European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro

# Melanin-concentrating hormone is necessary for olanzapine-inhibited locomotor activity in male mice

**Melissa J.S. Chee, Nicholas Douris, Avery B. Forrow, Arnaud Monnard[1], Shuangyu Lu[2], Stephen E. Flaherty III, Andrew C. Adams[3], Eleftheria Maratos-Flier***

*Division of Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA*

Received 17 January 2015; received in revised form 7 April 2015; accepted 25 May 2015

**KEYWORDS**
Anorexia;
Accumbens nucleus;
MCH;
Antipsychotic;
Locomotion;
Electrophysiology

**Abstract**
Olanzapine (OLZ), an atypical antipsychotic, can be effective in treating patients with restricting type anorexia nervosa who exercise excessively. Clinical improvements include weight gain and reduced pathological hyperactivity. However the neuronal populations and mechanisms underlying OLZ actions are not known. We studied the effects of OLZ on hyperactivity using male mice lacking the hypothalamic neuropeptide melanin-concentrating hormone (MCHKO) that are lean and hyperactive. We compared the *in vivo* effects of systemic or intra-accumbens nucleus (Acb) OLZ administration on locomotor activity in WT and MCHKO littermates. Acute systemic OLZ treatment in WT mice significantly reduced locomotor activity, an effect that is substantially attenuated in MCHKO mice. Furthermore, OLZ infusion directly into the Acb of WT mice reduced locomotor activity, but not in MCHKO mice. To identify contributing neuronal mechanisms, we assessed the effect of OLZ treatment on Acb synaptic transmission *ex vivo* and *in vitro*. Intraperitoneal OLZ treatment reduced Acb GABAergic activity in WT but not MCHKO neurons. This effect was also seen *in vitro* by applying OLZ to acute brain slices. OLZ reduced the frequency and amplitude of GABAergic activity that was more robust in WT than MCHKO Acb. These findings indicate that OLZ reduced Acb GABAergic transmission and that MCH is necessary for the hypolocomotor effects of OLZ.


*Corresponding author. Tel.: +1 617 7353289.
*E-mail address:* emaratos@bidmc.harvard.edu (E. Maratos-Flier).
[1]Current address: Institute of Neuropathology, University Hospital of Zurich, Zurich, Switzerland.
[2]Current address: Department of Human Anatomy, School of Medicine, Henan University, Kaifeng, Henan 475004, China.
[3]Current address: Diabetes Research, Lilly Research Laboratories, Indianapolis, IN 46285, USA.

http://dx.doi.org/10.1016/j.euroneuro.2015.05.010
0924-977X/

## 1. Introduction

Anorexia nervosa is characterized by an inability to maintain a minimally normal body weight, starvation behaviors and intense fear of weight gain. In *restricting* type anorexia, individuals limit the amount of food they eat while exercising excessively. This hyperactivity reflects the underlying anxiety and compulsivity driving destructive body image and restrictive behaviors (Davis and Kaptein, 2006) and plays a critical role in disorder progression (Davis et al., 1997).

Most second generation, atypical antipsychotics used to treat schizophrenia and other psychiatric disorders are associated with weight gain (Almandil et al., 2013). This led to the evaluation of antipsychotics for treating anorexia (Brewerton, 2012). Olanzapine (OLZ), more commonly linked with weight gain than other antipsychotics, is among the most effective drugs for treating anorexia (Brewerton, 2012). Some studies do not find a therapeutic effect of OLZ (Kishi et al., 2012) but indeed, some anorexic patients treated with OLZ gained weight, showed improved attitudes towards eating and reduced their activity levels (Dennis et al., 2006; Dunican and DelDotto, 2007). In rodent models of activity-based anorexia, where food-restricted rodents housed with running wheels develop paradoxical hyperactivity and severe weight loss (Epling et al., 1983), both acute (Prinssen et al., 2000) and chronic OLZ treatment (Albaugh et al., 2011; Hillebrand et al., 2005; Klenotich et al., 2012) reduced locomotor activity and prolonged survival (Hillebrand et al., 2005; Klenotich et al., 2012).

Mice deficient in melanin-concentrating hormone (MCH), a hypothalamic neuropeptide that stimulates food intake (Tritos et al., 1998) and weight gain (Della-Zuana et al., 2002; Gomori et al., 2003), may also represent one model of anorexia (Siegfried et al., 2003). MCHKO mice are lean, hypophagic (Shimada et al., 1998) and hyperactive (Kokkotou et al., 2005; Zhou et al., 2005). They do not elicit homeostatic increases in food intake despite a steadfast demonstration of hyperactivity and higher energy expenditure (Alon and Friedman, 2006; Kokkotou et al., 2005; Whiddon and Palmiter, 2013; Zhou et al., 2005). Interestingly, the orexigenic actions of OLZ and MCH can act synergistically, such as in the accumbens nucleus (Acb) (Guesdon et al., 2010). The Acb is a critical region controlling motivated behavior and motor control (Graybiel et al., 1994) and integrates both the feeding (Georgescu et al., 2005; Guesdon et al., 2009) and locomotor actions of MCH (Pissios et al., 2008).

OLZ may treat anorexia by reducing hyperactivity (Leggero et al., 2010) but its mechanisms of action are not known. Using the MCHKO as a mouse model of hyperactivity, we investigated the effect of OLZ to reduce locomotor activity. We demonstrated that the Acb partly mediates the hypolocomotor effects of OLZ, and then performed electrophysiological recordings from Acb medium spiny neurons (MSNs) to study the neuronal mechanisms underlying OLZ actions. OLZ treatment reduced GABAergic transmission at Acb MSNs. Both the behavioral and cellular actions of OLZ were less robust in MCHKO mice. These findings demonstrate that MCH is necessary for enabling the maximal effects of OLZ, which acts by suppressing local GABAergic transmission in the Acb and inhibit locomotor activity.

## 2. Experimental procedures

### 2.1. Animals

Male WT and MCHKO littermates were backcrossed onto the C57BL/6 background for >20 generations (Kokkotou et al., 2005; Shimada et al., 1998). All mice were housed in a 12-h light-dark cycle at 22 °C with *ad libitum* access to food (5058, LabDiet) and water. All procedures were conducted following accepted guidelines of the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committees.

### 2.2. Wheel-running activity

Baseline wheel-running activity of male WT and MCHKO littermates (11–16 weeks old) was averaged over 5 days after acclimation (7 days) to running wheels (11.5 cm diameter; Mini Mitter). The number of wheel revolutions was monitored using a magnetic reed switch and recorded using VitalView (Mini Mitter) data acquisition hardware and software. The effect of acute OLZ administration on wheel-running activity was assessed in WT and MCHKO mice injected intraperitoneally (ip) with vehicle (0.16% acetic acid (AcOH) in saline) or OLZ (2.5 mg/kg) 30 min before the onset of the dark cycle.

### 2.3. Intra-Acb administration

WT and MCHKO mice (12–16 weeks old) were anesthetized with ketamine (100 μg/kg, ip; Butler Schein)-xylazine (10 μg/kg, ip; Butler Schein) and a bilateral 4.2 mm guide cannula (Plastics One) was stereotaxically placed using coordinates: anterioposterior +1.20 mm, mediolateral ±0.60 mm, dorsoventral −4.10 mm (Paxinos and Franklin, 2001). The tip of the cannula was dorsomedial to the anterior commissure but within the medial Acb. After surgery, mice were singly-housed in a homecage free of suspended obstructions (*i.e.* food hoppers, wire mesh) to prevent the cannula from dislodging. Homecage locomotor activity was assessed over 24-h periods by an Opto-M3 infrared beam break monitoring system (Columbus Instruments) to detect the number of sequential beam breaks along the *x*-axis. A Multi Device Interface (v1.3; Columbus Instruments) recorded the number of X-ambulations. After one week to recover from surgery, each mouse was habituated to handling for 6 consecutive days before intra-Acb treatment.

All intra-Acb infusions began 30 min before the dark cycle. Using a 33 G bilateral injector, we bilaterally infused WT and MCHKO mice with 1 μl of vehicle (0.7% DMSO, 0.01% AcOH in ACSF [technical information, Alzet], pH 7) on Day 1 and then 1 μl of 0.74 mM OLZ (0.23 μg per side) on Day 2. Solutions are sequentially delivered over 4 min (0.25 μl/min) to each side of the Acb, waiting 2 min after each infusion before pulling out the injector to allow the solution to be absorbed by the Acb and prevent backflow up the cannula. All mice were returned to their respective homecage and locomotor activity was monitored for 4 h post-infusion.

At the end of the treatment period, bilateral cannula tips were coated with DiI stain (Life Technologies) to mark their placement in the Acb. The mice were deeply anesthetized with an overdose of ketamine-xylazine (ip) then transcardially perfused with chilled (4 °C) 0.9% NaCl saline followed with 10% buffered-formalin. The brains were removed, post-fixed, cryoprotected with 20% sucrose and 30 μm thick coronal sections were cut on a freezing microtome (SM2000R; Leica) into four equal series. We mounted one brain series to check the cannula placement. Results from mice where one or both cannula tips landed outside the medial Acb were excluded from analysis.

Please cite this article as: Chee, M.J.S., et al., Melanin-concentrating hormone is necessary for olanzapine-inhibited locomotor activity in male mice. European Neuropsychopharmacology (2015), http://dx.doi.org/10.1016/j.euroneuro.2015.05.010