Conditional deletion of melanin-concentrating hormone receptor 1 from GABAergic neurons increases locomotor activity

Melissa J. Chee1,2*, Alex J. Hebert1,2, Nadege Briançon1, Stephen E. Flaherty III1, Pavlos Pissios2, Eleftheria Maratos-Flier2

ABSTRACT

Objective: Melanin-concentrating hormone (MCH) plays a key role in regulating energy balance. MCH acts via its receptor MCHR1, and MCHR1 deletion increases energy expenditure and locomotor activity, which is associated with a hyperdopaminergic state. Since MCHR1 expression is widespread, the neurons supporting the effects of MCH on energy expenditure are not clearly defined. There is a high density of MCHR1 neurons in the striatum, and these neurons are known to be GABAergic. We thus determined if MCH acts via this GABAergic neurocircuit to mediate energy balance.

Methods: We generated a Mchr1-fox mouse and crossed it with the Vgat-cre mouse to assess if MCHR1 deletion from GABAergic neurons expressing the vesicular GABA transporter (vGAT) in female Vgat-Mchr1-KO mice resulted in lower body weights or increased energy expenditure. Additionally, we determined if MCHR1-expressing neurons within the accumbens form part of the neural circuit underlying MCH-mediated energy balance by delivering an adeno-associated virus expressing Cre recombinase to the accumbens nucleus of Mch-KO mice. To evaluate if a dysregulated dopaminergic tone leads to their hyperactivity, we determined if the dopamine reuptake blocker GBR12909 prolonged the drug-induced locomotor activity in Vgat-Mchr1-KO mice. Furthermore, we also performed amperometry recordings to test whether MCHR1 deletion increases dopamine output within the accumbens and whether MCH can suppress dopamine release.

Results: Vgat-Mchr1-KO mice have lower body weight, increased energy expenditure, and increased locomotor activity. Similarly, restricting MCHR1 deletion to the accumbens nucleus also increased locomotor activity. Vgat-Mchr1-KO mice show increased and prolonged sensitivity to GBR12909-induced locomotor activity, and amperometry recordings revealed that GBR12909 elevated accumbens dopamine levels to twice that of controls, thus MCHR1 deletion may lead to a hyperdopaminergic state that mediates their observed hyperactivity. Consistent with the inhibitory effect of MCH, we found that MCH acutely suppresses dopamine release within the accumbens.

Conclusions: As with established models of systemic MCH or MCHR1 deletion, we found that MCHR1 deletion from GABAergic neurons, specifically those within the accumbens nucleus, also led to increased locomotor activity. A hyperdopaminergic state underlies this increased locomotor activity, and is consistent with our finding that MCH signaling within the accumbens nucleus suppresses dopamine release. In effect, MCHR1 deletion may disinhibit dopamine release leading to the observed hyperactivity.

Keywords MCHR1; Accumbens nucleus; Locomotor activity; GABA; Neurocircuit; Dopamine

1. INTRODUCTION

Melanin-concentrating hormone (MCH) is produced in the lateral hypothalamus [1] and has emerged as a critical player in the regulation of central energy balance [2,3]. Acute MCH administration stimulates food intake [4], and chronic MCH infusion increases body weight gain [5]; thus MCH increases orexigenic drive and promotes positive energy balance. Furthermore, MCH gene expression is upregulated in mouse models of obesity like the leptin-deficient ob/ob mouse [4], and MCH-overexpressing mice are more prone to diet-induced obesity [6]. By contrast, the absence of MCH increases energy expenditure, thus promoting weight loss and negative energy balance [7]. MCH knockout (Mch-KO) mice are lean and have increased energy expenditure and locomotor activity [8,9]. Consistent with this, deletion of MCH in ob/ob mice attenuates their obesity [10]. Mch-KO mice also display prolonged hyperactivity when treated with a dopamine reuptake blocker and are more susceptible to amphetamine sensitization [11]. Moreover, amperometry recordings from the striatum show higher dopamine output from fresh Mch-KO brain slices [11]. These findings indicate that a hyperdopaminergic state in the striatum mediates the hyperactivity of Mch-KO mice.
In rodents, MCH acts exclusively via the MCH receptor MCHR1, which is a G protein coupled receptor [12–14]. Consistent with the effects of MCH deletion, MCHR1 knockout (Mchr1-KO) mice are also lean, resistant to diet-induced obesity, have increased energy expenditure, and exhibit pronounced hyperactivity [15,16]. As such, the leanness observed in Mch-KO and Mchr1-KO mice is largely attributed to their increased energy expenditure and/or hyperactivity. Furthermore, both MCH and MCHR1 deletion increase amphetamine-mediated hyperactivity [17–19], which is also recapitulated following the ablation of MCH neurons in adult mice [20], thereby implicating MCH as a regulator of the dopaminergic system.

MCH nerve terminals may regulate dopamine release in the striatum [21], a key integrative region for motor control. It has been shown that MCH infusion into the ventral striatum may also stimulate food intake [22,23]. Indeed, the most prominent expression of Mchr1 mRNA is in the striatum, which includes the caudate putamen and accumbens nucleus in the dorsal and ventral striatum, respectively [24]. These regions are almost entirely comprised of GABAergic medium spiny neurons or interneurons [25]. In aggregate, these data suggest that GABAergic neurons may mediate some actions of MCH, including dopamine-mediated locomotor activity.

To assess the actions of MCH on GABAergic neurons that effect energy expenditure and locomotor activity, we deleted MCHR1 from neurons that express the vesicular GABA transporter, vGAT, by generating the Mchr1-flox mouse and crossing it to the Vgat-cre mouse to enable cre-mediated deletion of MCHR1 from GABAergic neurons. Mice with the loss of MCHR1 in vGAT neurons were lean and display robust baseline locomotor activity and heightened sensitivity to dopamine-mediated hyperactivity. Cre-mediated deletion of MCHR1 from the accumbens nucleus using an adeno-associated virus to express Cre recombinase in Mchr1-flox mice also increased locomotor activity. Striatal brain slices following MCHR1 deletion have elevated dopaminergic levels when treated with a dopamine reuptake blocker during amperometry recordings. Moreover, MCH directly inhibits dopamine release to the accumbens nucleus in the dorsal and ventral striatum, respectively [24]. These data suggest that MCH regulates locomotor activity via GABAergic neurons in the accumbens nucleus, and that it does so in part by inhibiting dopamine release.

2. MATERIALS AND METHODS

2.1. Animals

Experiments were performed in female mice housed under a 12 h light, 12 h dark cycle, at 22 ± 2 °C, and provided with ad libitum access to water and standard lab chow (3.23 kcal/g, Formulab Diet 5008, LabDiet, St. Louis, MO). All procedures adhere to the National Institute of Health Guidelines for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee at the Beth Israel Deaconess Medical Center (Boston, MA).

2.1.1. Generation of Mchr1-flox mouse

We generated the Mchr1-flox mouse by designing a targeting vector that inserted loxP sites flanking exon2 of the Mchr1 gene. A 2.7 kb genomic fragment containing exon2 was PCR-amplified from murine genomic DNA and cloned at a SmaI site 5' of a neomycin resistance cassette flanked by Frt sites, and 3' of a loxP site previously inserted in the pBACe3.6 cloning vector (Sanger Institute, Hinxton, United Kingdom). A 4.95 kb genomic fragment containing exon1 of the Mchr1 gene was inserted at a NotI site immediately 5' of the above mentioned loxP site. Similarly, a 2.9 kb genomic fragment containing downstream intron2 sequences was introduced 3' of a second loxP site cloned immediately 3' of the Frt-flanked neomycin cassette. The cloned intronic sequence resided upstream of a diphtheria toxin A cassette used for selection of the ES cell clones that successfully underwent 3' homologous recombination. All genomic fragments were fully Sanger-sequenced after completion of the transgenic construct. Mice of the F1 generation were bred with Fip-expressing mice (#003946, Jackson Laboratory, Bar Harbor, ME) in order to eliminate the neomycin cassette, and one Frt site remained 5' of the 3'-most loxP site in intron2.

2.1.2. Generation of Vgat-Mchr1-KO mouse

We crossed our Mchr1-flox mouse with the Vgat-cre mouse (#016962, Jackson Laboratory) to produce the Vgat-Mchr1-KO mouse, in which the MCHR1 was deleted from GABAergic neurons expressing vGAT (Figure 1A). Both Mchr1-flox and Vgat-cre mice were bred onto the C57/BL6 background (#000664, Jackson Laboratory) for at least eight generations. The Mchr1-flox and Vgat-cre mouse lines were indistinguishable from their respective wild-type littermates by body weight, food intake, or locomotor activity (Supplemental Tables 1 and 2). We chose Vgat-cre mice as the control group for our experiments due to potential metabolic differences caused by the expression of Cre recombinase [26].

2.2. Gene expression

2.2.1. In situ hybridization

The Vgat-cre and Vgat-Mchr1-KO mouse brains were perfused and sliced into five series of 30 μm coronal sections as previously described [24]. A [35S]-labeled antisense Mchr1 riboprobe comprising nucleotide 30–1061 of the rat Mchr1 mRNA was used to detect MCHR1 mRNA as previously described [24]. In brief, free-floating brain sections were pretreated with sodium citrate buffer (pH 6.0, 5 min, 90 °C), incubated with the Mchr1 riboprobe for 18 h at 60 °C, and then washed by sequentially increasing the stringency of sodium citrate buffer exchanges. The brain slices were mounted, dehydrated, and delipidated before placing in X-ray film cassettes with BioMax MR film (Kodak, Rochester, NY) for five days.

2.2.2. Quantitative RT-PCR analysis

Microdissected brain tissue samples from the caudate putamen, accumbens nucleus, hippocampus, and cerebellum were flash-frozen in liquid nitrogen. We first isolated total RNA from these tissue samples using the Direct-zol RNA MiniPrep Kit (Zymo Research, Irvine, CA) and concentrated the resulting RNA solutions via a glycogen-ethanol precipitation using 20 μg/μl UltraPure Glycogen (Invitrogen, Carlsbad, CA), as necessary. Total RNA (0.5 μg) was reverse transcribed to synthesize cDNA using the QuantTect Reverse Transcription Kit (Qiagen, Germantown, MD). We performed quantitative RT-PCR via SYBR Green master mix (Applied Biosystems, Foster City, CA) using the 7900HT thermal cycler (Applied Biosystems). Relative mRNA expression was calculated via delta delta Ct analysis and normalized to expression levels of the housekeeping gene cyclophilin. Custom primers for cyclophilin (CypB) and Mchr1 were obtained from Invitrogen (Carlsbad, CA) using the following sequences: CypB forward, 5'–GGTGGAGGACGACCAAGACAGA-3'; CypB reverse, 5'–GCCGGAGTCGACAATGATG-3'; Mchr1 forward, 5'–CACTGCGAACAACTCTCC-3'; Mchr1 reverse, 5'–ACCAAAAACACGAAAGGCATGA-3'.
2.3. Metabolic and behavioral analysis

2.3.1. Food intake
Food intake was measured by weighing food in the hoppers of individually-housed mice for either one day or over several days as indicated.

2.3.2. Body composition
Body composition was determined using EchoMRI (Echo Medical Systems, Houston, TX) at 12 weeks of age.

2.3.3. Energy expenditure
Energy expenditure was assessed in 12 week old mice by measuring oxygen consumption (VO₂) using the Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH). Mice were acclimated for two days before two days of active data collection.

2.3.4. Locomotor activity
All mice were habituated to single housing for at least 24 h before initiating locomotor recordings. We calculated locomotor activity using the Opto-M3 infrared beam break monitoring system (Columbus...

Figure 1: Cre-mediated deletion of Mchr1 from GABAergic neurons expressing the vesicular GABA transporter. Vgat-Mchr1-KO mice were generated by crossing the Vgat-cre mouse to the Mchr1-flox mouse to delete exons2, flanked by loxP sites, of the Mchr1 gene (A). Autoradiographs of 35S-labeled hybridization signal for Mchr1 mRNA in the Vgat-cre (Bi) and Vgat-Mchr1-KO (Bii) mouse brain tissue demonstrated the absence of 35S-Mchr1 hybridization in GABAergic striatal regions (black solid line), but not glutamatergic pyramidal layer of the cortex or hippocampus (gray dashed line) (B). qPCR analysis from the striatum, hippocampus, and cerebellum collected from the same mice shows the loss of Mchr1 mRNA in the striatum of Vgat-Mchr1-KO mice only (C). Ordinary one-way ANOVA: **, p < 0.01; ***, p < 0.001.
2.5. Amperometry as described in section 2.2.2.

were rapidly removed from the skull for quantitative RT-PCR analysis software (Olympus), and we viewed and exported the images off-BX61VS microscope (Olympus, Tokyo, Japan) running VS-ASW-FL acquired stitched epi-fluorescence images with a fully motorized B6X1VS microscope (Olympus, Tokyo, Japan) running VS-ASW-FL software (Olympus), and we viewed and exported the images offline using OlyVIA software (Olympus). Brains from remaining mice were rapidly removed from the skull for quantitative RT-PCR analysis of Mchr1 mRNA from the caudate putamen and accumbens nucleus, as described in section 2.2.2.

2.4. Stereotaxic injections

Mchr1-flox mice (4—6 week old) were anesthetized with a ketamine (100 µg/kg)-xylazine (10 µg/kg) mixture and placed in a mouse stereotaxic frame. We bilaterally delivered a total of 800 nl (400 nl per hemisphere) of an adeno-associated viral vector (University of North Carolina Gene Therapy Center, Chapel Hill, NC) encoding either Cre recombinase-mCherry (AAV8-hSyn-CRE-mCherry, 2.8 × 10^{12} genomic copies/ml) or mCherry only (AAV8-hSyn-mCherry, 8.0 × 10^{12} genomic copies/ml) to the rostral (anteroposterior (AP) +1.60, mediolateral (ML) ±0.60 and ± 1.40, dorsoventral (DV) −4.25 and −4.75) and caudal accumbens (AP +1.10, ML ±0.60 and ± 1.50, DV −4.30 and −4.80) using mouse atlas coordinates [27]. All mice were returned to their home cage and received buprenorphine (0.1 mg/kg, i.p.) analgesia for 24 h post-surgery. We determined their home cage locomotor activity after viral transfection and surgery recovery for seven weeks.

After the completion of the experiments, one mouse each from the control and experimental groups was deeply anesthetized and sacrificed by transcardiac perfusion with saline and 10% formalin for histological analysis to determine the spread of the virus throughout the striatum. Brains were sliced into 30 µm coronal sections to determine native mCherry-fluorescence at the injection site. We acquired stitched epifluorescence images with a fully motorized B6X1VS microscope (Olympus, Tokyo, Japan) running VS-ASW-FL software (Olympus), and we viewed and exported the images offline using OlyVIA software (Olympus). Brains from remaining mice were rapidly removed from the skull for quantitative RT-PCR analysis of Mchr1 mRNA from the caudate putamen and accumbens nucleus, as described in section 2.2.2.

2.5. Amperometry

Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and transcardially perfused with ice-cold, carbogenated (95% O₂, 5% CO₂)-ACSF containing (in mM): 110 choline chloride, 25 NaHCO₃, 2.5 KCl, 7 MgCl₂, 0.5 CaCl₂, 1.25 NaH₂PO₄, 25 glucose, 11.6 ascorbic acid, 3.1 pyruvic acid (pH 7.35, 305 mOsmlL). The brain was rapidly removed from the skull and coronal brain slices (350 µm) were sliced (4°C), then incubated in the choline-based ACSF for an absolute 5 min (37°C). These acute brain slices were recovered in a carbogenated bath ACSF containing (in mM): 124 NaCl, 2.5 KCl, 1.24 NaH₂PO₄, 1.3 MgCl₂, 2.0 glucose, 26 NaHCO₃, 2.5 CaCl₂ (300 mOsml) for 10 min (37°C) before resting at room temperature (22°C, >1 h) until used for recordings.

Each slice (between Bregma +1.35 mm to +0.60 mm) was transferred to the recording chamber and maintained by constant perfusion of carbogenated bath ACSF at 32°C. A concentric bipolar stimulating microelectrode (CBBP100, FHC, Bowdoin, ME) was placed at the brain tissue surface in the medial accumbens nucleus and a carbon fiber electrode (Carbostar-1 E1011-7, 100 µm tip length, Kation Scientific, Minneapolis, MN) was positioned within 100 µm of the stimulating electrode at approximately 50 µm beneath the tissue surface. The electrode used in each recording was calibrated with fresh 5 µM dopamine standards by sampling the current amplitude detected at one-minute intervals while holding at +600 mV in order to calculate the conversion of current amplitude to extracellular dopamine concentration. Dopamine (Sigma—Aldrich, St. Louis, MO) standard stock solutions (5 mM) were prepared in 500 mM sodium metabisulfite and stored as frozen aliquots at −20°C.

To evoke dopamine release, we delivered a single 1 ms rectangular pulse (80 µA) to the stimulating electrode at 5-minute intervals with a constant current stimulus isolator (World Precision Instruments, Sarasota, FL) while applying a constant voltage of +600 mV to the carbon fiber tip with a Multiclamp 700B amplifier (Molecular Devices, San Jose, CA). We sampled the amperometric readings using Clampfit 10 software (Molecular Devices) and maintained a stable dopamine output, where peak amplitudes were within 10% of each other, for at least 25 min before drug application. Each pharmacological compound was prepared immediately before use by dilution in carbogenated bath ACSF, then bath applied to the recording chamber for 15—20 min.

2.6. Statistical analysis

We analyzed amperometry data using Clampfit 10.7 software (Molecular Devices) and all other data sets using Excel (Microsoft Corporation, Redmond, WA). All data are reported as group mean ± SEM, with the number of mice or slices per group included in parentheses within each figure. We used Prism 6 (GraphPad, La Jolla, CA) to determine statistical significance for group means using either an ordinary one-way ANOVA with post-hoc Bonferroni test or an unpaired Student’s t-test, where appropriate, with *, p < 0.05; **, p < 0.01; and ***, p < 0.001. We also used a repeated measures two-way ANOVA with post-hoc Bonferroni test to compare entire data sets over time, with |, p < 0.05 and ||, p < 0.01.

3. RESULTS

3.1. Conditional MCHR1 deletion from GABAergic neurons produced lean, hyperactive mice

We generated Mchr1-flox mice and mated them to Vgat-cre mice to delete MCHR1 from GABAergic neurons in Vgat-Mchr1-KO mice (Figure 1A). Following in situ hybridization, a comparison of autoradiographic images from Vgat-cre control and Vgat-Mchr1-KO brain tissue labeled with an isotopic [35S]Mchr1 riboprobe show a distinct absence of Mchr1 hybridization in GABAergic regions like the striatum but not glutamatergic pyramidal neurons throughout the hippocampus or cortex (Figure 1B). We quantified the extent of MCHR1 deletion by RT-qPCR analysis and show a complete loss of Mchr1 mRNA from Vgat-Mchr1-KO striatal tissue relative to Vgat-cre and Mchr1-flox controls (Figure 1C). Meanwhile, Mchr1 mRNA expression levels remain unchanged in the hippocampus, where MCHR1-expressing neurons are on glutamatergic pyramidal neurons [24,28], or in the cerebellum, where MCHR1 expression is minimal.

Compared to Vgat-cre controls, adult Vgat-Mchr1-KO mice weighed 10% less (Figure 2A) and had a lower body fat percentage (Figure 2B) while consuming the same number of calories (12.6 ± 7 kcal, n = 8; Vgat-cre: 14.2 ± 7.0 kcal, n = 8; p = 0.133). MCHR1 deletion in vGAT neurons increased VO₂ consumption (Figure 2C) and Vgat-Mchr1-KO mice showed a 93% increase in home cage locomotor activity (Figure 2D). These findings indicate that cre-mediated deletion of

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Figure 2: Mchr1 deletion from GABAergic neurons resulted in lean and hyperactive mice with increased dopamine-mediated locomotor activity. At 12 weeks of age, Vgat-Mchr1-KO (cKO) mice weighed 10% less (Ai), consistent with lower body fat (B). They also have increased oxygen consumption (Ci) and home cage locomotor activity (Di). Systemic administration of GBR12909 (GBR, 20 mg/kg, i.p.) in cKO mice produced a longer-lasting increase in home cage locomotor activity (Ei). Repeated measures two-way ANOVA: ¶, p < 0.05 vs. Vgat-cre; Ci, Di; †, p < 0.05 vs. Vgat-cre GBR; El. Bonferroni multiple comparisons post-test: *, p < 0.05; **, p < 0.01; ***; p < 0.001 (Di). Ordinary two-way ANOVA (Eii). Student’s t test: *, p < 0.05; **, p < 0.01 (Ai, B, Ci, Di).
Mchr1 from GABAergic neurons produces lean mice that have increased energy expenditure.

Considering the baseline hyperactivity of Vgat-Mchr1-KO mice, we next examined how these mice would respond to a paradigm of hyperactivity induced by the blockade of dopamine reuptake [11]. Systemic treatment of Vgat-cre and Vgat-Mchr1-KO mice with the dopamine reuptake inhibitor GBR12909 (20 mg/kg) produced a pronounced and sustained increase in the locomotor activity of all mice relative to vehicle treatment. Interestingly, conditional MCHR1 deletion heightened the sensitivity of Vgat-Mchr1-KO mice to GBR12909 and increased their induced hyperactivity compared to Vgat-cre controls. GBR12909-mediated ambulation peaked 90 minutes post-injection for all mice (Figure 2Ei), but this increase was greater in Vgat-Mchr1-KO mice. Vgat-cre mice returned to baseline ambulation levels within four hours, while the locomotor activity of Vgat-Mchr1-KO mice remained sustained and elevated for at least five hours (Figure 2Ei). In effect, the cumulative ambulatory count of Vgat-Mchr1-KO mice treated with GBR12909 was two-fold higher than the treated Vgat-cre mice (Figure 2Eii). These findings suggest that a dysregulation of the dopaminergic system at least in part contributes to the hyperactivity displayed by Vgat-Mchr1-KO mice.

3.2. Deletion of MCHR1 from the accumbens nucleus increased locomotor activity

In order to identify the candidate GABAergic MCHR1 neurons that contribute to the hyperactivity of Vgat-Mchr1-KO mice, we bilaterally deleted Mchr1 mRNA from the accumbens nucleus of Mchr1-flox mice by stereotaxic delivery of an AAV encoding Cre-mCherry. Our viral delivery confined mCherry expression to the accumbens nucleus (Figure 3A), and we evaluated Mchr1 expression in the accumbens nucleus compared to the dorsally-located caudate putamen to verify the efficacy and specificity of this cre-mediated deletion. Additionally, we also injected Mchr1-flox mice with an AAV encoding mCherry only to control for decreases in Mchr1 levels following AAV delivery. Mchr1-flox mice transfected with Cre-mCherry showed a 50% reduction of Mchr1 mRNA in the accumbens nucleus, while Mchr1-flox mice transfected with mCherry expressed similar Mchr1 mRNA levels between the accumbens and caudate putamen (Figure 3B). Although our AAV cre-mediated strategy only produced a partial deletion of

![Figure 3: Deletion of Mchr1 in the accumbens nucleus increased locomotor activity.](image-url)
Since both transmission in the accumbens nucleus 3.3. Conditional MCHR1 deletion dysregulates dopamine Mch-KO [11] and Vgat-Mchr1-KO (Figure 2E) mice show an enhanced sensitivity to dopamine-mediated hyperactivity, we sought to determine if MCH signaling directly interacts with the dopamine system of the accumbens nucleus. In order to measure dopamine release, we performed in vitro amperometry recordings in fresh brain slices containing the striatum and determined the peak current amplitude evoked by a stimulating electrode every five minutes. There were no differences in the kinetics of the evoked dopamine current from Vgat-cre and Vgat-Mchr1-KO mice, which had similar rise times (Vgat-cre: 64.9 ± 11.7 ms, n = 5; Vgat-Mchr1-KO: 105.4 ± 19.1 ms, n = 6; p = 0.119) and decay times (Vgat-cre: 273.5 ± 42.3 ms; Vgat-Mchr1-KO: 360.2 ± 46.0 ms; p = 0.206). Bath application of GBR12909 (3 μM) increased dopamine release within acute brain slices from both Vgat-Mchr1-KO and Vgat-cre mice. Notably, GBR12909 elicited a two-fold greater increase in dopamine release from Vgat-Mchr1-KO striatal slices (Figure 4A), thus confirming that the loss of MCHR1 signaling increased the sensitivity of Vgat-Mchr1-KO mice to dopaminergic dysregulation. In order to determine if MCH acutely inhibits dopamine release, we performed in vivo amperometry recordings in fresh brain slices from both Vgat-Mchr1-KO and Vgat-cre mice. Representative traces from an amperometry recording of mCherry (Figure 3C). There was no differences in their body weight (mCherry: 25.4 ± 1.3 g, n = 3; Cre-mCherry: 22.6 ± 0.9 g, n = 5; p = 0.116) or daily food intake (mCherry: 11.1 ± 2.0 kcal, n = 3; Cre-mCherry: 10.7 ± 2.2 kcal, n = 5; p = 0.903). This suggested that MCHR1 neurons within the accumbens nucleus mediate the effects of MCH on locomotor activity.

4. DISCUSSION

Multiple studies have implicated the MCH system in the regulation of energy homeostasis. Systemic deletion of MCH [8,9] or MCHR1 [15,16,29] in rodents results in increased energy expenditure and locomotor activity, in part by regulating dopaminergic tone [11]. This hyperactivity phenotype is also seen following the ablation of MCH neurons both in early life [20] or adult animals [30]. Consistent with the role of MCH in suppressing energy expenditure and locomotor activity, direct chemogenetic activation of these neurons also leads to decrease spontaneous locomotor activity [31]. The accumbens is a potential target for MCH action, which additionally includes its effects on food intake [22,32] and mood [33–35]. However, the identities of downstream neurons that mediate MCH actions on energy expenditure were not well described. In order to identify these neurons, we used a transgenic model to delete MCHR1 specifically from GABAergic neurons marked by the expression of vGAT, which includes the accumbens. This recapitulated the phenotype following systemic MCH or MCHR1 deletion, as we observed an increase in energy expenditure and a two-fold increase in spontaneous locomotor activity. An elevated dopaminergic tone contributes to this hyperactivity, and we localized this effect to the accumbens by site-specific MCHR1 deletion.

Interestingly, although MCH has been reported to stimulate food intake [4,22,23], MCHR1 deletion from vGAT neurons in our studies did not alter food intake. However, it should be noted that the effects of MCH on food intake in mice are potentially problematic. While intra-cerebroventricular (ICV) administration of MCH to rats leads to rapid

Figure 4: Reversible MCH-mediated inhibition of dopamine release in the accumbens nucleus. Representative traces from an amperometry recording of dopamine current before (black) and after a 20-minute bath application of 3 μM GBR12909 (GBR, red) to freshly prepared striatal brain slices from Vgat-cre (Aii) or Vgat-Mchr1-KO mice (Aiii). GBR produced a greater increase in dopamine current amplitude from the accumbens of Vgat-Mchr1-KO mice (Aiii). Representative amperometric traces of dopamine current before (Bii), immediately following a 15-minute bath application of 3 μM MCH (Bii), and after its washout (Biii), MCH elicited a reversible inhibition of dopamine current in striatal slices from control but not Vgat-Mchr1-KO mice (Biii). Repeated measures two-way ANOVA: *, p < 0.05; **, p < 0.01. Bonferroni multiple comparisons post-test: *, p < 0.05; **, p < 0.01.
and substantive increases in food intake [4,23,36], the effects of ICV administration in mice is minimal, thus either repetitive or chronic MCH administration is required to increase food intake [5]. In transgenic models, global MCh deletion initially decreased food consumption [8,37], but this effect did not persist over time [9]. Furthermore, in contrast to MCH deletion, global MCHR1 deletion produces hyperphagia [15,16], which was significantly more robust in male than female mice [15]. The absence of a food intake effect in our model could relate to our use of females [36,38] or to a less robust effect of MCH on food intake in mice.

Increased locomotor activity is associated with increased dopaminergic tone. For example, deletion of the dopamine transporter that produces a hyperdopaminergic state is associated with spontaneous locomotor activity [39,40]. Previous reports suggest that MCH is a regulator of dopaminergic tone. A comparison of accumbens dopamine release in mice that lack MCH [11] revealed elevated dopamine release compared to wildtype mice. Similar results were also noted in rats that from mice that lack MCH [11,20]. We found that in addition to recapitulating the hyperactivity in global MCH- or MCHR1-deficient mice, MCHR1 deletion from vGAT neurons in our mice also increased dopaminergic tone, and they were more sensitive to the hyperlocomotor effects of GBR12909.

Since the absence of MCH signaling results in enhanced dopaminergic tone, MCH itself would have an inhibitory effect on the dopamine system. Consistent with this, we found that MCH acutely suppressed dopamine release in accumbens brain slices, an effect that was abolished following MCHR1 deletion in vGAT neurons. The pathways downstream of the incumbent GABAergic MCHR1 neurons in the accumbens that mediate this response have not yet been defined. In the intact animal, MCH may directly inhibit striatal inputs from dopaminergic efferents that originate in the ventral tegmental area or downstream of the incumbent GABAergic MCHR1 neurons in the brain, thus site-specific MCHR1 deletion at other discrete neuroanatomical sites would provide additional insights to elucidate the full range of MCH effects.

5. CONCLUSION

Transgenic models of MCH or MCHR1 deletion produce robust and consistent effects of increase energy expenditure and locomotor activity. While the role of MCH in the regulation of energy homeostasis is well known, the neuronal population supporting the actions of MCH are not well defined. We found that MCH acts via a GABAergic neurocircuit to regulate energy homeostasis. Furthermore, we also show that MCH regulates dopamine release, and the interaction between dopaminergic and GABAergic systems in the accumbens is a critical pathway contributing to the effects of MCH on energy expenditure.

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CONFLICTS OF INTEREST

None.

APPENDIX

### Supplemental Table 1 – Baseline characterization of Mchr1-flx mice

|                | Wildtype (5) | Mchr1-flx (5) | p value<sup>4</sup> |
|----------------|-------------|--------------|--------------------|
| Body weight (g)<sup>1</sup> | 19.0 ± 1.0 | 18.4 ± 1.4 | 0.749              |
| Food intake (kcal)<sup>2</sup> | 64.0 ± 3.5 | 66.0 ± 5.7 | 0.770              |
| Ambulation (counts)<sup>3</sup> | 97,027 ± 9,740 | 83,083 ± 9,616 | 0.338          |

### Supplemental Table 2 – Baseline characterization of Vgat-cre mice

|                | Wildtype (6) | Vgat-cre (5) | p value<sup>4</sup> |
|----------------|-------------|--------------|--------------------|
| Body weight (g)<sup>1</sup> | 19.7 ± 0.6 | 20.0 ± 0.7 | 0.799              |
| Food intake (kcal)<sup>2</sup> | 62.5 ± 2.8 | 63.2 ± 3.2 | 0.855              |
| Ambulation (counts)<sup>3</sup> | 79,915 ± 18,344 | 80,934 ± 18,585 | 0.970          |

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