

# The Effects of the Monoamine Stabilizer (–)-OSU6162 on Binge-Like Eating and Cue-Controlled Food-Seeking Behavior in Rats

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Binge-eating disorder (BED) is characterized by recurring episodes of excessive consumption of palatable food and an increased sensitivity to food cues. Patients with BED display an addiction-like symptomatology and the dopamine system might be a potential treatment target. The clinically safe monoamine stabilizer (–)-OSU6162 (OSU6162) restores dopaminergic dysfunction in long-term alcohol-drinking rats and shows promise as a novel treatment for alcohol use disorder. Here, the effects of OSU6162 on consummatory (binge-like eating) and appetitive (cue-controlled seeking) behavior motivated by chocolate-flavored sucrose pellets were evaluated in non-food-restricted male Lister Hooded rats. OSU6162 significantly reduced binge-like intake of chocolate-flavored sucrose pellets without affecting prior chow intake. Furthermore, OSU6162 significantly reduced the cue-controlled seeking of chocolate-flavored sucrose pellets under a second-order schedule of reinforcement before, but not after, the delivery and ingestion of reward, indicating a selective effect on incentive motivational processes. In contrast, the dopamine D2/D3 receptor antagonist raclopride reduced the seeking of chocolate-flavored sucrose pellets both pre- and post reward ingestion and also reduced responding under simpler schedules of seeking behavior. The D1/5 receptor antagonist SCH23390 had no effect on instrumental behavior under any reinforcement schedule tested. Finally, local administration of OSU6162 into the nucleus accumbens core, but not dorsolateral striatum, selectively reduced cue-controlled sucrose seeking. In conclusion, the present results show that OSU6162 reduces binge-like eating behavior and attenuates the impact of cues on seeking of palatable food. This indicates that OSU6162 might serve as a novel BED medication.

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## INTRODUCTION

Binge-eating disorder (BED) is characterized by persistent uncontrollable episodes of eating large amounts of palatable food, and an increased responsivity to food-associated cues (Schienle *et al*, 2009) inducing food cravings and overeating (Curtis and Davis, 2014; Sobik *et al*, 2005). These behaviors resemble symptoms of substance use disorders, including loss of control over drug intake, drug cue sensitivity, and craving (Cassin and von Ranson, 2007). The mesocortico-limbic dopamine system may be implicated in the overlapping neural mechanisms between the two disorders, as well as the reinforcing effects of both palatable foods and drugs of abuse (Gonzales *et al*, 2004; Wise, 2006). In fact, intake of both palatable food and drugs of abuse (Ericson *et al*, 1998; Ito *et al*, 2000; Rada *et al*, 2005) and cues

associated with these reinforcers (Di Ciano *et al*, 1998; du Hoffmann and Nicola, 2014) release dopamine in the nucleus accumbens (NAc) in rodents. Similarly, in both BED (Wang *et al*, 2011) and substance use disorders (Volkow *et al*, 2006), dopamine is released in the striatum following the presentation of food- and drug-associated cues, respectively. Moreover, like chronic drug intake, chronic binge-like intake of palatable food induces addictive-like behaviors and dopaminergic changes in the striatum (Avena, 2010; Naef *et al*, 2015), including decreased NAc dopamine D2 receptor levels in rodents (Avena, 2007; Bello and Hajnal, 2010; Colantuoni *et al*, 2001). In addition, genetic studies have associated binge-eating pathology in humans with polymorphisms of the dopamine transporter and D2 receptor (for review see Bello and Hajnal, 2010). Finally, the monoamine-releasing compound lisdexamfetamine is the only FDA-approved BED medication (Davis and Attia, 2017). However, because of the medication's side effects and abuse potential, improved treatment options are needed.

The monoamine stabilizer (–)-OSU6162 (OSU6162) (Sonesson *et al*, 1994) is a clinically safe compound (Johansson *et al*, 2012; Khemiri *et al*, 2015; Kloberg *et al*,

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2014) with the ability to increase and decrease dopaminergic activity depending on the prevailing dopaminergic tone (Rung *et al*, 2008; Tedroff *et al*, 1998). Instead of partial agonism, antagonism at both extrasynaptic dopamine D2 auto- and heteroreceptors (Tolboom *et al*, 2014) has been hypothesized to underlie this effect. We recently identified OSU6162 as a potential treatment for alcohol use disorder by showing that it attenuates alcohol-mediated behaviors (Steenland *et al*, 2012) and restores striatal dopaminergic deficits (Feltmann *et al*, 2016) in alcohol-drinking rats, as well as attenuates alcohol craving in alcohol-dependent humans (Khemiri *et al*, 2015).

Based on the behavioral and neurochemical similarities between BED and substance use disorders, together with our observation that OSU6162 reduced sucrose drinking in rats (Steenland *et al*, 2012), we here explored the potential of OSU6162 as a novel BED treatment using relevant rodent models. The effect of OSU6162 on food consumption was investigated using a model of binge-like intake of highly palatable food (Cottone *et al*, 2008; Giuliano *et al*, 2012) and the ability of OSU6162 to affect the impact of food-associated cues was investigated using food seeking under a second-order schedule of reinforcement (Giuliano *et al*, 2012). In the latter procedure, animals respond on a fixed-ratio schedule (FR10) for brief, contingent presentations of reward-associated conditioned stimuli (CS), acting as conditioned reinforcers, during two 15 min intervals. Each interval is terminated by reward delivery (20 chocolate-flavored sucrose pellets). This procedure was used to investigate whether OSU6162, compared with dopamine D1 and D2 receptor antagonists, affects high-incentive food reward seeking directly (first interval, before reward delivery) or indirectly via alteration of the hedonic impact of food ingestion (second interval, after reward delivery) (Giuliano *et al*, 2012). Furthermore, to evaluate whether potential effects would be specific to prolonged seeking and CS responding, the effects of the compounds were also investigated under fixed interval (FI) schedules: one involving frequent reward delivery (1 min, FI1) and one similar to the second-order schedule but without contingent CS presentations (FI15). Finally, to identify brain areas involved in mediating the systemic effects of OSU6162 on the second-order schedule, local bilateral OSU6162 infusions were given into the NAc core and dorsolateral striatum (DLS). These two structures were targeted as they, in contrast to the NAc shell, have been consistently shown to be implicated in conditioned reinforcement and in the effects of conditioned reinforcers on instrumental seeking behavior in a second-order schedule of reinforcement (Ito *et al*, 2000); for example, the integrity of the NAc core is necessary for the expression of conditioned reinforcement and the acquisition of a novel response for a food- or cocaine-paired conditioned reinforcer (Di Ciano, 2008; Di Ciano and Everitt, 2004; Parkinson *et al*, 1999).

## MATERIALS AND METHODS

Detailed descriptions of animals (male Lister Hooded rats (Charles River, Margate, UK)), housing conditions, intracranial guide cannula implantation and drug infusion, histological assessment of cannula placement, and drugs and chemicals are available in Supplementary Information.

All procedures were conducted according to the UK Animal (Scientific Procedures) Act 1986 Amendment Regulations 2012, following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB).

### Binge-Like Eating of Highly Palatable Food

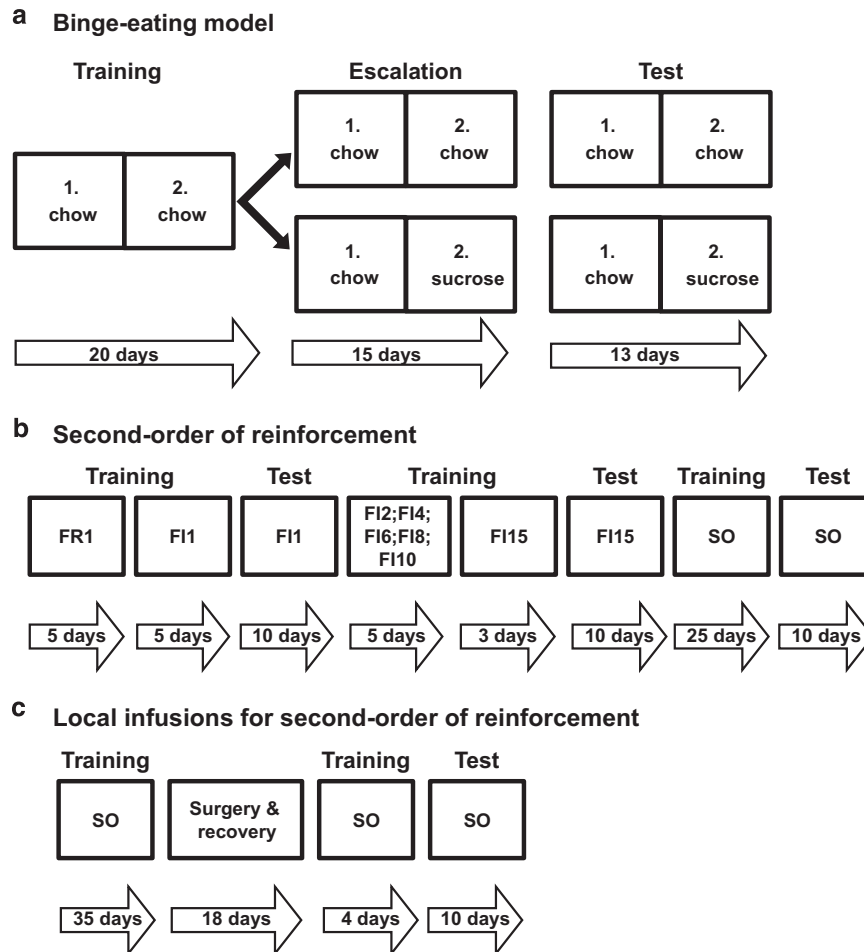
**Paradigm.** The binge-eating procedure was used to induce a binge-like hyperphagia of palatable food and a prior chow hypophagia in anticipation of the palatable food (negative anticipatory contrast).

The procedure was performed as described previously (Cottone *et al*, 2008; Giuliano *et al*, 2012) but minor modifications were made. In brief, male rats with free access to rodent chow in the home cage throughout the experiment were transferred daily to behavioral chambers. After 2 h of food deprivation, rats had access to chow in one (first) and then another (second) feeder for 10 min in each feeder. After 20 sessions rats were divided into two groups, both of which had access to chow from the first feeder and chocolate-flavored sucrose pellets (chow/sucrose group,  $n=10$ ) or chow (chow/chow group,  $n=10$ ) from the second feeder, respectively (Figure 1a).

**Treatment.** To ensure binge-like eating in the chow/sucrose group, testing began at least 15 sessions after the chocolate-flavored sucrose pellets had been introduced in the second feeder (Figure 1a). Thereafter, OSU6162 (5, 10, 15, and 30 mg/kg, subcutaneously (i.p.)) or vehicle were administered 60 min before giving access to the first feeder using a Latin square design with 2 days of re-baselining in between injections. One rat in the binge-eating paradigm was excluded from analysis because of ill health.

### Seeking of Highly Palatable Food: Second-Order Schedule of Reinforcement

**Paradigm.** The second-order schedule of high-incentive food reinforcement procedure was performed as previously described (Giuliano *et al*, 2012), except that rats in the present experiment were given unlimited access to food in the home cages throughout the experiment. Briefly, 24 rats were initially trained in operant chambers to press a lever under a FR1 schedule for delivery of chocolate-flavored sucrose pellets, paired with a 20 s light stimulus (CS). Following five sessions on FR1 (Figure 1b), sessions under FI schedules were introduced during which pellet delivery and 20 s CS presentation was restricted to the first lever press after the end of each 1 min interval (FI1). After ~15 sessions on FI1, the length of the intervals was progressively increased up to 15 min (FI15) during six sessions (FI2, FI4, FI6, FI8, FI10, and FI15), whereas the maximum total number of 40 pellets/session remained constant (ie, during a FI15 session, 20 pellets were delivered together with a 20 s CS at the end of each of the two intervals). Following 13 sessions on FI15, a second-order schedule of reinforcement was introduced, differing only from the FI15 schedule in the presentation of a brief 1 s CS together with every tenth lever press during each interval. Pellet consumption was assessed by checking for any remaining pellets in the food tray at the end of each session.



**Figure 1** Timeline of experimental procedures. (a) In the binge-eating paradigm, rats were trained daily in behavioral chambers, including access to food in feeder 1, followed by access to feeder 2 (10 min per feeder). During the training phase (20 days), all rats got access to chow in both feeders. Thereafter, rats were divided into a control group, continuing the chow/chow access and one group receiving chow from the first, and chocolate-flavored sucrose pellets from the second feeder. After 15 days, food intake in each group had stabilized and drug testing was conducted. (b) To train rats in the second-order schedule of reinforcement, rats were initially trained on a fixed ratio (FR) 1 schedule, followed by fixed interval (FI) schedules of increasing length ranging from 1 min (FI1) to 15 min (FI15, second-order). During FI1, FI15, and second-order (SO) schedules, drug testing was performed. (c) To test the effects of drugs administered locally into the brain, rats that had been trained under the second-order schedule (SO) of reinforcement for 35 days were implanted with guide cannulae. Following 2 weeks of recovery, rats were retrained for 4 days before testing of local infusions started. (a–c) Drug testing was conducted using a Latin square design, ie, each rat received each dose, with 2 days of rebaselining in between injections. The period of each training and testing stage is indicated.

**Treatment.** The animals were divided into three groups (matched for baseline FI1 responding,  $n=8$  per group) and treated with OSU6162 (5, 10, and 15 mg/kg, s.c.), raclopride (0.01, 0.03, and 0.1 mg/kg, s.c.), or SCH23390 (0.003, 0.01, and 0.03 mg/kg, intraperitoneally (i.p.)), respectively. Testing was conducted within each treatment group after reaching stable baseline performance on FI1, FI15, and second-order schedules, respectively, using a Latin square design within each schedule with at least 2 days of rebaselining in between injections.

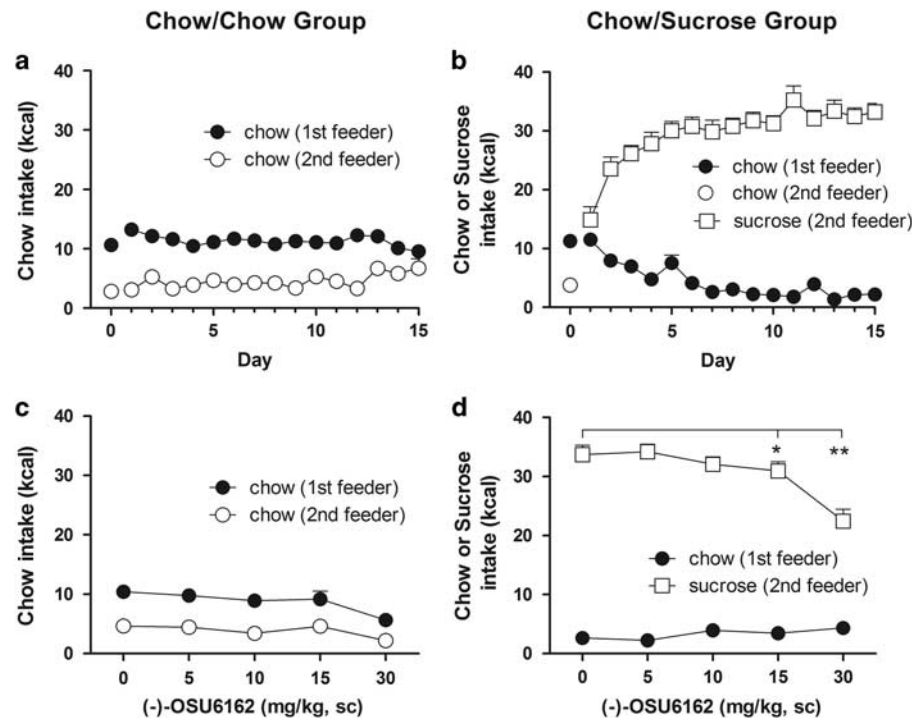
**Surgical procedures and intracerebral infusions.** Two cohorts of rats trained on the second-order schedule of reinforcement (Figure 1c) were implanted bilaterally with guide cannulae above the NAc core (AP +1.7, ML  $\pm$ 1.9, DV -1.5) or DLS (AP +1.2, ML  $\pm$ 3.0, DV -2.0). The first cohort was the same as in the previous experiment (systemic injections of OSU6162, raclopride, or

SCH23390); surgery took place within 2 weeks after the last injection on the second-order schedule. The second group was prepared using a similar training procedure with the exception of shorter FI1 and FI15 schedules as no drug testing was conducted.

OSU6162 (0.5, 1.5, and 5  $\mu$ g/0.5  $\mu$ l/side, Latin square design) was bilaterally infused into the NAc core or DLS 5 min before the start of the session. Only animals with cannula placements in the target areas were included in the statistical analysis (see details in Supplementary Information).

### Statistical Analysis

To identify overall effects on acquisition of the binge-like eating behavior, food intake (kcal) was analyzed using repeated-measures analysis of variance (ANOVA) with diet (group: chow/chow, chow/sucrose) as the between-subject



**Figure 2** OSU6162 reduces intake of highly palatable food in a binge-like eating paradigm. The binge-eating procedure used consisted of three contiguous phases: 2 h food deprivation, first feeder: 10 min access to chow (closed circles), second feeder: 10 min access to chow (open circles) or chocolate-flavored sucrose pellets (open squares). Values represent the mean  $\pm$  SEM food intakes in kcal ( $n = 9-10$ /group). The chow/chow-fed rats (a) displayed stable intake of chow over 15 sessions. The chow/sucrose-fed rats (b) escalated in sucrose intake while reducing prior chow intake ('anticipatory negative contrast'). Following the 15 sessions (in a, b), OSU6162 (0, 5, 10, 15, and 30 mg/kg) was tested for effects on food intake in the chow/chow (c) and the chow/sucrose (d) group. Within each group, each rat received each dose and thus served as its own control. In the chow/chow-fed rats (c), OSU6162 (10 and 30 mg/kg) significantly reduced chow intake compared with vehicle upon analysis of both feeders together due to lack of a significant session  $\times$  feeder interaction (repeated-measures ANOVA and within-subject contrast to vehicle). In the chow/sucrose-fed rats (d), significant effects of treatment compared with vehicle are displayed ( $*p < 0.05$ ,  $**p < 0.01$ , repeated-measures ANOVA and within-subject contrast to vehicle).

factor and day and feeder as within-subject factors. Significant main effects of diet were followed by repeated-measures ANOVA with day and feeder as within-subject factors for each diet group separately. Significant feeder  $\times$  session interactions were followed by repeated-measures ANOVA within each feeder. A significant main effect of day was followed by Sidak *post hoc* to evaluate significant changes against day 1.

To detect overall effects of OSU6162 treatment in the binge-like eating model, food intake (kcal) was analyzed using repeated-measures ANOVA with diet group as the between-subject factor and feeder and treatment as within-subject factors. Upon confirmation of a significant main effect of diet, repeated-measures ANOVA with treatment and feeder as within-subject factors were performed for each diet group separately and significant feeder  $\times$  treatment interactions were followed by repeated-measures ANOVA within each feeder. Significant main effect of treatment was followed by within-subject contrasts to vehicle.

Effects of systemic and intracerebral drug treatments on operant food seeking under F11, F15, and second-order schedules were analyzed using separate one-way repeated-measures ANOVAs for each drug and schedule as well as within each 15 min interval. Data of the F15 and second-order schedule were not normally distributed and therefore square root transformed before analysis. Note that the non-

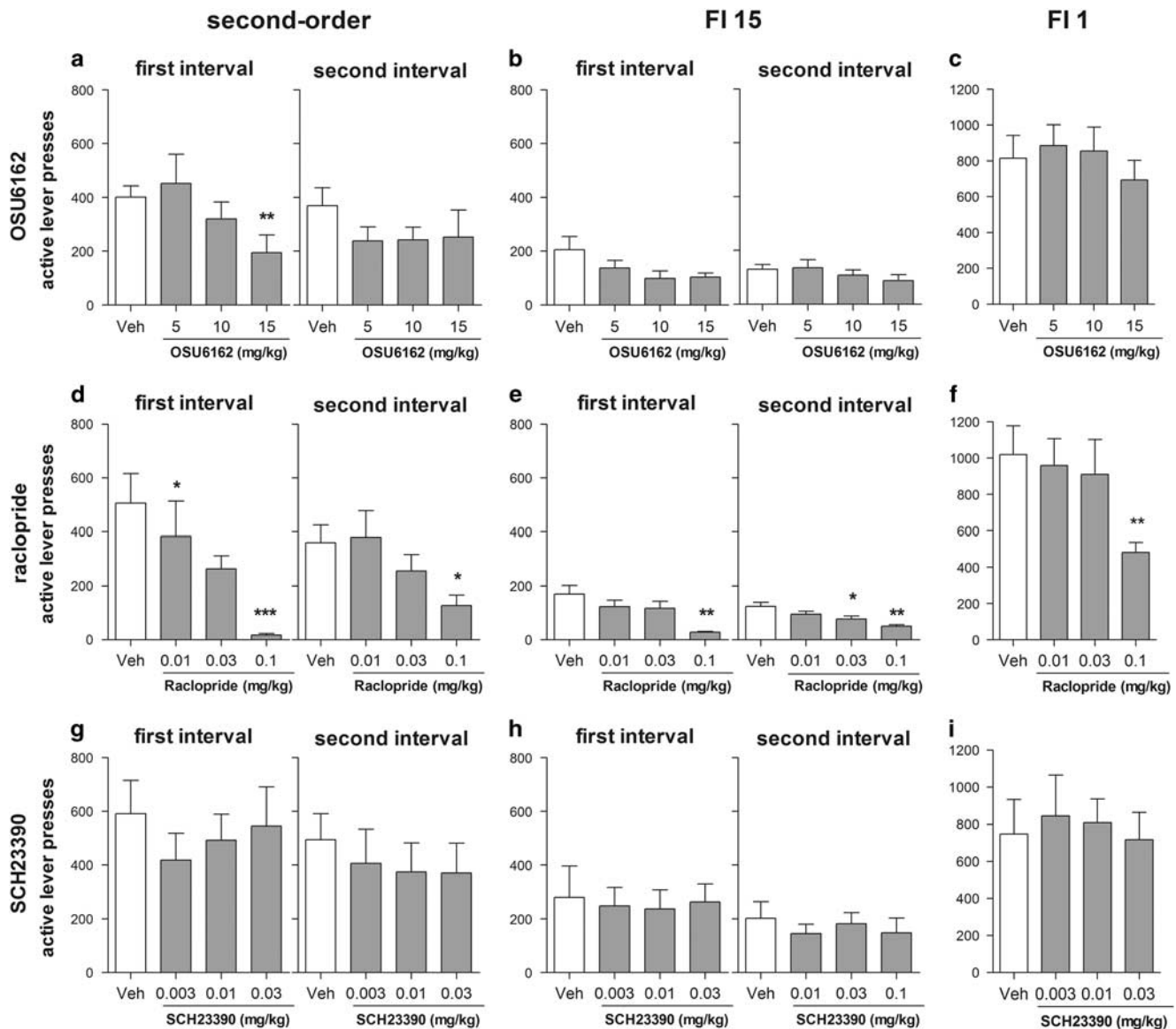
transformed number of lever presses is presented in the figures for the sake of clarity. Significant treatment effects were followed by within-subject contrasts to vehicle.

Statistical analyses were performed using the software SPSS (version 22, Chicago, IL) and all values were reported as mean  $\pm$  SEM. The significance level was set at  $\alpha \leq 0.05$ .

## RESULTS

### Binge-like Eating Is Induced by Limited Access to Chocolate-Flavored Sucrose Pellets

A binge-like eating pattern (ie, escalation of sucrose intake over time) was achieved by giving rats daily exposure to the following paradigm during 15 days of training: 2 h food deprivation, 10 min access to chow in the first feeder, followed by a 10 min access to either chow (chow/chow group) or chocolate-flavored sucrose pellets (chow/sucrose group) in the second feeder. There were significant overall main effects of diet group (ie, chow/chow vs chow/sucrose;  $F_{1,17} = 118.3$ ,  $p < 0.001$ ), session ( $F_{14,238} = 18.6$ ,  $p < 0.001$ ), and feeder ( $F_{1,17} = 100.6$ ,  $p < 0.001$ ), as well as a diet  $\times$  session  $\times$  feeder interaction ( $F_{14,238} = 17.9$ ,  $p < 0.001$ ). Within-group analyses revealed significant session  $\times$  feeder interactions in both diet groups (chow/chow:  $F_{14,112} = 2.9$ ,  $p < 0.01$ ; chow/sucrose:  $F_{14,126} = 42.5$ ,  $p < 0.001$ ). In the chow/chow group (Figure 2a) there was a stable chow intake



**Figure 3** Effects of OSU6162, raclopride, and SCH23390 on seeking for highly palatable food under a fixed interval (FI) 1 and 15 min and a second-order schedule of reinforcement. Effects of OSU6162 (0, 5, 10, and 15 mg/kg, a–c), raclopride (0, 0.01, 0.03, 0.1 mg/kg, d–f), and SCH23390 (0, 0.003, 0.01, and 0.03 mg/kg, g–i) ( $n=8$ /group) on seeking for highly palatable food under a second-order (left panel) schedule and fixed interval schedules of 15 min (FI15, central panel) and 1 min (FI1, right panel) of reinforcement are displayed. In both the second-order and FI15 schedule, 20 pellets (chocolate-flavored sucrose) together with a 20 s conditioned stimuli (CS) were delivered at the end of each 15 min interval, leading to the first interval (before reward delivery) and second interval (after reward delivery). The second-order schedule differed from FI15 by presentation of a contingent 1 s CS upon every tenth active lever presses during an interval. In the FI1 schedule, 1 pellet and a 20 s CS were delivered upon an active lever press first after 1 min had elapsed, leading to 40 pellets delivered in total. Values represent mean  $\pm$  SEM number of active lever presses during each interval. Within each treatment group, each rat received each dose and thus served as its own control. Significant effects of treatment on active lever presses compared with vehicle are displayed (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , repeated-measures ANOVA and within-subject contrast to vehicle).

in both feeders during the 15 sessions. The *post hoc* analysis revealed that the chow/sucrose group significantly increased the sucrose consumption to binge-like levels on each session compared with the first session (Figure 2b) and significantly decreased the chow intake starting from session 6, ie, they displayed an anticipatory negative contrast effect, under-eating the first presented food in expectation of access to the more highly preferred chocolate-flavored sucrose pellets (Giuliano *et al*, 2012). Sucrose intake was similar to the binge-like levels previously reported in female

Lister Hooded (Giuliano *et al*, 2012) and Wistar rats (Cottone *et al*, 2008).

### OSU6162 Reduced Binge-Like Eating of Chocolate-Flavored Sucrose Pellets

OSU6162 significantly reduced binge-like intake of chocolate-flavored sucrose pellets, but did not significantly affect the negative anticipatory contrast.

The effect of OSU6162 (5, 10, 15, and 30 mg/kg, s.c.) on binge-like eating was evaluated after 15 sessions in the

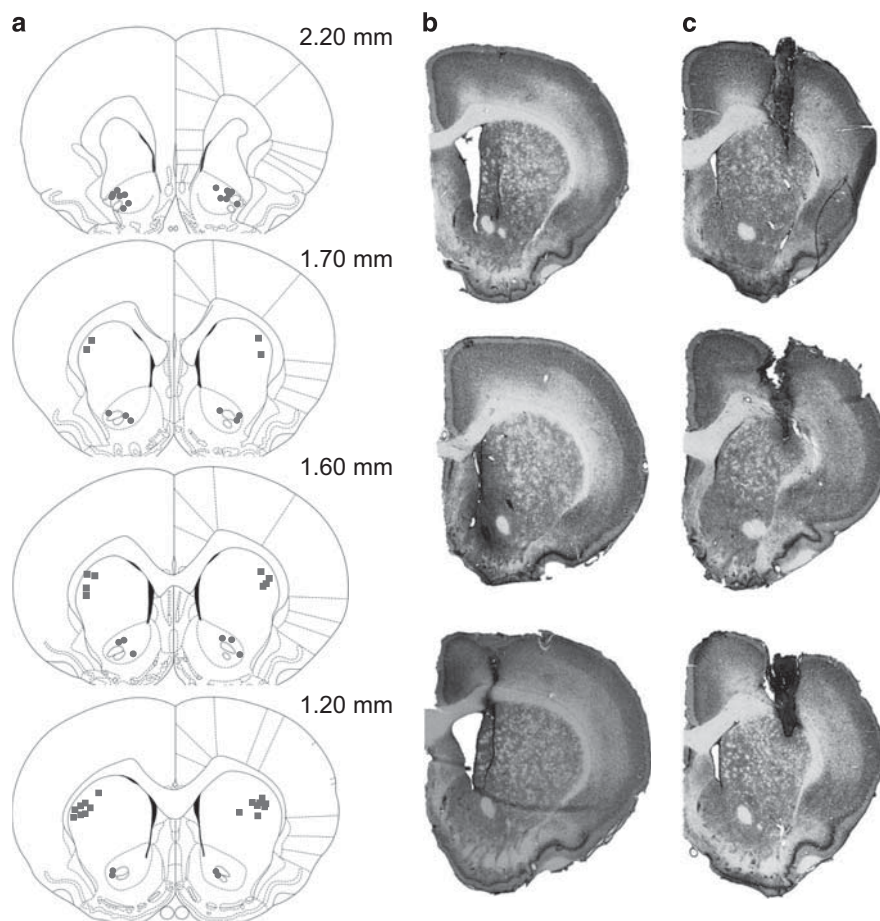
binge-eating paradigm. OSU6162 treatment significantly affected food intake, as indicated by a significant main effect of treatment ( $F_{4, 48} = 16.2, p < 0.001$ ), and also affected food intake differently in each diet group, depending on the feeder, as indicated by significant main effects of diet group ( $F_{1, 17} = 227.8, p < 0.001$ ), feeder ( $F_{1, 17} = 144.5, p < 0.001$ ), and a diet group  $\times$  treatment  $\times$  feeder interaction ( $F_{3.3, 56.8} = 16.5, p < 0.001$ ). In the chow/chow group (Figure 2c), there was a significant main effect of treatment ( $F_{4, 32} = 7.7, p < 0.001$ ) but no feeder  $\times$  treatment interaction ( $F_{4, 32} = 0.9, \text{NS}$ ). Hence, analyzing both feeders together, within-subject contrast to vehicle revealed that OSU6162 at 10 ( $p < 0.05$ ) and 30 mg/kg ( $p < 0.001$ ) significantly reduced chow intake. In the chow/sucrose group (Figure 2d), there was a significant main effect of treatment ( $F_{4, 36} = 9.7, p < 0.001$ ) and a significant feeder  $\times$  treatment interaction ( $F_{2.1, 19.1} = 22.9, p < 0.001$ ). Within-subject contrasts to vehicle for each feeder revealed that 15 and 30 mg/kg OSU6162 significantly reduced sucrose, but did not significantly affect chow intake. Hence, there was no significant effect of OSU6162 on the anticipatory negative contrast.

### OSU6162 Reduced Cue-Controlled Seeking for Chocolate-Flavored Sucrose Pellets

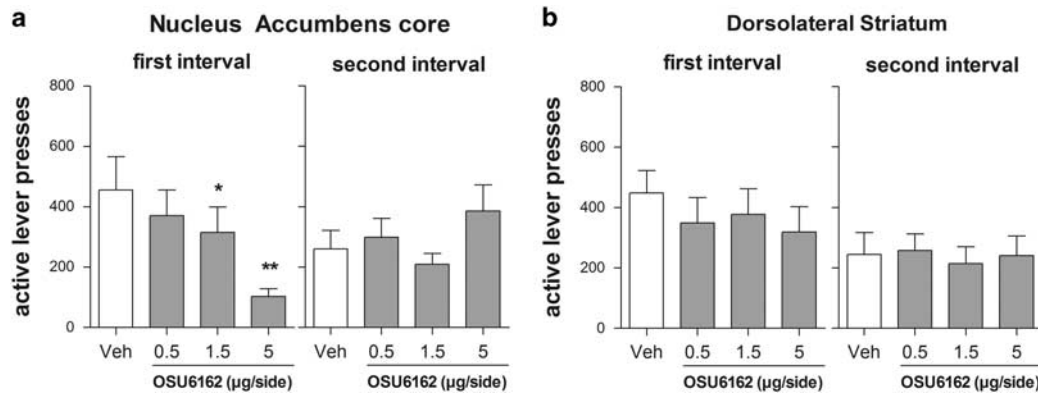
The effects of OSU6162 (5, 10, and 15 mg/kg, s.c.), raclopride (0.01, 0.03, and 0.1 mg/kg, s.c.), and SCH23390 (0.003, 0.01, and 0.03 mg/kg, i.p.) were evaluated on seeking behavior motivated by chocolate-flavored sucrose pellets under the second-order schedule of reinforcement and during prior training stages (FI1 and FI15).

OSU6162 treatment significantly affected seeking behavior under the second-order schedule (Figure 3a) during the first interval (before sucrose ingestion) ( $F_{3, 21} = 4.1, p < 0.05$ ), but not during the second interval (after sucrose ingestion) ( $F_{3, 21} = 1.1, \text{NS}$ ). Within-subject contrasts to vehicle revealed that OSU6162 (15 mg/kg) significantly reduced the responding on the active lever during the first interval only, before delivery of food. Under the FI15 schedule (Figure 3b), OSU6162 treatment produced an overall main effect during the first ( $F_{3, 21} = 3.3, p < 0.05$ ) but not the second interval ( $F_{3, 21} = 1.5, \text{NS}$ ). However, within-subject contrast to vehicle revealed no significant effects of any dose. Seeking under FI1 was not affected by OSU6162 (Figure 3c,  $F_{3, 21} = 2.0, \text{NS}$ ).

The D2-receptor antagonist raclopride significantly reduced sucrose-seeking behavior under the second-order



**Figure 4** Histological illustration of locations of infusions into nucleus accumbens core and dorsolateral striatum. Guide cannulas were implanted above the nucleus accumbens (NAc,  $n = 15$ ) core or dorsolateral striatum (DLS,  $n = 14$ ). Coronal sections from +2.2 to +1.2 mm anteroposterior from bregma represent histological verified locations of infusions into NAc core (filled circles) and DLS (filled squares) (a). Representative photomicrographs of Cresyl violet-stained coronal sections of NAc core- (b) and DLS- (c) cannulated rat brains, showing the guide cannula damage above and the infusion track into the targeted areas.



**Figure 5** The role of the ventral and dorsal striatum in the effects of OSU6162 on seeking for highly palatable food under a second-order schedule of reinforcement. Effects of OSU6162 (0, 0.5, 1.5, and 5 µg/side) infused into either the (a) nucleus accumbens (NAc) core ( $n = 15$ ) or (b) dorsolateral striatum (DLS,  $n = 14$ ) on seeking for highly palatable food under a second-order schedule of reinforcement are displayed. At the end of each 15 min interval, 20 pellets (chocolate-flavored sucrose) together with a 20 s conditioned stimuli (CS) were delivered, leading to the first interval (before reward delivery) and second interval (after reward delivery). During each interval, contingent 1 s CS were presented upon every tenth active lever press. Values represent mean  $\pm$  SEM number of active lever presses during each interval. Within each treatment group, each rat received each dose and thus served as its own control. Significant effects of treatment on active lever presses compared with vehicle are displayed (\* $p < 0.05$ , \*\* $p < 0.01$ , repeated-measures ANOVA and within-subject contrast to vehicle).

(Figure 3d, first interval:  $F_{1,4,10.5} = 15.5$ ,  $p < 0.01$ ; second interval:  $F_{3,21} = 4.8$ ,  $p < 0.05$ ), the FI15 (Figure 3e, first interval:  $F_{3,21} = 12.9$ ,  $p < 0.001$ ; second interval:  $F_{3,21} = 8.4$ ,  $p < 0.01$ ), and the FI1 (Figure 3f,  $F_{3,21} = 5.1$ ,  $p < 0.01$ ) schedules. Within-subject contrasts to vehicle revealed that raclopride at the highest dose (0.1 mg/kg) significantly reduced lever pressing during all schedules and both intervals. Raclopride 0.01 mg/kg significantly reduced responding in the first interval of the second-order schedule compared with vehicle and at a dose of 0.03 mg/kg significantly reduced lever pressing during the second interval of the FI15 schedule.

SCH23390 treatment did not affect seeking behavior under either the second-order (Figure 3g, first interval:  $F_{3,21} = 1.0$ , NS; second interval:  $F_{3,21} = 0.9$ , NS), the FI15 (Figure 3h, first interval:  $F_{3,21} = 0.2$ , NS; second interval:  $F_{3,21} = 1.7$ , NS), or the FI1 (Figure 3i,  $F_{3,21} = 0.7$ , NS) schedule.

None of the evaluated compounds affected pellet consumption in any schedule compared with vehicle; all rats consumed all pellets at the end of each interval during testing.

### Effects of Local Infusion of OSU6162 into the NAc Core or DLS on Cue-Controlled Seeking of Chocolate-Flavored Sucrose Pellets

To evaluate the involvement of the NAc core and DLS in the effects of OSU6162 on cue-controlled sucrose seeking under a second-order schedule of reinforcement, OSU6162 (0.5, 1.5, and 5 µg/side) was bilaterally infused in either brain area before testing (Figure 4). OSU6162 infused into the NAc core (Figure 5a,  $n = 15$ ) reduced responding for sucrose in the first (before sucrose ingestion) ( $F_{3,42} = 9.2$ ,  $p < 0.001$ ) but not in the second interval (after sucrose ingestion) ( $F_{3,42} = 1.4$ , NS). Within-subject contrasts to vehicle revealed that animals treated with OSU6162 at 1.5 and 5 µg/side significantly reduced active lever pressing in the first interval. In contrast, OSU6162 infused into the DLS (Figure 5b,  $n = 14$ ), had no effect on responding for food in either the first ( $F_{3,39} = 1.4$ , NS) or the second interval ( $F_{3,39} = 0.07$ , NS).

### DISCUSSION

The monoamine stabilizer OSU6162 reduced binge-like consumption of chocolate-flavored sucrose pellets. Moreover, systemic as well as local administration of OSU6162 into the NAc core, but not the DLS, reduced cue-controlled seeking behavior for these pellets under a second-order schedule of reinforcement. Thus, OSU6162 affects both consummatory and appetitive behaviors of relevance to BED.

OSU6162 reduced sucrose intake in rats that had been trained to consume binge-like quantities of chocolate-flavored sucrose pellets during brief daily access in behavioral chambers. However, OSU6162 did not significantly alter chow intake from the first feeder (before sucrose access). These results suggest that the monoamine-stabilizing effects of OSU6162 suppress overeating of palatable food in a model of BED directly during consumption without affecting prior anticipation. Furthermore, we previously reported that another potential treatment for BED, the selective  $\mu$ -opioid receptor antagonist GSK1521498, decreased bingeing on chocolate-flavored sucrose pellets and increased prior chow intake, suggestive of a decreased anticipatory negative contrast (Giuliano *et al*, 2012). Hence, we suggested that GSK1521498 has a specific effect on the impact of the hedonic value of the palatable food (Giuliano *et al*, 2012), whereas we found no evidence for an effect on anticipatory negative contrast after OSU6162 treatment in the current study. Together, these findings could indicate that opioid mechanisms are more important than dopamine signaling in anticipatory negative contrast or that any dopaminergic influence on this phenomenon is not blocked by OSU6162.

OSU6162 reduced chow intake in the chow/chow group (control group for the sucrose bingeing rats) in contrast to our previous study where GSK1521498 did not produce this effect (Giuliano *et al*, 2012). This indicates that OSU6162 is not generally specific to palatable food and can reduce intake of bland food. This apparent discrepancy to the effects in the sucrose-bingeing rats might be because of the fact that rats in the chow/chow group consumed higher amounts of chow from the first feeder than rats in the chow/sucrose group.

These results, together with the attenuation of sucrose binge-like eating, suggest that OSU6162 moderates intake only if the amounts of food consumed are relatively high, regardless of palatability. This suggestion is further supported by the finding that OSU6162 attenuated binge-like eating ( $34 \pm 2$  kcal sucrose) but did not affect sucrose consumption in the seeking procedures (40 pellets = 0.15 kcal). A similar selective, decreasing effect of OSU6162 only when the baseline is high, has previously been reported for locomotor activity (Natesan *et al*, 2006; Rung *et al*, 2008), alcohol intake (Steenland *et al*, 2012) and striatal L-[ $^{11}\text{C}$ ]DOPA influx rate (Tedroff *et al*, 1998).

The mechanisms behind the monoamine-stabilizing effects of OSU6162 are not fully understood, but there are several plausible explanations for its effects on food intake in the present study. First, intake of palatable food, including sucrose, induces a peak in dopamine levels in the NAc, potentially coding for the motivational value of the food (Bassareo *et al*, 2002; Rada *et al*, 2005; Wilson *et al*, 1995). Thus, based on our previous finding showing that OSU6162 diminishes alcohol-induced NAc dopamine output in alcohol-naive rats (Steenland *et al*, 2012), we speculate that OSU6162 might attenuate this sucrose-induced dopamine peak, reducing the motivation to consume sucrose. Second, it is possible that an OSU6162-induced increase in dopaminergic tone before food presentation might underlie the reduction in food intake. This hypothesis is supported by rodent studies showing that: (1) OSU6162 slowly increases and maintains a stable elevated NAc dopamine output for several hours (Feltmann *et al*, 2016; Steenland *et al*, 2012); (2) optogenetically induced stable NAc dopamine release reduces voluntary sucrose intake (Mikhailova *et al*, 2016); (3) the dopamine-releasing compound lisdexamfetamine attenuates binge-like eating of chocolate (Vickers *et al*, 2015); and (4) the dopamine-releasing compound methylphenidate reduces food consumption in humans and rodents (Davis *et al*, 2012; Thanos *et al*, 2015). Nevertheless, further studies are needed to elucidate the mechanism underlying the effect of OSU6162 to reduce food consumption.

In the cue-controlled sucrose-seeking (second-order schedule of reinforcement) procedure, OSU6162 significantly reduced responding during the first interval (before sucrose consumption), but not the second interval (after sucrose consumption). These results indicate that OSU6162 reduced incentive responding for the reward without affecting its hedonic value, consistent with the theory that the dopamine system is primarily involved in incentive motivation, but not hedonic aspects of food reward (Barbano and Cador, 2007; Berridge, 2007; Kelley *et al*, 2002). This effect is again in contrast to our previous findings with GSK1521498, which reduced seeking during both intervals (Giuliano *et al*, 2012), suggesting that  $\mu$ -receptor antagonism attenuates both the incentive motivational and hedonic impact of palatable food reward (for a review, see (Giuliano and Cottone, 2015)).

The traditional dopamine D2 receptor antagonist raclopride, in contrast to OSU6162, reduced sucrose seeking under all schedules (F11, F115, and second-order), indicating that raclopride has a more general effect on seeking than OSU6162. These results indicate that although D2 dopamine receptors seem to be implicated in the effects of OSU6162 on

cue-controlled sucrose seeking, the mechanism of action might differ between OSU6162 and raclopride. Accordingly, and in contrast to traditional D2 receptor antagonists, OSU6162 does not induce catalepsy at high (80%) striatal D2/D3 occupancies in rodents (Natesan *et al*, 2006; Rung *et al*, 2008) or primates (Ekesbo *et al*, 1999). Moreover, the recent finding that OSU6162 occupies a maximum of 40% of striatal D2/D3 receptors in humans led to the hypothesis that OSU6162 might preferentially antagonize extrasynaptic, relative to synaptic, D2 receptors (Tolboom *et al*, 2014), potentially explaining the lack of extrapyramidal side effects (Johansson *et al*, 2012; Khemiri *et al*, 2015; Kloberg *et al*, 2014).

Intracranial infusion of OSU6162 into the NAc core recapitulated the systemic effects of the compound, resulting in decreased sucrose seeking under the second-order schedule of reinforcement during the first (pre-reward), but not second, interval. The NAc core is an important site mediating the capacity of food-associated conditioned reinforcers to control seeking behavior (Parkinson *et al*, 1999). Specifically, dopamine receptors within the NAc mediate sucrose seeking (Ikemoto and Panksepp, 1996), cue-induced reinstatement of food seeking (Floresco *et al*, 2008), and CS-dependent Pavlovian-Instrumental Transfer (Lex and Hauber, 2008). Whereas both systemic and local treatment with OSU6162 reduced seeking under the second-order schedule of reinforcement, none of the OSU6162 doses tested significantly reduced seeking under the F115 schedule compared with vehicle, suggesting that OSU6162 interacts specifically with the conditioned reinforcing effects of food-associated cues. This might be particularly important in BED, where environmental cues such as commercials or smells often induce food cravings that are alleviated by seeking and ingestion of the food (Curtis and Davis, 2014; Sobik *et al*, 2005).

Infusions of OSU6162 into the DLS did not affect responding under the second-order schedule of reinforcement for sucrose, indicating that OSU6162 does not influence the stimulus-response mechanism located in this striatal domain. These results appear to be in contrast to previous studies showing that the mixed D1/D2 receptor antagonist  $\alpha$ -flupenthixol infused into the DLS reduced habitual cocaine seeking under this schedule (Belin and Everitt, 2008; Murray *et al*, 2012). This discrepancy might reflect a primary involvement of D1 signaling in the DLS, although this suggestion is contradicted by the present lack of effects of systemic SCH23390 administration. Alternatively, if there is a primary involvement of D2 signaling, this discrepancy could also reflect the suggested differences in the mechanisms of action of OSU6162 compared with traditional D2 receptor antagonists. Nevertheless, the involvement of the DLS and the effects of dopaminergic manipulations in the DLS on food seeking under the second-order schedule require further investigation.

In conclusion, the present study demonstrates that the monoamine stabilizer OSU6162 has the ability to reduce both binge-like eating and cue-controlled food seeking in rats. Together with a favorable side-effect profile in humans (Johansson *et al*, 2012; Khemiri *et al*, 2015; Kloberg *et al*, 2014), these results warrant further investigation of the potential of the monoamine stabilizer OSU6162 as a novel treatment for BED.



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