

# Chronic food restriction enhances dopamine-mediated intracranial self-stimulation

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Dopamine-mediated reinforcement and behavioral adaptation is essential to survival. Here, we test the effects of food restriction on dopamine-mediated learning and reinforcement using optical intracranial self-stimulation (oICSS), an optogenetic version of conventional electrical ICSS (also known as brain stimulation reward, BSR). Using mouse genetic lines to express channelrhodopsin selectively in midbrain dopamine neurons, we demonstrate that genetically expressed channelrhodopsin can mediate optically evoked dopamine release and support self-stimulation in a lever-pressing paradigm. Using this midbrain dopamine oICSS preparation, we compare acquisition and rate of pressing in ad libitum versus food restricted mice. Food restriction facilitated both more rapid acquisition of self-stimulation behavior and higher rates of responding; reversing food status after acquisition modulated response vigor in already established behavior. These data suggest that food restriction enhances both the acquisition and expression of dopamine-reinforced

self-stimulation responding. These data demonstrate the utility of oICSS for examining changes in reinforcement learning concomitant to neuroadaptations induced in dopamine signaling by experimental manipulations such as food restriction. *NeuroReport* 32: 1128–1133 Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.

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## Introduction

The ability to adapt to different environmental conditions determines survival. Among evolutionary challenges, adapting to food scarcity is fundamental. In many organisms, dopamine plays a central role in mediating reinforcement learning and modulating motivated behaviors that facilitate adaptive behavior. Consistent with a role for dopamine in adapting to environmental conditions, such as food scarcity, the midbrain dopamine system changes under chronic food restriction [1–4]. Under food restriction, animals exhibit lower tonic dopamine transmission [5–7], increased dopamine D1-expressing medium spiny neuron excitability [7,8], and increased levels of dopamine transporter (DAT) [5,9], yielding a sensitized dopamine system. These food restriction induced alterations sensitize the brain to drugs of abuse [2,10,11]. For example, food restriction increases the rate of drug self-administration in rodents [10,12,13].

Investigating how food restriction alters dopamine and dopamine-mediated behavior can be challenging because animal behavioral tasks typically use food restriction to motivate the animal to participate in the task. As a consequence, there is a confound between the increased value of the reinforcer (i.e. the food) to a hungry animal and changes to the dopamine system itself. One approach

to circumvent this challenge is to test dopamine and reinforcement learning in the absence of food reward through intracranial self-stimulation (ICSS) paradigms, also known as brain stimulation reward (BSR) [14,15]. Traditional ICSS studies using electrical stimulation have demonstrated that food restriction lowers the stimulation threshold required for reward [15–17] and increases animals' willingness to work harder for self-stimulation [18,19]. However, the electrical stimulation used in ICSS excites all neurons in its vicinity indiscriminately, making it difficult to specifically assess dopamine reinforcement. Here, we selectively stimulated dopamine neurons in the ventral tegmental area using optical intracranial self-stimulation (oICSS) by selectively expressing channelrhodopsin-2, a light-activated sodium channel, in dopamine cells [20,21].

## Methods

### Animals

Thirteen mice of 270–300 days old were kept on a 12-h light/dark cycle. Crossing mice homozygote for floxed channelrhodopsin (ChR2<sup>+/+</sup>; Jackson 012569) with mice expressing cre-recombinase (Cre) under the control of the DAT promoter (DAT<sup>cre/wt</sup>; JAX 020080) produced mice heterozygous for both ChR2 and DAT-Cre

(ChR2<sup>+/-</sup>;DAT<sup>cre/wt</sup>), selectively expressing ChR2 in dopamine cells. Animal protocols were approved by the Institutional Animal Care and Use Committee of Queens College, City University of New York.

### Diet

Mice (seven males and six females) were assigned to either *ad libitum* or food restricted groups counterbalanced by initial weight and sex. Food restricted mice were maintained at 85% of their baseline weight by providing 4 h of free access to chow starting 2 h after each daily session during the light cycle. Food restriction began 4 days prior to the start of behavioral testing. When the animals' feeding conditions were reversed, they were maintained under the new conditions for 4 days prior to resuming behavioral testing to allow stabilization in consumption and body weight. Sample size did not have sufficient power to detect sex differences and therefore sexes were collapsed in the analysis.

### Fiber optic surgery

Mice were given an intraperitoneal injection of ketamine/xylazine (50 mg/kg and 5 mg/kg, respectively) for placement into stereotaxis and then maintained under isoflurane anesthesia (4% induction, 1.5% maintenance). Coordinates targeting the ventral tegmental area (VTA) were AP: -3.16 mm, lateral (LAT): +0.55 mm, dorsal-ventral (DV): -4.6 mm relative to bregma. A fiber optic (FP200URT; Thorlabs, Newton, New Jersey, USA) attached to a ferrule (CFLC230-10; Thorlabs) was slowly lowered into the brain. The ferrule was fixed to the skull with C&B Metabond Quick Adhesive Cement System (Parkell Inc., Brentwood, New York, USA).

### Fast-scan cyclic voltammetry

Mice were given an intraperitoneal injection of urethane (1.8 g/kg) and placed in a stereotaxic frame. A carbon fiber microelectrode was lowered into the nucleus accumbens at the following coordinates, AP: +1.3 mm, LAT: +1.1 mm, DV: -4.4 mm relative to bregma and a chloride-coated silver wire reference electrode was implanted and secured contralateral to the microelectrode using a stainless-steel screw and dental cement. A fiber optic attached to a ferrule was lowered directly above the VTA using the VTA coordinates noted above. A cycling potential was applied to the carbon fiber microelectrode (-0.4 V to 1.3 V and back) at a scan rate of 400 V/s while being held at -0.4 V between scans. To stabilize background current, the microelectrode was cycled at 60 Hz for 15 min then 10 Hz for 10 min prior to starting experimental stimulation protocols. Optical stimulation power was 20 mW. Evoked dopamine data were collected sampling at 10 Hz for 15 s. In each recording, background was digitally subtracted using 10 scans immediately preceding optical stimulation. After establishing an optimal dopamine signal, dopamine was evoked using 5, 10, 20 pulses at 5 and

40 Hz, with 2 min between each optical test stimulation. Oxidation and reduction peaks at 0.6 V and -0.2 V was identified using a cyclic voltammogram. Microelectrodes were calibrated following experiments using a micro flow cell and a 1- $\mu$ M dopamine solution [22].

### Behavioral testing

Mice were singly housed during the course of the experiments. Operant chambers were made from black, opaque plastic boxes (22 cm  $\times$  35 cm  $\times$  23 cm). Chambers included an active and inactive lever and a pellet dispenser (Med-Associates, St. Albans, Vermont, USA). Above each chamber was a PlexBright Compact LED module attached to a PlexBright LED Commutator which allowed the fiber optic cable to spin freely as the animals moved in the chambers (Plexon, Dallas, Texas, USA). Mice were weighed daily immediately prior to testing. 60-min sessions began after the fiber optic was attached and the animal placed in the chambers. Mice were allowed to press both the active and inactive lever at will during the session.

### Optogenetics

A blue 465 nm  $\lambda$  LED light (Plexon) was used to excite ChR2. Each lever press yielded a 500 ms burst of 20 pulses lasting 10 ms each at 40 Hz delivered by a Plexon controller (OPTMN0002e; PlexBright 4 Channel Optogenetic Controller; Plexon Inc, Brentwood, New York, USA). Light intensity at the tip of the ferrule was  $\sim$ 5 mW, tested daily prior to starting sessions.

### Imaging

Brains were perfused and fixed in 4% paraformaldehyde and placed in a 25% sucrose solution for 48 h. Frozen brains were cut using a cryostat (Leica Biosystems, Buffalo Grove, Illinois, USA) in 50  $\mu$ m sections. Channelrhodopsin was visualized via coexpression of enhanced yellow fluorescent protein (EYFP) and imaged with an Olympus Fluoview FV10i confocal microscope. The sections were also used to verify correct placement of the fiber in the VTA (Fig. 1).

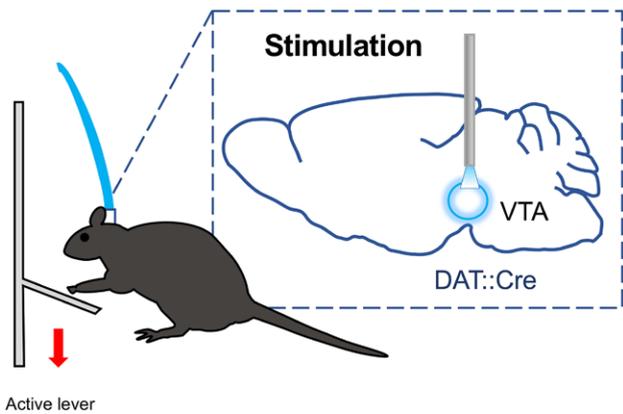
## Results

### Genetically expressed channelrhodopsin mediates optically stimulated dopamine release

Commonly, expression of channelrhodopsin in dopamine cells is achieved via viral expression, resulting in high expression levels [20,23]. Here, we use genetically expressed channelrhodopsin. Mice heterozygous for ChR2 and DAT-Cre (ChR2<sup>+/-</sup>; DAT<sup>cre/wt</sup>) express channelrhodopsin in the midbrain as reflected by coexpressed EYFP (Fig. 2a). The DAT<sup>cre</sup> line has previously been demonstrated to be selective for dopamine cells [24,25]. We tested optical stimulation of dopamine cells using fast-scan cyclic voltammetry, optically stimulating in the VTA at 5 and 40 Hz with 5, 10, and 20 pulses and



Fig. 3

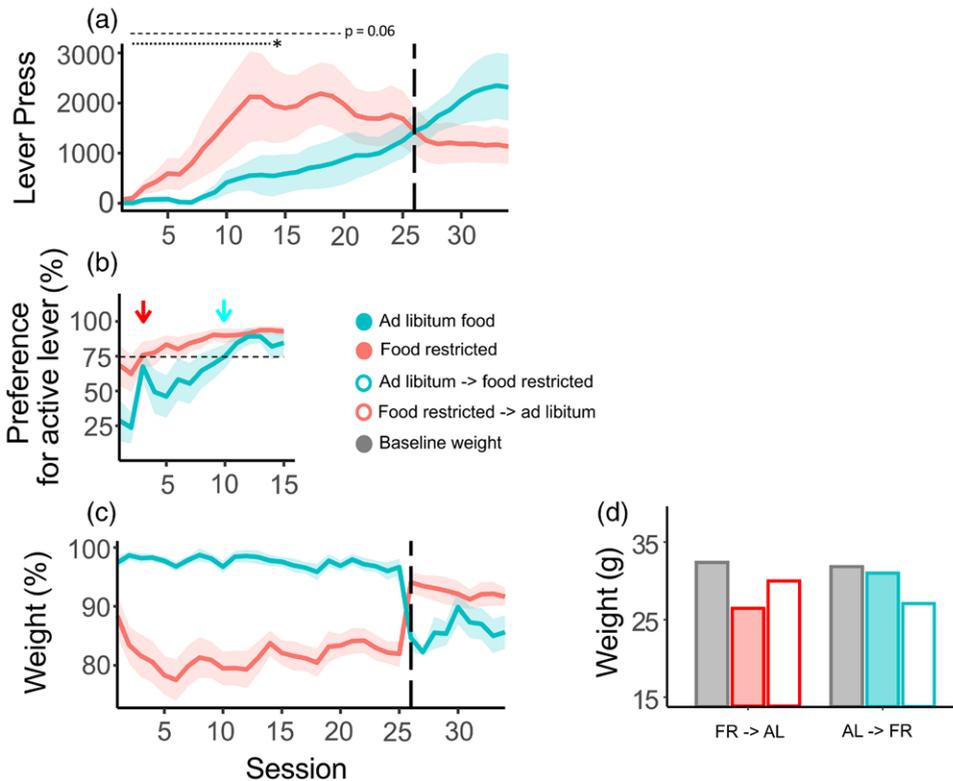


Schematic of oICSS behavioral paradigm. A press on the active lever is followed by optical stimulation of VTA dopamine cells in  $ChR2^{+/-};DAT^{Cre/wt}$  mice. DAT, dopamine transporter; oICSS, optical intracranial self-stimulation; VTA, ventral tegmental area.

**Chronic food restriction facilitates rapid acquisition of optical self-stimulation**

Mice were maintained on either food restriction (3 h/day access) or *ad libitum* access to food and tested in optical self-stimulation paradigm where every press on the active lever activated an LED that selectively stimulated midbrain dopamine cells (Fig. 3). In terms of day to day increases in lever-pressing, acquisition of oICSS was enhanced in food restriction mice compared to *ad libitum* mice (Fig. 4a, first 14 days, main effect,  $F_{(1,9)} = 4.95, P = 0.05$ ; group  $\times$  day,  $F_{(1,165)} = 18.83, P < 0.001$ ). The food restriction group reached asymptotic pressing by 14 days, exhibiting a group average of 1950 presses per session (Fig. 4a, days 12–14,  $1950 \pm 415$ ). In contrast, in the *ad libitum* group, the much slower session by session increases never reached asymptote, achieving a maximal average of only 1012 per session (Fig. 4a, days 23–25,  $1012 \pm 200$ ). Another measure of learning is the proportion of pressing on the active versus the inactive lever, where the percentage of presses on the active lever should increase substantially with reinforcement via dopamine stimulation. The

Fig. 4



Optical self-stimulation of ventral tegmental area dopamine restricted and ad libitum fed mice. (a) Mean daily responding on the active lever for each group across sessions. Dashed vertical line indicates reversal of feeding conditions. (b) Preference for active lever compared to inactive lever. Arrowheads indicate when each group reached 75% preference, dashed horizontal line indicates 75% preference. (c) Average weight from baseline by group across sessions, dashed vertical line indicates reversal of feeding conditions. (d) Average weights by group and condition.  $N=6, 7$  for food restricted and ad libitum, respectively.  $*P=0.05$ , Error bars, SEM.

food restriction mice as a group reached 75% preference for active lever by day 3 compared to the *ad libitum* mice that did not reach an equivalent preference until day 10 (Fig. 4b,  $t_{(1,11)} = -2.05$ ,  $P = 0.066$ ). These data suggest that chronic food restriction increases the efficacy of stimulated dopamine release in reinforcing an instrumental action in the absence of external reward such as food.

### Current food restriction status modulates the vigor of responding for optical self-stimulation

While the data for initial acquisition indicate a difference in rate of learning, or reinforcement efficacy, to test whether the vigor of the acquired response is modulated by food availability, we reversed feeding conditions. The *ad libitum* group were placed on 3 h of access per day (*ad libitum* → food restriction) while the food restriction group were provided *ad libitum* access to chow (food restriction → *ad libitum*). After 4 days for weight adaptation and stabilization, testing continued as before (in plots, open symbols reflect period after reversal of feeding conditions). The change in feeding conditions induced a significant weight adjustment for both groups (Fig. 4c and d, food restriction → *ad libitum*,  $F_{(1,60)} = 672.29$ ,  $P < 0.01$ ; *ad libitum* → food restriction,  $F_{(1,71)} = 94.78$ ,  $P < 0.01$ ). The food restriction → *ad libitum* group decreased lever pressing approximately 67% (Fig. 4a, days 23–25 vs. 32–34, 1690 → 1132), while the *ad libitum* → food restriction group increased pressing and stabilized at a group average of 2316 presses per session (Fig. 4a, days 23–25 vs. 34–35, 1247 → 2316). After reversal, the now food restricted *ad libitum* → food restriction mice pressed 205% more than the now *ad libitum* food restriction → *ad libitum* mice, reflecting a statistical trend (Fig. 4a, days 32–34, main effect,  $F_{(1,9)} = 3.60$ ,  $P = 0.09$ ).

### Discussion

Chronic food restriction increased both learning rate and vigor of responding for oICSS in the absence of food reward. Food restriction mice were able to distinguish between active and nonactive levers faster and more rapidly reached peak levels of lever pressing than *ad libitum* mice. In addition, food restriction increased the overall number of lever presses per session. The finding that food restriction increases vigor of responding is consistent with previous studies showing that food restriction increases the amount of effort an animal is willing to expend for ICSS [18,19]. Altogether, these findings reflect the body of evidence showing that food restriction causes neuroadaptations that increase the efficacy of dopamine reinforcement [3,10,26].

Our behavioral paradigm measures the rate of acquisition of self-stimulation, which contrasts with typical ICSS studies that measure stimulation threshold by varying either strength or frequency of electrical stimulation. By calculating stimulation threshold, conventional ICSS is used to determine how putative drugs of abuse administered

acutely change responding for self-stimulation, assessing how drugs alter self-stimulation dose-response (electrical ‘dose’ to lever pressing) curves, quantified as a single read-out of threshold. It is difficult, however, to assess rates of acquisition of learning in paradigms with constantly shifting levels of stimulation. By providing a constant level of optical stimulation, our simple paradigm offers an intuitive assessment of the efficacy of dopamine-mediated reinforcement under different environmental and organismal conditions. Consistent with prior work by others, these data demonstrate that chronic food restriction alters the dopamine system, highlighting that studying dopamine in animals under food restriction does not necessarily reflect ‘normal’ reward function [1–4]. Use of oICSS paradigms can provide a route to examine dopamine-mediated motivation and learning without the confounding effects of chronic food restriction and associated chronic stress.

Although oICSS selectively stimulates dopamine neurons, we cannot conclude with certainty the observed effects are mediated by dopamine *per se*. Specifically, glutamate can be released by dopamine cells and has been shown to mediate reinforcement independent of dopamine [27,28]. Thus, we cannot determine here whether increased reinforcement efficacy under food restriction arises from altered release of dopamine, glutamate, or both from dopamine cells in the midbrain.

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### Conflicts of interest

There are no conflicts of interest.

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