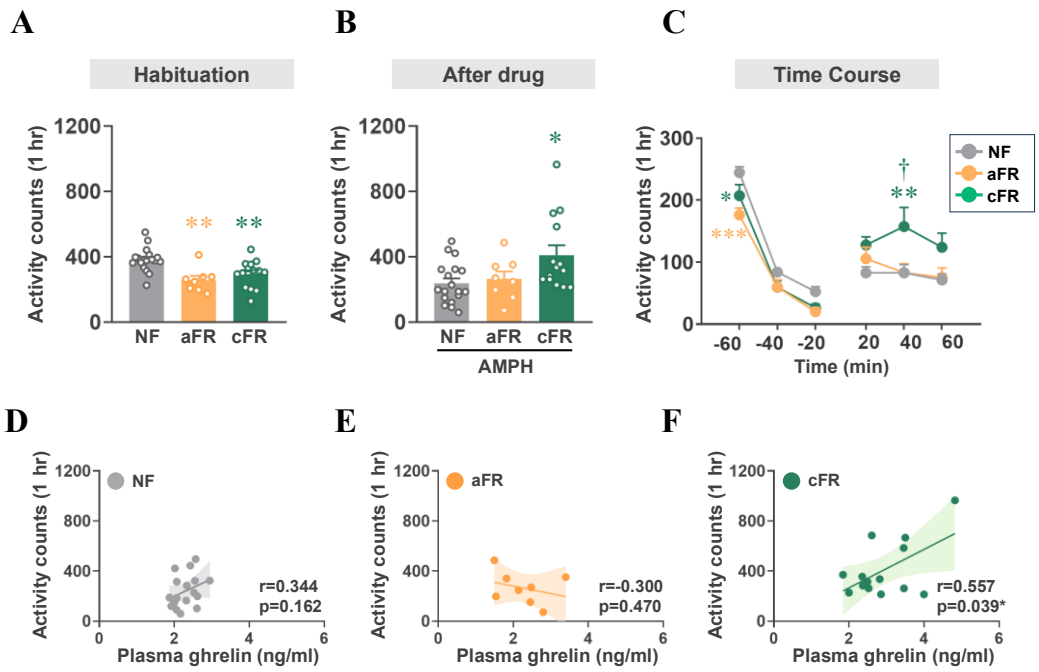

Figure 3. Differential responses in locomotor activity to acute AMPH. A. Habituation to a new environment, measured over an hour, shows the average locomotor activity levels for each group. **P<0.01: Notably lower activity counts in the aFR and cFR groups compared to NF group. **B.** The graph shows the overall locomotor activity counts during 60-minute test following an AMPH challenge, presented as the average for each group mean (+SEM) *P<0.05, a significant increase in activity in the cFR group versus the NF group. **C.** Time-course data are shown as group mean (+SEM) locomotor activity counts at 20-minute intervals obtained during the 1 hour preceding (-60 through 0 min) and the 1 hour following AMPH IP injection (0 to 60 min). Statistical analysis was performed by post hoc Bonferroni comparisons following two-way repeated measures ANOVA. Significant differences are marked as follows: ***P<0.001, **P<0.01, *P<0.05 for higher counts in the aFR and cFR compared to the NF group over time, and †P<0.05 for higher counts in the cFR group versus the aFR group. **D-F.** Data showing the correlation between plasma ghrelin concentrations on day 14 of food restriction and locomotor activity 60 minutes after AMPH injection. While there was no significant correlation between ghrelin levels and locomotor activity counts in NF and aFR groups, there was a significant positive correlation observed in cFR group (*P<0.05).

3.3. cFR alters the D1R system in the NAcc core

Previous studies have shown that systemically or centrally injected ghrelin can modulate dopamine signaling in the NAcc[33,65] and that the combined effects of ghrelin and D1R agonists in the NAcc core synergistically enhance AMPH responses.[37] Given that increased plasma ghrelin in the cFR group correlated with AMPH-induced locomotor activity, it was hypothesized that the cFR-induced enhancement of AMPH-induced locomotor activity is mediated by changes in the ghrelin and dopamine systems in the NAcc core. Therefore, to elucidate the effects of food restriction on AMPH responses, the expression of GHSR, D1R, and D2R in the NAcc were measured using brain samples collected after administering acute AMPH and measuring locomotor activity for 1 hour following food restriction. GHSR levels were notably increased in the aFR group compared to the NF and cFR groups, as revealed by one-way ANOVA [$F_{2,37} = 16.104$, $P < 0.001$] and subsequent Bonferroni post hoc tests (aFR vs. NF, $P < 0.001$, aFR vs. cFR, $P < 0.001$, Figure 4A), but returned to basal levels in the cFR group, suggesting a possible adaptation to prolonged food restriction. Furthermore, D1R levels were significantly higher in the cFR group compared to both NF and aFR groups, as revealed by one-way ANOVA ($F_{2,37} = 6.291$, $P < 0.01$, Figure 4B), with Bonferroni post hoc tests clarifying differences between the groups (cFR vs. NF $P < 0.05$; cFR vs aFR $P < 0.01$). These results suggest an enhanced reactivity of D1R to dopamine. Conversely, D2R expression did not differ significantly between groups (Figure 4C), indicating a selective effect of food restriction on dopaminergic receptor subtypes within the NAcc core.

Administration of AMPH following cFR is known to further increase dopamine release in the NAcc.[46] Similarly, systemic administration of both ghrelin and AMPH has been to shown to enhance dopamine release in the NAcc.[45,65] Given these effects, under cFR conditions, the rise in plasma ghrelin and the increase in D1R expression in the NAcc are proposed to synergistically enhance D1R signaling, potentially influencing D1R recycling during AMPH exposure.[66] Further analysis on the fractionation of the NAcc tissue samples revealed differential distribution of D1R within cellular compartments by comparing its localization in the plasma membrane to the total cellular membrane, which includes both plasma and organelle membranes. Unpaired t-tests revealed significant differences between NF and cFR groups. D1R levels in the plasma membrane were significantly decreased in cFR group ($t_9 = 2.870$, $P < 0.05$), as shown in Figure 5A. In contrast, D1R levels in the total cellular membrane were significantly increased in cFR group ($t_9 = -2.531$, $P < 0.05$), as

illustrated in Figures 5B. Additionally, the ratio of D1R in the plasma membrane to total cellular membrane, indicating the possibility of receptor translocation, was significantly reduced in the cFR group compared to NF (t_9= 2.993, $P < 0.05$, Figure 5C).

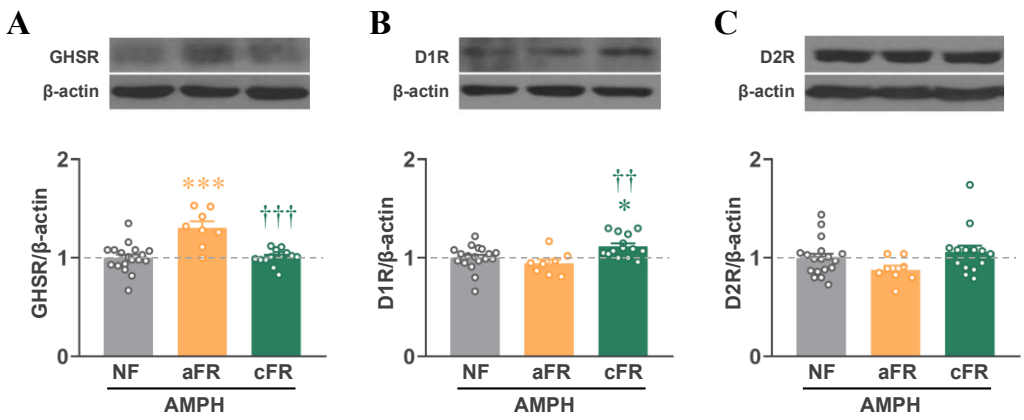

Figure 4. The expression of GHSR, D1R, and D2R in the NAcc core. A-C. Quantification of GHSR, D1R, and D2R levels with representative Western blot bands are shown. Band intensities were normalized to β-actin for each group and expressed as standard error of the mean (+SEM). Statistical significance was determined using the one-way ANOVA followed by Bonferroni post-hoc tests. **A.** GHSR levels were significantly higher in the aFR group than in both the NF and cFR groups (***P<0.001 aFR vs. NF; †††P<0.001 aFR vs. cFR) **B.** D1R levels were notably higher in the cFR group compared to both the NF group (*P<0.05) and the aFR group (††P<0.01). **C.** No significant differences in D2R levels were observed between the groups.

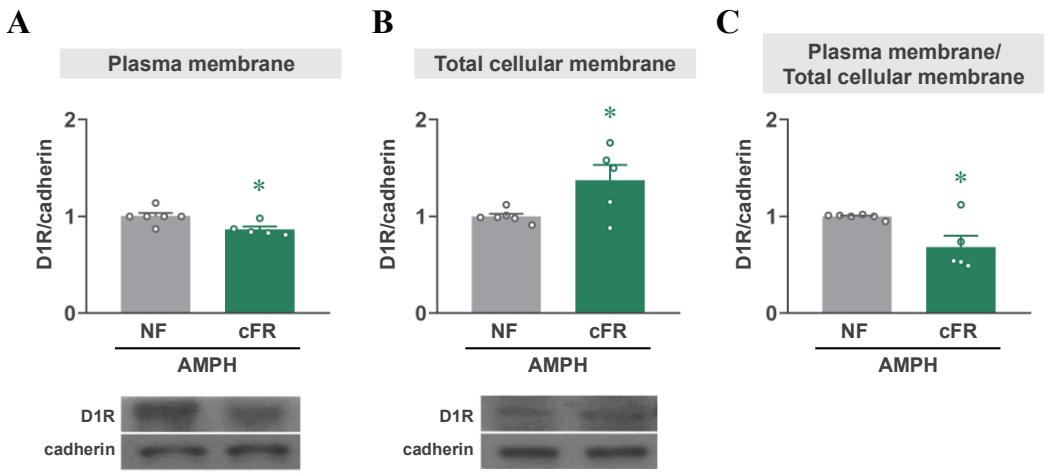

Figure 5. D1R expression in plasma membrane and total cellular membrane in NF and cFR groups following AMPH administration. **A-C.** Analysis of D1R expression in the plasma membrane and total cellular membrane. Results were normalized to cadherin levels for each group, presented as mean values with standard error of the mean (+SEM). Statistical significance was assessed using t-test with Bonferroni correction. Group sizes are as follows: NF (n=6), cFR (n=5). **A.** D1R levels at the plasma membrane markedly lower in the cFR group compared to the NF group (*P<0.05). **B.** The cFR group exhibited a notable elevation in D1R levels across the total cellular membrane, which encompasses both the organelle and plasma membranes, in contrast to the NF group (*P<0.05). **C.** The ratio of D1R levels in the plasma membrane to total cellular membrane was substantially dropped in the cFR group relative to the NF group. (*P<0.05)

3.4. Enhanced AMPH-induced locomotor activity in cFR rats was inhibited by systemic administration and microinjection into NAcc core of GHSR and D1R antagonists

To investigate systemic the role of increased plasma ghrelin (Figure 2C) in cFR-induced AMPH responses, along with the quantitative changes of D1R in the NAcc core (Figure 4B), rats subjected to acute or chronic food restriction were systemically administered a GHSR antagonist (JMV2959) or a D1R antagonist (SCH23390). The concentrations of antagonists were selected based on previous studies to ensure they did not alter basal locomotion.[65,67] As depicted in Figure 6A, this protocol was employed to assess the effect of GHSR and D1R antagonists on AMPH-induced locomotor activity. In the post-drug phase, no significant differences in locomotor activity were detected in the aFR rats treated with different doses of JMV2959 or SCH23390 compared to the VEH group, as indicated by one-way ANOVA [$F_{4,37} = 1.916$, $P = 0.128$] (Figure 6B). Conversely, in the cFR group, a significant decrease in locomotor activity was observed at a higher doses of JMV2959 (6 mg/kg) and SCH23390 (0.03 mg/kg) in comparison to the VEH group, as revealed by one-way ANOVA [$F_{4,42} = 5.556$, $P < 0.01$] followed by Bonferroni post hoc tests (VEH vs. JMV2959 6 mg/kg, $P < 0.05$; VEH vs. SCH23390 0.03 mg/kg, $P < 0.01$, Figure 6C).

Prior observations shown in Figure 4 indicated changes in D1R and GHSR levels in the NAcc core after AMPH administration under food-restricted conditions. This led to the hypothesis that these pathways in the NAcc core are crucial for the enhanced AMPH effects post-food restriction. Therefore, it was questioned how administering D1R and GHSR antagonists in the NAcc core affects the enhanced AMPH response under cFR conditions. To explore this, following cannulation surgery and one-week recovery period, rats underwent two weeks of food restriction. The antagonists were administered directly into the NAcc core of cFR rats before systemic injection of AMPH, and locomotor activity was subsequently measured (Figure 7A). In the post-drug phase, significant differences in locomotor activity were detected in cFR rats treated with different doses of JMV2959 or SCH23390 compared to VEH group, as indicated by one-way ANOVA [$F_{4,33} = 18.364$, $P<0.001$]. Specifically, a high dose of JMV2959 (10 μg/side) and both doses of SCH23390 (0.1 μg/side and 1 μg/side) resulted in a significantly reduced of locomotor activity in cFR rats compared to VEH-cFR counterparts, with Bonferroni post hoc tests affirming the significance of these decreases (VEH vs.

JMV2959 10 μg/side, $P < 0.01$; VEH vs. SCH23390 0.1 μg/side, $P < 0.001$; VEH vs. SCH23390 1 μg/side, $P < 0.001$) (Figure 7C). These results indicate that the effect of cFR on AMPH responses was mediated by ghrelin and dopamine signaling system in the NAcc core.

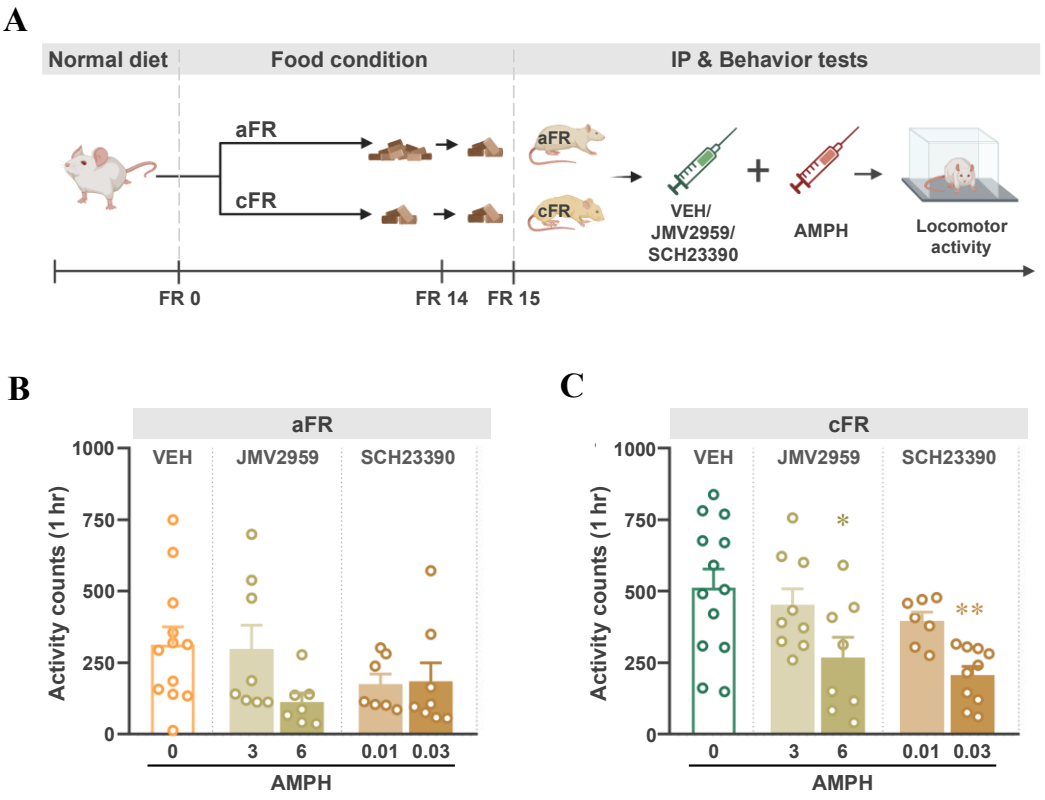

Figure 6. Systemic effects of GHSR and D1R antagonism on AMPH-induced locomotor activity in food-restricted rats. A. A schematic representation of experimental design. On day 15 food restriction (FR 15), IP injections of VEH, JMV2959, or SCH23390 were made followed by AMPH administration and subsequent locomotor activity measurement. Created with Biorender.com. **B.** The average activity counts for each group are presented with the standard error of the mean (+SEM). In aFR rats, locomotor activity was not significantly changed after AMPH administration. Group sizes are as follows: VEH (n=12), JMV2959 3 mg/kg (n=8), JMV2959 6 mg/kg (n=7), SCH23390 0.01 mg/kg (n=7), SCH23390 0.03 mg/kg (n=8). **C.** In cFR rats, AMPH-induced locomotor activity was significantly inhibited with high doses of JMV2959 and SCH23390. Statistically lower activity levels were marked with *P<0.05, **P<0.01 compared to the VEH group, as determined by one-way ANOVA with Bonferroni post-hoc analysis. Group sizes are as follows: VEH (n=13), JMV2959 3 mg/kg (n=9), JMV2959 6 mg/kg (n=8), SCH23390 0.01 mg/kg (n=7), SCH23390 0.03 mg/kg (n=10).

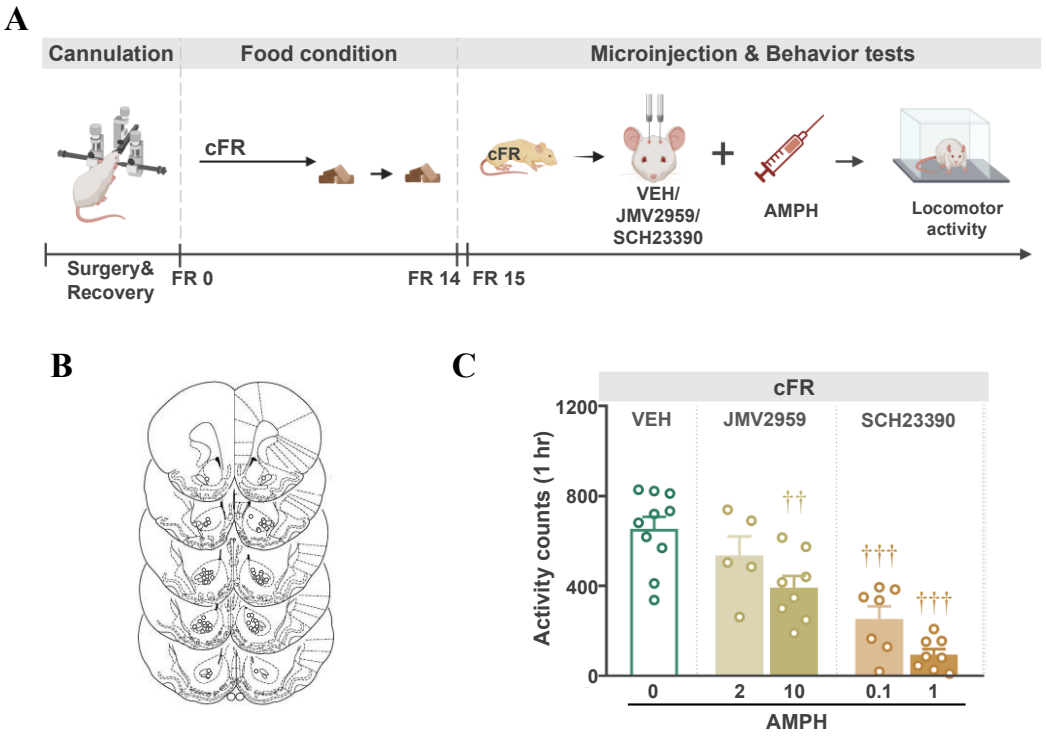

Figure 7. The effects of microinjection into the NAcc of GHSR and D1R antagonism on AMPH-induced locomotor activity in cFR rats. A. A schematic representation of experimental design. On FR 15, rats received microinjections of either VEH, JMV2959, or SCH23390 directly into the NAcc core, followed by AMPH administration and subsequent locomotor activity measurement. Created with Biorender.com. **B.** Verification of the precise placement of cannula tips in the NAcc core, as guided by Paxions & Watson (1997). **C.** Activity counts for each group are displayed with standard error of the mean (+SEM). Compared to the cFR-VEH group, significant inhibition was observed at a high dose of JMV2959 (††P<0.01), while SCH23390 blockade effects were significant at both low and high doses (†††P<0.001), as determined by one-way ANOVA with Bonferroni post-hoc analysis. Group sizes are as follows: cFR-VEH (n=10), cFR-JMV2959 2 μg/side (n=5), cFR-JMV2959 10 μg/side (n=8), cFR-SCH23390 0.1 μg/side (n=7), cFR-SCH23390 1 μg/side (n=8).

3.5. Food restriction after AMPH pre-treatment induces behavioral sensitization via the NAcc core D1R system

These experiments aim to investigate whether food restriction after AMPH pre-treatment can induce behavioral enhancement via the D1R system in the NAcc core, similar to the responses to AMPH treatment after food restriction. After cannula surgery, rats were pre-exposed to saline or AMPH four times, followed by food restriction for a withdrawal period. Their locomotor responses were assessed following either VEH or the D1R agonist, SKF81297 (0.5 μg/0.5 μl/side), administered directly into the NAcc core (Figure 8A). A counterbalancing procedure was employed across groups to alternate between SKF81297 and VEH treatments, with a three-day break between sessions to minimize potential biases.

The results revealed pronounced differences in locomotor activity, particularly showing that a sensitized locomotor activity was observed in AMPH pre-exposed rats to the combined effects of food restriction and SKF81297 administration compared to their saline pre-exposed counterparts. Analysis of the saline pre-exposed groups revealed significant variations in 1 hour locomotor activity measurements (Figure 8C). Two-way repeated measures ANOVA revealed significant effects in the food restriction conditions [$F_{2,37} = 7.435$, $P < 0.01$], and in the interaction between the food restriction condition and the SKF81297 administration status [$F_{2,37} = 7.133$, $P < 0.01$]. Specifically, the cFR group treated with SKF81297 showed a significant increase in locomotor activity compared to both the NF group receiving SKF81297 ($P < 0.001$), and the cFR group treated with VEH ($P < 0.001$). These result show that cFR by itself has the ability to produce an enhanced locomotor response to D1R activation in the NAcc core without the help of AMPH pre-exposure.

Further examination on AMPH pre-exposure groups revealed significant enhancements in locomotor activity (Figure 8E), with two-way repeated measures ANOVA showing significant effects of food restriction conditions [$F_{2,45} = 13.827$, $p < 0.001$], SKF81297 injection [$F_{1,45} = 75.728$, $p < 0.001$], and their interaction [$F_{2,45} = 11.595$, $P < 0.001$]. Bonferroni post hoc tests highlighted these enhancements, demonstrating a pronounced increase in locomotor activity ($P < 0.001$) for both the aFR and cFR groups treated with SKF81297 relative to the NF group receiving SKF81297. Furthermore, differences within each dietary condition were pronounced, with Bonferroni post hoc tests confirming significant differences in locomotor responses within aFR and cFR conditions ($P <$

0.001).

Prior results showed that an increased level of plasma ghrelin was detected by cFR and it played a significant role in the enhancement of AMPH-induced locomotor activity by making interaction with D1R system in the NAcc core (Figures 2~7). Therefore, it was hypothesized that the observed increase in locomotor activity by aFR in AMPH pre-exposed group in the presence of D1R agonist could be also related with the increase of plasma ghrelin levels. To examine this hypothesis, blood samples from both saline and AMPH pre-exposed groups were collected at five time points: D1+ (the next day after pre-exposure day 1), D4+ (the next day after pre-exposure day 4), WD 7 (the 7th day of withdrawal), WD 14 (the 14th day of withdrawal), and aFR+ (the next day of acute food restriction).

The two-way repeated measures ANOVA identified significant effects of the pre-exposure condition [$F_{1,12} = 5.040$, $P < 0.05$] and the day of the blood collection [$F_{4,48} = 5.761$, $P < 0.001$]. Specifically, in the AMPH pre-exposure group, plasma ghrelin levels showed a notable increase on aFR+ compared to D1+ levels within the same group ($P < 0.05$, by post hoc Bonferroni comparison). Furthermore, ghrelin concentrations in the AMPH pre-exposure group were significantly elevated compared to the saline pre-exposure group at aFR+ ($P < 0.05$, as determined by post hoc Bonferroni comparisons), indicating that aFR combined with AMPH pre-exposure causes a significant increase in plasma ghrelin levels.

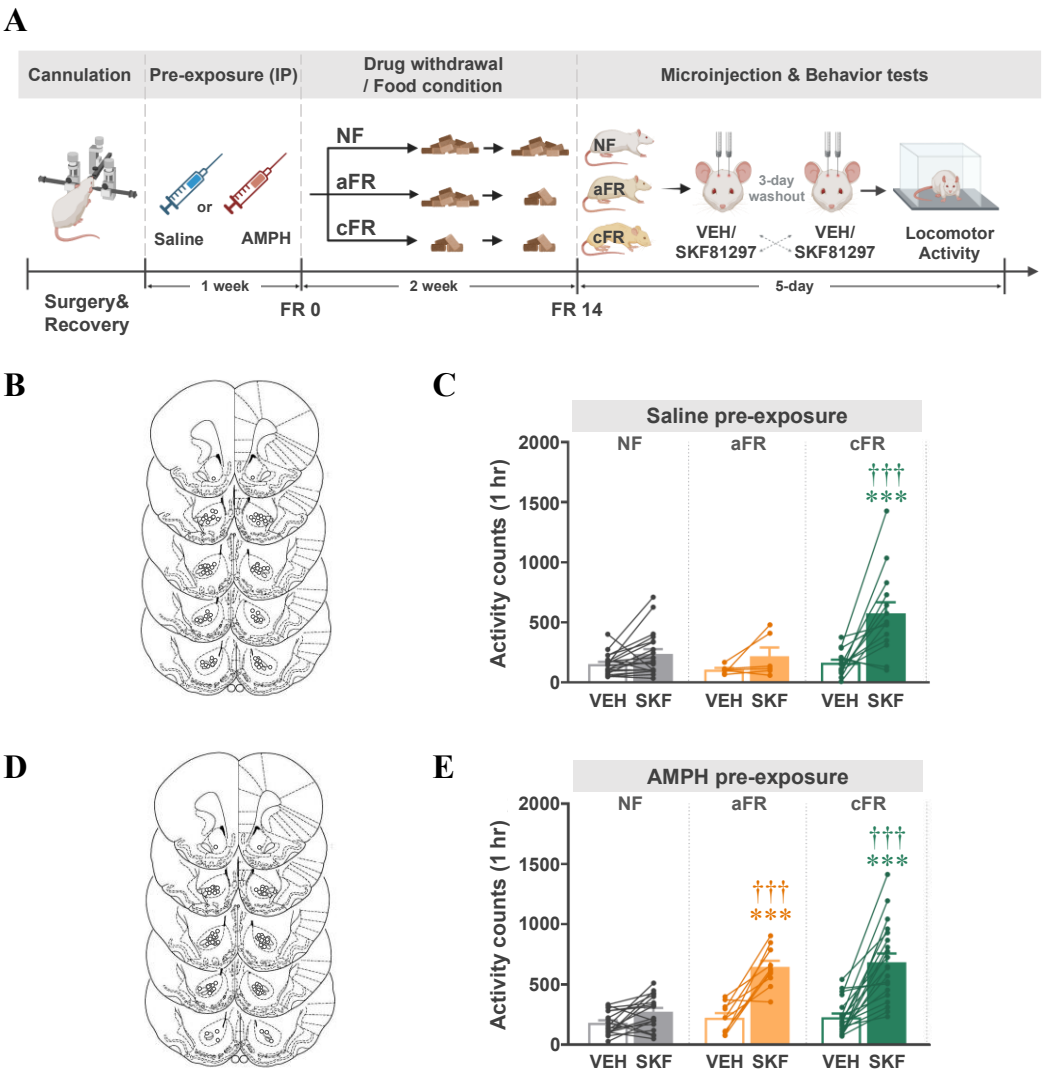

Figure 8. Combined effects of food restriction and dopamine D1R agonist in the NAcc core on locomotor enhancement. A. A schematic representation of experimental design. Rats underwent surgery for cannulation followed by pre-exposure to either saline or AMPH and were then subjected to food restriction conditions: NF, aFR or cFR. After drug withdrawal and food restriction, the rats received injections of either VEH or the D1R agonist SKF81297 (SKF) directly into the NAcc core, followed by locomotor activity measurements. Created with Biorender.com. **B-C** Saline pre-exposure effects: **B.** Verification of the precise placement of cannula tips in the NAcc core, as guided

by Paxions & Watson (1997). **C.** Presentation of 1 hour locomotor activity in saline pre-exposed rats across the dietary groups, with significant differences noted: ***P<0.001 for NF-SKF vs. cFR-SKF and †††P<0.001 for cFR-VEH vs. cFR-SKF, determined through two-way repeated measures ANOVA with Bonferroni post hoc tests, data shown as mean activity counts +SEM. Group sizes are as follows: NF-VEH (n=20), NF-SKF (n=20), aFR-VEH (n=6), aFR-SKF (n=6), cFR-VEH (n=14), and cFR-SKF (n=14). **D-E**. AMPH pre-exposure effects: **D.** Verifies the precise placement of cannula tips in the NAcc core, as guided by Paxions & Watson (1997). **E.** Details of post-AMPH exposure effects across dietary groups, showing significant locomotor activity enhancements in the aFR-SKF and cFR-SKF groups, ***P<0.001 compared to NF-SKF, and marked differences within the aFR and cFR conditions, †††P<0.001. Group sizes are as follows: NF-VEH (n=18), NF-SKF (n=18), aFR-VEH (n=10), aFR-SKF (n=10), cFR-VEH (n=20), cFR-SKF (n=20).

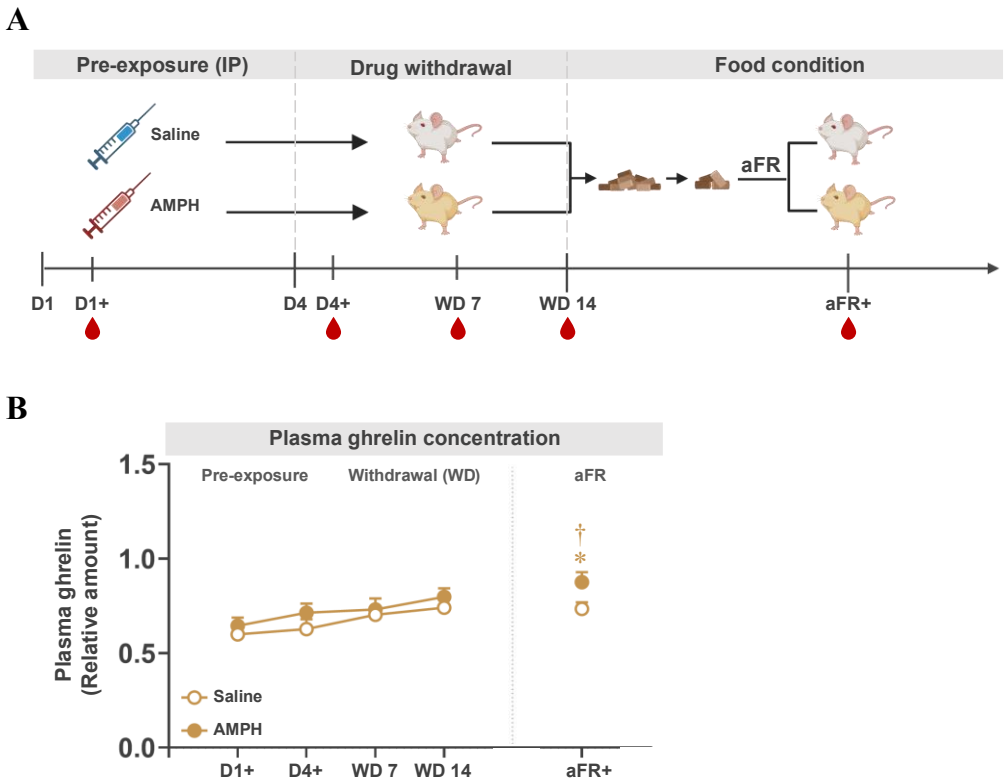

Figure 9. Changes in plasma ghrelin levels following aFR after saline or AMPH pre-exposure. **A.** A schematic representation of experimental design. Rats underwent IP pre-exposure (saline or AMPH for four days), followed by drug withdrawal and aFR. Blood samples were collected on D1+ (the next day after pre-exposure day 1), D4+ (the next day after pre-exposure day 4), WD 7 (the 7th day of withdrawal), WD 14 (the 14th day of withdrawal), and aFR+ (the next day of acute food restriction). Created with Biorender.com. **B.** Data is presented as the mean for each group (+SEM). Symbols denote statistically significant differences as determined by post hoc Bonferroni comparisons following two-way repeated measures ANOVA. *P<0.05 indicates significantly higher ghrelin levels on aFR+ in the AMPH pre-exposure group compared to their own baseline. †P<0.05 indicates significantly higher ghrelin levels in AMPH pre-exposure group compared to the saline pre-exposure group on aFR+. Group sizes are as follows: Saline pre-exposure (n=8), AMPH pre-exposure (n=6).

4. Discussion

This study demonstrates that the effects of food restriction on psychostimulant-induced behaviors like those elicited by AMPH are influenced by both the duration and timing of restriction. It further identifies the involvement of changes in plasma ghrelin levels and dopamine signaling in the NAcc core in mediating these processes, highlighting food conditions as a significant factor in regulating addictive behaviors.

It was visually demonstrated, for the first time to the best of my knowledge, the expression of GHSRs at the protein level in the NAcc. Further, it was revealed that most GHSRs exist in MSNs (Figure 1E-F). Given that almost 95% of neurons in the NAcc are classified as MSNs,[64] it is well established that the modulation of reward-related behaviors is linked to the specific dopamine receptor subtypes present on these cells.[68] The present findings suggest that ghrelin may have a regulatory role in mediating these processes by interacting with dopamine receptors on MSNs. Moreover, the formation of heterodimers between GHSRs and dopamine receptors has been identified in various brain regions, including the cerebral cortex, hippocampus, substantia nigra, midbrain, and ventral tegmental area.[69] This widespread presence suggests that similar interactions likely occur in the NAcc, supporting the idea that ghrelin modulates dopamine signaling to influence reward behavior.

Ghrelin, an appetite regulating hormone, is known to fluctuate in circadian rhythms in both humans and rodents.[70] Its levels increase during the active phase, when eating or other activities occur, and remains low during the resting phase.[71] Further, rather than a steady rise when hungry, ghrelin rises sharply when anticipating a meal and falls down following a period with no food.[72] In this study, food restriction groups of rats were offered restricted amounts of food at a certain designated time and blood samples were collected simultaneously to reflect ghrelin's daily rhythmic fluctuations. Accordingly, the finding that cFR group showed a significant increase in ghrelin levels (Figure 2C) may result from rats anticipating food at the same time daily and physiological shift in ghrelin's circadian rhythm. However, only with a short-term food restriction of one or two days in the aFR group may be insufficient to show ghrelin change by altering the natural hormonal cycle as shown in Figure 2C. Similar findings have been reported in other studies, which consistently show that long-term food restriction of two weeks significantly increases ghrelin levels,[48,49] whereas short-

term restriction of one or two days does not.[73] Although some studies have observed ghrelin increases following short-term, these results were based on conditions where rats were entirely deprived of food for two days, and the timing of blood collection was not clearly specified.[74] Such differences in experimental protocols may account for the inconsistencies.

This change in ghrelin levels in the cFR group appears to influence the responses to AMPH. Acute AMPH-induced locomotor activity was significantly enhanced in cFR group compared to the aFR and NF groups (Figure 3), showing a strong correlation with plasma ghrelin concentration (Figure 4C). Systemically administered ghrelin has been shown to alter plasma ghrelin levels and increase dopamine activity in the NAcc, leading to psychostimulant-induced behavioral reinforcement.[74,75] Therefore, the significant positive correlation between plasma ghrelin levels and AMPH-induced locomotor activity observed only under conditions of increased plasma ghrelin in cFR group emphasizes the pivotal role of plasma ghrelin in this effect.

Previous studies have shown that D1R agonist induces stronger locomotor and rewarding effects in 2 weeks food-restricted subjects compared to controls, with marked increases in c-fos immunostaining observed specifically in the NAcc.[11,42] These effects are not observed with D2R agonists, emphasizing the unique role of D1R signaling in enhancing these responses under the conditions of food restriction. Interestingly, in this study, the expression level of D1R, but not D2R, in the NAcc was found increased in cFR (Figure 4E, F). Consequently, a dopamine surge induced by AMPH may have synergistic interactions with increased D1R levels in cFR group of rats, potentially strengthening D1R signaling. Notably, one hour after AMPH administration in cFR rats, plasma membrane D1R levels decreased while total cellular D1R increased (Figure 5). This pattern raises the possibility that D1R internalization might have occurred in the cFR group upon AMPH exposure, serving as an indirect indication that D1R activation actually occured.[76] However, this study did not directly demonstrate a reduction in plasma membrane D1R levels in the cFR group as a result of internalization. Therefore, while our interpretation suggests this possibility, additional research is required to confirm the exact changes in D1R distribution under these conditions. Additionally, research has shown that GHSR significantly affects dopamine signaling by making heterodimers with D1R.[69] For example, it has been demonstrated that treating cultured hippocampal neurons with ghrelin and D1R agonist, SKF81297, increases colocalization and heterodimerization of GHSR-D1R, which enhancing Ca2+ influx via D1Rs and thereby amplifying dopamine

signaling.[77] These results suggest that administering AMPH in the presence of elevated ghrelin, as seen in cFR conditions, may evoke synergistic interaction between ghrelin and the D1R system in the NAcc resulting in enhanced locomotor activity. The blockade effects of enhanced locomotor activity in cFR by administration of GHSR and D1R antagonists both systemically (Figure 6C) and directly into the NAcc core (Figure 7C) support this hypothesis.

Different from the effects of AMPH exposure on locomotor activity under different pre-existing food restriction conditions, the effects of food restriction on locomotor activity in pre-existing AMPH exposure conditions were additionally examined in this study. Psychostimulants like AMPH are known not only for their appetite-regulating effects but also for their influence on the expression of their appetite-regulating peptides.[23-25] Based on these findings, it was expected that AMPH pre-exposure would also influence the effects of food restriction. To confirm this, the effects of food restriction were evaluated during the withdrawal period following AMPH exposure. It was found that D1R agonist-induced locomotor activity was significantly increased only in cFR in saline pre-exposed rats (Figure 8C), whereas it was significantly enhanced in both aFR and cFR conditions in AMPH pre-exposed rats (Figure 8E). In the literature, it was previously found that co-administration of ghrelin and a D1R agonist directly into the NAcc core, compared to the administration of either one alone, increases locomotor activity in saline pre-exposed rats, while it was manifested as more amplified in AMPH pre-exposed rats.[37] As cFR was shown to increase plasma ghrelin (Figure 2C), the present results showing that D1R agonist combined with cFR produces increased locomotor activity (Figure 8C, E) are consistent with previous findings.

In addition, the present results also showed that D1R agonist-induced locomotor activity was significantly increased even in aFR group in AMPH pre-exposed rats (Figure 8E), suggesting that pre-existing AMPH pre-exposure condition may have synergistically enhanced the ghrelin system to respond to D1R activation even in aFR group. This is supported by additional findings that plasma ghrelin levels in aFR group were significantly higher in AMPH pre-exposed compared to saline pre-exposed rats (Figure 9B). Previous studies have also found that total ghrelin blood levels positively correlate with cue-induced cocaine-seeking behavior, indicating a close link between ghrelin and the anticipation of rewards.[78] Furthermore, repeated cocaine exposure causes repeated temporary elevations in ghrelin levels, and even after unexpected saline administration, ghrelin levels rise due to the anticipation of cocaine's effects.[25] When cocaine methiodide, however, which does not cross

the blood-brain barrier, is used instead, this effect is significantly reduced.[79] Taken together, these observation suggest that psychostimulant-induced changes in ghrelin are mediated through central nervous system stimulation. Repeated exposure to psychostimulants can create a state of heightened reward expectation, where even a small energy deficit, such as aFR, or minor reward-related cues, can lead to elevated ghrelin levels. In this context, AMPH pre-exposure promotes ghrelin release even in aFR condition, subsequently interacting with D1R activation in the NAcc core to intensify locomotor activity.

This study demonstrates that cFR and AMPH pre-exposure share a common mechanism in enhancing plasma ghrelin-D1R-mediated locomotor responses. These findings suggest that the sensitization of the reward system by psychostimulants and energy deficit signals are interconnected via the dopamine-ghrelin pathway. This interplay underscores how food restriction can influence psychostimulant sensitivity and, conversely, how psychostimulant exposure can modulate physiological adaptations to energy deficits. While this study contributes to the growing field of research linking drug addiction and eating behavior, further investigations are needed to delineate the precise downstream mechanisms through which dopamine and ghrelin, or other appetite-regulating hormones, interact. Elucidating these pathways could provide critical insights into developing novel therapeutic strategies for addressing both drug addiction and eating-related conditions.

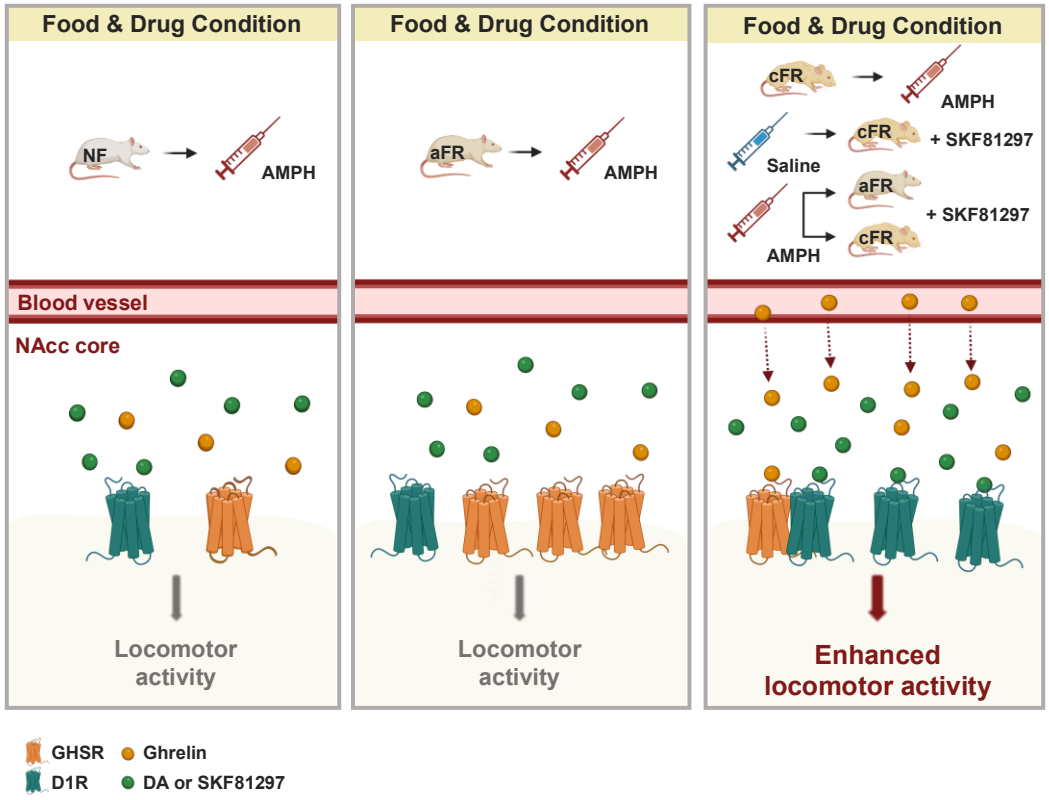

Figure 10. An overview of reciprocal effects of food restriction and amphetamine exposure. This figure illustrates how food restriction influences AMPH-induced effects and how AMPH pre-exposure modifies responses to subsequent food restriction. Food restriction, both before and after AMPH pre-exposure, sensitized the D1R system in the NAcc, enhancing behavioral responses. cFR elevated plasma ghrelin and D1R expression, significantly amplifying AMPH-induced locomotor activity, while aFR had no such effect despite increased GHSR levels in the NAcc core. Furthermore, following AMPH pre-exposure, FR enhanced D1R-mediated behavioral sensitization. In the cFR group, locomotor activity increased regardless of whether the animals were pre-exposed to saline or AMPH, driven by elevated plasma ghrelin and enhanced D1R activation. In contrast, while aFR alone did not alter plasma ghrelin levels, aFR following AMPH pre-exposure significantly increased plasma ghrelin, likely enhancing D1R agonist-induced locomotor activity. Created with Biorender.com.

5. Conclusion

This research reveals that endogenous ghrelin, elevated by food restriction, works in conjunction with D1R activation in the NAcc core to boost the effects of AMPH. Importantly, the timing and duration of these dietary changes were shown to influence the behavioral responses to psychostimulants, suggesting that the combined impact of eating status on psychostimulants, and the manifestation of addictive behaviors can be quite significant. Consequently, these findings imply that food conditions and targeted dietary interventions should be key factors when developing strategies for addiction treatment.

References

1. Kruger J, Galuska DA, Serdula MK, Jones DA. Attempting to lose weight: specific practices among U.S. adults. Am J Prev Med 2004;26:402-6.
2. Bish CL, Blanck HM, Serdula MK, Marcus M, Kohl HW, 3rd, Khan LK. Diet and physical activity behaviors among Americans trying to lose weight: 2000 Behavioral Risk Factor Surveillance System. Obes Res 2005;13:596-607.
3. Stice E, Davis K, Miller NP, Marti CN. Fasting increases risk for onset of binge eating and bulimic pathology: a 5-year prospective study. J Abnorm Psychol 2008;117:941-6.
4. Pietiläinen KH, Saarni SE, Kaprio J, Rissanen A. Does dieting make you fat? A twin study. Int J Obes (Lond) 2012;36:456-64.
5. Krahn D, Kurth C, Demitrack M, Drewnowski A. The relationship of dieting severity and bulimic behaviors to alcohol and other drug use in young women. J Subst Abuse 1992;4:341-53.
6. Cochrane C, Malcolm R, Brewerton T. The role of weight control as a motivation for cocaine abuse. Addict Behav 1998;23:201-7.
7. Volkow ND, Wang GJ, Fowler JS, Telang F. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philos Trans R Soc Lond B Biol Sci 2008;363:3191-200.
8. Volkow ND, Wang GJ, Tomasi D, Baler RD. Obesity and addiction: neurobiological overlaps. Obes Rev 2013;14:2-18.
9. Holderness CC, Brooks-Gunn J, Warren MP. Co-morbidity of eating disorders and substance abuse review of the literature. Int J Eat Disord 1994;16:1-34.
10. Cabeza de Vaca S, Carr KD. Food restriction enhances the central rewarding effect of abused drugs. J Neurosci 1998;18:7502-10.
11. Carr KD, Kim GY, Cabeza de Vaca S. Rewarding and locomotor-activating effects of direct dopamine receptor agonists are augmented by chronic food restriction in rats. Psychopharmacology (Berl) 2001;154:420-8.
12. Carr KD. Chronic food restriction: enhancing effects on drug reward and striatal cell signaling. Physiol Behav 2007;91:459-72.
13. Verdejo-Garcia A, Crossin R. Nutritional and metabolic alterations arising from stimulant

use: A targeted review of an emerging field. Neurosci Biobehav Rev 2021;120:303-6.

14. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656-60.
15. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, et al. Ghrelin increases food intake in obese as well as lean subjects. Int J Obes (Lond) 2005;29:1130-6.
16. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab 2008;7:400-9.
17. Goldstone AP, Prechtl CG, Scholtz S, Miras AD, Chhina N, Durighel G, et al. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. Am J Clin Nutr 2014;99:1319-30.
18. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50:1714-9.
19. Gil-Campos M, Aguilera CM, Cañete R, Gil A. Ghrelin: a hormone regulating food intake and energy homeostasis. Br J Nutr 2006;96:201-26.
20. Egecioglu E, Jerlhag E, Salomé N, Skibicka KP, Haage D, Bohlooly YM, et al. Ghrelin increases intake of rewarding food in rodents. Addict Biol 2010;15:304-11.
21. Naleid AM, Grace MK, Cummings DE, Levine AS. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 2005;26:2274-9.
22. Jang JK, Kim WY, Cho BR, Lee JW, Kim JH. Microinjection of ghrelin in the nucleus accumbens core enhances locomotor activity induced by cocaine. Behav Brain Res 2013;248:7-11.
23. Crowley WR, Ramoz G, Keefe KA, Torto R, Kalra SP, Hanson GR. Differential effects of methamphetamine on expression of neuropeptide Y mRNA in hypothalamus and on serum leptin and ghrelin concentrations in ad libitum-fed and schedule-fed rats. Neuroscience 2005;132:167-73.
24. Kobeissy FH, Jeung JA, Warren MW, Geier JE, Gold MS. Changes in leptin, ghrelin, growth hormone and neuropeptide-Y after an acute model of MDMA and methamphetamine exposure in rats. Addict Biol 2008;13:15-25.
25. You ZB, Wang B, Gardner EL, Wise RA. Cocaine and cocaine expectancy increase growth

hormone, ghrelin, GLP-1, IGF-1, adiponectin, and corticosterone while decreasing leptin, insulin, GIP, and prolactin. Pharmacol Biochem Behav 2019;176:53-6.

26. Wellman PJ, Davis KW, Nation JR. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. Regul Pept 2005;125:151-4.
27. Wellman PJ, Hollas CN, Elliott AE. Systemic ghrelin sensitizes cocaine-induced hyperlocomotion in rats. Regul Pept 2008;146:33-7.
28. Davis KW, Wellman PJ, Clifford PS. Augmented cocaine conditioned place preference in rats pretreated with systemic ghrelin. Regul Pept 2007;140:148-52.
29. Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, Jerlhag E. The role of the central ghrelin system in reward from food and chemical drugs. Mol Cell Endocrinol 2011;340:80-7.
30. Panagopoulos VN, Ralevski E. The role of ghrelin in addiction: a review. Psychopharmacology (Berl) 2014;231:2725-40.
31. Ferrini F, Salio C, Lossi L, Merighi A. Ghrelin in central neurons. Curr Neuropharmacol 2009;7:37-49.
32. Skibicka KP, Shirazi RH, Rabasa-Papio C, Alvarez-Crespo M, Neuber C, Vogel H, et al. Divergent circuitry underlying food reward and intake effects of ghrelin: dopaminergic VTA-accumbens projection mediates ghrelin's effect on food reward but not food intake. Neuropharmacology 2013;73:274-83.
33. Jerlhag E. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict Biol 2008;13:358-63.
34. Olsen CM. Natural rewards, neuroplasticity, and non-drug addictions. Neuropharmacology 2011;61:1109-22.
35. Salamone JD, Pardo M, Yohn SE, López-Cruz L, SanMiguel N, Correa M. Mesolimbic Dopamine and the Regulation of Motivated Behavior. Curr Top Behav Neurosci 2016;27:231-57.
36. Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JA. Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict Biol 2007;12:6-16.
37. Jang JK, Kim WY, Cho BR, Lee JW, Kim JH. Locomotor sensitization is expressed by

ghrelin and D1 dopamine receptor agonist in the nucleus accumbens core in amphetamine pre-exposed rat. Addict Biol 2018;23:849-56.

38. Bell SM, Stewart RB, Thompson SC, Meisch RA. Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology (Berl) 1997;131:1-8.
39. Fulton S, Woodside B, Shizgal P. Modulation of brain reward circuitry by leptin. Science 2000;287:125-8.
40. Zheng D, Cabeza de Vaca S, Carr KD. Food restriction increases acquisition, persistence and drug prime-induced expression of a cocaine-conditioned place preference in rats. Pharmacol Biochem Behav 2012;100:538-44.
41. Cabeza De Vaca S, Holiman S, Carr KD. A search for the metabolic signal that sensitizes lateral hypothalamic self-stimulation in food-restricted rats. Physiol Behav 1998;64:251-60.
42. Carr KD, Tsimberg Y, Berman Y, Yamamoto N. Evidence of increased dopamine receptor signaling in food-restricted rats. Neuroscience 2003;119:1157-67.
43. Haberny SL, Berman Y, Meller E, Carr KD. Chronic food restriction increases D-1 dopamine receptor agonist-induced phosphorylation of extracellular signal-regulated kinase 1/2 and cyclic AMP response element-binding protein in caudate-putamen and nucleus accumbens. Neuroscience 2004;125:289-98.
44. Carr KD. Modulatory Effects of Food Restriction on Brain and Behavioral Effects of Abused Drugs. Curr Pharm Des 2020;26:2363-71.
45. Cone JJ, McCutcheon JE, Roitman MF. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. J Neurosci 2014;34:4905-13.
46. Pothos EN, Creese I, Hoebel BG. Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. J Neurosci 1995;15:6640-50.
47. Branch SY, Goertz RB, Sharpe AL, Pierce J, Roy S, Ko D, et al. Food restriction increases glutamate receptor-mediated burst firing of dopamine neurons. J Neurosci 2013;33:13861-72.
48. Zheng D, Liu S, Cabeza de Vaca S, Carr KD. Effects of time of feeding on psychostimulant reward, conditioned place preference, metabolic hormone levels, and nucleus accumbens

biochemical measures in food-restricted rats. Psychopharmacology (Berl) 2013;227:307-20.

49. Tezenas du Montcel C, Cao J, Mattioni J, Hamelin H, Lebrun N, Ramoz N, et al. Chronic food restriction in mice and increased systemic ghrelin induce preference for running wheel activity. Psychoneuroendocrinology 2023;155:106311.
50. Banks WA, Burney BO, Robinson SM. Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. Peptides 2008;29:2061-5.
51. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 1993;18:247-91.
52. Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci Biobehav Rev 2004;27:827-39.
53. Anagnostaras SG, Schallert T, Robinson TE. Memory processes governing amphetamine-induced psychomotor sensitization. Neuropsychopharmacology 2002;26:703-15.
54. Cador M, Bjijou Y, Stinus L. Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience 1995;65:385-95.
55. Zahm DS. An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neurosci Biobehav Rev 2000;24:85-105.
56. Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 2005;58:751-9.
57. Sellings LH, Clarke PB. Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. J Neurosci 2003;23:6295-303.
58. Li Y, Acerbo MJ, Robinson TE. The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci 2004;20:1647-54.
59. Landgren S, Engel JA, Hyytiä P, Zetterberg H, Blennow K, Jerlhag E. Expression of the gene encoding the ghrelin receptor in rats selected for differential alcohol preference. Behav Brain Res 2011;221:182-8.
60. Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL. Ghrelin directly

targets the ventral tegmental area to increase food motivation. Neuroscience 2011;180:129-37.

61. Lee S, Cai WT, Yoon HS, Kim JH. Visualization of the existence of growth hormone secretagogue receptor in the rat nucleus accumbens. Mol Brain 2024;17:37.
62. Carroll ME, Meisch RA. The effects of feeding conditions on drug-reinforced behavior: maintenance at reduced body weight versus availability of food. Psychopharmacology (Berl) 1980;68:121-4.
63. Carroll ME, Meisch RA. Determinants of increased drug self-administration due to food deprivation. Psychopharmacology (Berl) 1981;74:197-200.
64. Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., et al. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 1990;250:1429-32.
65. Edvardsson CE, Vestlund J, Jerlhag E. A ghrelin receptor antagonist reduces the ability of ghrelin, alcohol or amphetamine to induce a dopamine release in the ventral tegmental area and in nucleus accumbens shell in rats. Eur J Pharmacol 2021;899:174039.
66. Kotowski SJ, Hopf FW, Seif T, Bonci A, von Zastrow M. Endocytosis promotes rapid dopaminergic signaling. Neuron 2011;71:278-90.
67. Chausmer AL, Katz JL. Comparison of interactions of D1-like agonists, SKF 81297, SKF 82958 and A-77636, with cocaine: locomotor activity and drug discrimination studies in rodents. Psychopharmacology (Berl) 2002;159:145-53.
68. Soares-Cunha C, Coimbra B, Sousa N, Rodrigues AJ. Reappraising striatal D1- and D2-neurons in reward and aversion. Neurosci Biobehav Rev 2016;68:370-86.
69. Jiang H, Betancourt L, Smith RG. Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers. Mol Endocrinol 2006;20:1772-85.
70. Bodosi B, Gardi J, Hajdu I, Szentirmai E, Obal F, Jr., Krueger JM. Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. Am J Physiol Regul Integr Comp Physiol 2004;287:R1071-9.
71. Espelund U, Hansen TK, Højlund K, Beck-Nielsen H, Clausen JT, Hansen BS, et al. Fasting unmasks a strong inverse association between ghrelin and cortisol in serum: studies in obese and normal-weight subjects. J Clin Endocrinol Metab 2005;90:741-6.

72. Kulkarni SS, Singh O, Zigman JM. The intersection between ghrelin, metabolism and circadian rhythms. Nat Rev Endocrinol 2024;20:228-38.
73. Nørrelund H, Hansen TK, Ørskov H, Hosoda H, Kojima M, Kangawa K, et al. Ghrelin immunoreactivity in human plasma is suppressed by somatostatin. Clin Endocrinol (Oxf) 2002;57:539-46.
74. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908-13.
75. Cadoni C, Solinas M, Valentini V, Di Chiara G. Selective psychostimulant sensitization by food restriction: differential changes in accumbens shell and core dopamine. Eur J Neurosci 2003;18:2326-34.
76. Hansen HD, Schain M, Deng HP, Mandeville JB, Rosen BR, Sander CY. Differential D₁ and D₂ receptor internalization and recycling induced by amphetamine in vivo. bioRxiv 2022:2022.06.10.493955.
77. Kern A, Mavrikaki M, Ullrich C, Albarran-Zeckler R, Brantley AF, Smith RG. Hippocampal Dopamine/DRD1 Signaling Dependent on the Ghrelin Receptor. Cell 2015;163:1176-90.
78. Tessari M, Catalano A, Pellitteri M, Di Francesco C, Marini F, Gerrard PA, et al. Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. Addict Biol 2007;12:22-9.
79. You ZB, Galaj E, Alén F, Wang B, Bi GH, Moore AR, et al. Involvement of the ghrelin system in the maintenance and reinstatement of cocaine-motivated behaviors: a role of adrenergic action at peripheral β1 receptors. Neuropsychopharmacology 2022;47:1449-60.

Abstract in Korean

먹이제한과 암페타민 행동민감화의 상호작용에 미치는 측좌핵 내 그렐린과 도파민 시스템의 역할

음식과 같은 자연적 보상이나 중독성 약물에 의해 활성화되는 중변연계 도파민 시스템은 식욕과 중독성 행동을 조절하는 중격측좌핵(nucleus accumbens)의 공통 신경 경로를 공유한다. 이에 따라 최근 연구에서는 중독성 약물과 식욕을 조절하는 호르몬 간의 양방향 상호작용에 대한 연구가 주목받고 있다. 배고픔 신호 전달 기능으로 잘 알려진 그렐린(ghrelin)은 도파민과 함께 작용하여 이러한 과정을 조절함으로써 중독성 행동에도 영향을 미친다고 밝혀졌다. 먹이제한(food restriction)은 혈장 그렐린 수치를 상승시키며, 그렐린으로 유발된 약물 추구 행동과 정신 자극제에 대한 민감도가 증가와 유사한 효과를 보인다. 그러나 그렐린의 직접 주입이 아닌 먹이 제한에 의해 자연적으로 상승하는 내인성 그렐린이 중격측좌핵 내의 도파민 신호와 상호작용하여 암페타민(amphetamine)의 강화 효과를 유발하는지, 먹이 제한과 암페타민 노출 시기와 기간이 이러한 경로에 어떠한 영향을 미치는지는 아직 명확하게 알려진 바가 없다. 따라서 본 연구에서는 그렐린 신호와 중격측좌핵 중심층내의 도파민 시스템의 역할에 초점을 맞춰 암페타민 처리 전후의 먹이제한 기간과 시기가 암페타민에 의한 행동 반응에 어떤 영향을 미치는지에 대한 연구를 진행하였다. 첫 번째 실험에서는 사전 먹이제한이 암페타민에 의한 운동 반응에 영향을 미치는지에 초점을 맞추었다. 쥐를 정상 먹이(Normal feeding) 집단, 급성 먹이제한(acute food restriction; 1~2일) 집단, 만성 먹이제한(chronic food restriction; 2주) 집단의 세 집단으로 분류하였다. 조건별 먹이제한 이후에, 암페타민을 투여하고 보행성 활동량을 측정한 결과, 만성 먹이제한 집단에서 보행성 활동량이 크게 증가하였다. 이 집단에서는 혈장 그렐린이 유의미하게 증가하였고,

중격측좌핵 내 도파민 D1 수용체(dopamine D1 receptor) 발현 역시 다른 집단에 비해 높게 나타났다. D1 수용체 및 그렐린 수용체의 길항제를 전신 또는 중격측좌핵 중심층에 특이적으로 투여하여 만성 먹이 제한 집단에서 암페타민으로 인해 증가했던 보행성 활동량이 감소하였고, 이는 혈장 그렐린과 D1 수용체의 작용이 만성 먹이제한으로 인해 강화된 암페타민 반응을 조절하는 역할을 하는 것을 의미한다. 이러한 결과를 바탕으로 다음 실험에서는 암페타민 전처리 후의 먹이제한이 암페타민 처리 전에 먹이제한을 했을 때 관찰된 효과와 유사하게 중격측좌핵 도파민 D1 시스템을 통해 행동 민감화를 유도할 수 있는지 확인하고자 하였다. 식염수에 사전 노출된 쥐의 경우, 중격측좌핵 중심층에 D1 수용체의 작용제를 주입하면 만성 먹이제한 집단에서만 보행성 활동량이 증가하였다. 그러나 암페타민에 사전 노출된 쥐에서는 음식 제한 기간에 관계없이 D1 수용체 작용제에 대한 민감도가 향상되는 것이 관찰되었다. 급성 먹이제한 집단의 혈장 그렐린 수치는 식염수 사전 노출 쥐에 비해 암페타민 사전 노출 쥐에서 유의미하게 높았으며, 이는 암페타민 사전 노출 조건이 단기 먹이제한 집단에서도 D1 수용체 활성화에 대한 그렐린 시스템의 반응을 상승시켰을 가능성을 시사한다. 본 연구는 약물로 인한 행동을 조절하는 데 있어 식이 상태의 중요한 역할을 강조하며, 먹이 제한 시기나 기간이 중격측좌핵에서 그렐린과 도파민 시스템간의 상호작용을 조절할 수 있음을 밝혀 중독 치료를 위한 잠재적 전략에 새로운 통찰력을 제공한다.

핵심되는 말: 먹이제한, 그렐린, 도파민 D1 수용체, 중격측좌핵, 암페타민