Dopamine and μ-opioid receptor dysregulation in the brains of binge-eating female rats – possible relevance in the psychopathology and treatment of binge-eating disorder

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Abstract
Adult, female rats given irregular, limited access to chocolate develop binge-eating behaviour with normal bodyweight and compulsive/perseverative and impulsive behaviours similar to those in binge-eating disorder. We investigated whether (a) dysregulated central nervous system dopaminergic and opioidergic systems are part of the psychopathology of binge-eating and (b) these neurotransmitter systems may mediate the actions of drugs ameliorating binge-eating disorder psychopathology. Binge-eating produced a 39% reduction of striatal D₁ receptors with 22% and 23% reductions in medial and lateral caudate putamen and a 22% increase of striatal μ-opioid receptors. There was no change in D₁ receptor density in nucleus accumbens, medial prefrontal cortex or dorsolateral frontal cortex, striatal D₂ receptors and dopamine reuptake transporter sites, or μ-opioid receptors in frontal cortex. There were no changes in ligand affinities. The concentrations of monoamines, metabolites and estimates of dopamine (dopamine/dihydroxyphenylacetic acid ratio) and serotonin/5-hydroxyindolacetic acid ratio turnover rates were unchanged in striatum and frontal cortex. However, turnover of dopamine and serotonin in the hypothalamus was increased ~20% and ~15%, respectively. Striatal transmission via D₁ receptors is decreased in binge-eating rats while μ-opioid receptor signalling may be increased. These changes are consistent with the attenuation of binge-eating by lisdexamfetamine, which increases catecholaminergic neurotransmission, and nalmeine, a μ-opioid antagonist.

Keywords
Binge-eating, D₁ and D₂ receptors, dopamine reuptake transporter sites, μ-opioid receptors, striatum

Introduction
Binge-eating disorder (BED) is a psychiatric condition characterised by compulsive episodes of excessive consumption of highly palatable foods (binges). Binge-eating episodes are often accompanied by intense anxiety and guilt, and in the period following these events, a compensatory reduction of food intake may also occur. Unlike other eating disorders the symptoms of BED do not include compensatory purging. BED occurs in 2–5% of the adult population (de Zwaan, 2001; Dingemans et al., 2002; Kessler et al., 2013). Although BED is associated with obesity (body mass index (BMI)>30 kg/m²) (Fairburn et al., 2000; Hudson et al., 2007; Goldschmidt et al., 2011), a significant proportion of subjects (17–30%) have normal body weights (BMI 18.0–25 kg/m²) (Fairburn et al., 2000; Goldschmidt et al., 2011) and ~60% are in the normal weight/overweight categories (BMI 18.5–29.9 kg/m²) (Hudson et al., 2007). A loss of impulse control and compulsivity may be causal in BED (Colles et al., 2008; Galanti et al., 2007; McElroy et al., 2015b; Schag et al., 2013; Wu et al., 2013). Evidence has also emerged to indicate that attention deficit hyperactivity disorder (ADHD) symptomatology is present in a significant proportion of subjects with eating disorders including BED (Cortese et al., 2007; Docet et al., 2012; Fernández-Aranda et al., 2013).

Lisdexamfetamine dimesylate is a d-amphetamine prodrug that is approved to treat ADHD (see Hutson et al., 2014). Lisdexamfetamine has been shown to be effective in managing BED in clinical trials (Citrome, 2015; McElroy et al., 2015a, 2016) and was approved in the USA for the treatment of moderate to severe BED in adults (FDA News Release, 2015). McElroy et al. (2015b) have recently reported that lisdexamfetamine reduced various measures of impulsivity, compulsivity and obsessiveness in subjects with BED, demonstrating its effectiveness in treating the underlying psychopathology of the disorder. Ziauddeen et al. (2013) reported results from a multicentre,
double-blind, placebo-controlled, phase II trial showing that compared with placebo GSK1521498, a novel µ-opioid receptor antagonist, significantly reduced the rewarding effect of various palatable foods and their consumption by subjects with BED.

Based on the work of Corwin (2004), we developed a model of binge-eating in which adult, female rats were given unpredictable, limited access to chocolate (Vickers et al., 2015). These rats showed compulsive and perseverative chocolate consumption when given access to it (Heal et al., 2016) and increased cognitive impulsivity in a chocolate-rewarded delay discounting task (Hutson et al., 2015; Vickers et al., 2017). Thus, the model mimics some of the underlying psychopathology of BED. Lisdexamfetamine markedly reduced chocolate binging by the rats without suppressing the consumption of normal chow; an effect that was partly mediated through indirect activation of α1-adrenoceptors, and possibly also, dopamine D1 receptors Vickers et al. (2015). Consistent with its therapeutic effects on the psychopathology of BED, lisdexamfetamine decreased the compulsive and perseverative chocolate consumption by binge-eating rats (Heal et al., 2016) and abolished their impulsive responding in delay discounting (Hutson et al., 2015; Vickers et al., 2017). Binge-eating behaviour in rats was also dose-dependently reduced by the µ-opioid receptor antagonist, nalmefene (Vickers et al., 2015).

Eating disorders have been linked with dopaminergic dysregulation in the central nervous system (CNS) (Geiger et al., 2009; Johnson and Kenny, 2010; Pothos et al., 1995). Endogenous opioids are important in the motivational aspects of feeding, and these regulatory systems in the CNS may also be dysregulated in eating disorders like BED and bulimia (Bencherif et al., 2005; Davis et al., 2009; Nathan and Bullmore, 2009). Brain regions implicated are the striatum, including the ventral striatum and nucleus accumbens (NAC), which play a pivotal role in motivation, emotional responding and reward processing (Delgado, 2007; Balleine et al., 2007), the prefrontal cortex, which mediates attention cognitive function and decision-making (Arnsten, 2001, 2011; Robbins and Arnsten, 2009), and is anatomically linked to the striatum via the striato-cortical pathway (Arnsten, 2001), and finally, the hypothalamus, which integrates central and peripheral signals that regulate ingestive behaviour and thermogenesis (Clapham, 2012; Harrold et al., 2012; Parker and Bloom, 2012).

When these different strands of research are drawn together, the evidence supports the view that our freely-fed/intermittent, limited chocolate access model exhibits some of the key psychopathology of BED and there is good translational validity between the effects of drugs in this model and clinical outcomes. The findings tentatively indicate that the dopaminergic and µ-opioid receptor systems of the brain may be part of the psychopathology of binge-eating and also may serve as pharmacological mediators of the actions of drugs that are clinically effective in treating the psychopathology of BED. Therefore, the aim of this study was to investigate the dopaminergic and µ-opioidergic systems in the brains of binge-eating and control rats. We measured the density (Bmax) and affinity of D1 and D2 receptors, and dopamine reuptake transporter (DAT) sites in the striatum, and in the light of the findings, performed D1 receptor autoradiography to examine potential changes in receptor density in medial and lateral caudate-putamen (CP-M and CP-L, NAc, medial prefrontal cortex (MPC) and dorsolateral frontal cortex (DFC). We also quantified the concentrations of dopamine, noradrenaline and serotonin (5-HT), the dopamine metabolites, dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA) and the 5-HT metabolite, 5-hydroxyindolacetic acid (5-HIAA), in various brain regions. The density and affinity of µ-opioid receptors in the striatum and prefrontal cortex of binge-eating rats were also measured using [3H]-D-Ala2, N-Me-Phe4, Gly5-ol ([3H]DAMGO) (Ko et al., 2003).

Materials and methods

Animals and environment

The study was conducted in three separate cohorts of lean, female Wistar rats (200–250 g; Charles River, UK) with rats trained to binge eat chocolate or non-binge controls as previously described by Vickers et al. (2015) and in the following section on the establishment of binge-eating.

Cohort 1 was used for the determination of Bmax and affinity by radioligand saturation binding to D1, D2 and DAT sites, and for the measurement of dopamine neurochemistry (n=20 rats for the binge-eating group (10 rats for the binding experiments and 10 rats for the neurochemistry); n=20 rats for the non-binge-eating control group (10 rats for the binding experiments and 10 rats for the neurochemistry)).

Cohort 2 was used for the determination of Bmax and affinity of µ-opioid receptors by radioligand saturation binding analysis (n=10 rats for the binge-eating group; n=10 rats for the non-binge-eating control group).

Cohort 3 was used for the measurement of D1 receptors using the technique of autoradiography (n=8 rats for the binge-eating group; n=8 rats for the non-binge-eating control group).

All rats were singly housed in polypropylene cages (45×28×20 cm) with wire grid floors to enable the food intake of each rat to be recorded. Each cage contained a small amount of soft paper to provide bedding for warmth, environmental enrichment and an area for rats to rest away from the wire grid floor. Polypropylene trays with cage pads were placed beneath each cage to detect any food spillage. Rats were maintained on a reverse phase light-dark cycle (lights off for eight hours from 10:30–18:30) so that the dark phase where food intake was expected to be elevated was during the working day. Rats were kept at a temperature of 21±4°C and 55±20% humidity. The room was illuminated with red light during the dark phase. Rats had free access to standard powdered diet (Harlan Teklad 2018) at all times unless stated. Animals were acclimatised to the facility for a two-week period and were weighed once weekly. All experiments were performed in strict accordance with Home Office Guidelines and licensed under the Animals (Scientific Procedures) Act 1986.

Establishment of binge-eating

Following the two-week acclimatisation period, rats were allocated into treatment groups based on body weight. All rats had 24 h ad libitum access to standard powdered diet (Harlan Teklad 2018; 13.0 kJ/g) and tap water throughout the study. The diet was contained in a glass feeding jar with an aluminium lid (Solmedia Laboratory Suppliers, Romford, Essex, UK). Each lid
had a 3–4 cm hole cut in it to allow access to the food. On binge days, an additional jar containing ground chocolate (Cadbury’s Dairy Milk; 23.44 kJ/g) or an empty jar (control) was placed in each cage for a two-hour period. The diet and chocolate were provided as powdered diet to reduce spillage and make accurate recording of daily food intakes rapid and simple and to control, as far as possible, for differences in physical form which may have affected dietary preference. Food was supplied dry so that grams consumed could be accurately converted to kJ to allow total food intake to be measured. Rats were allowed to binge-eat on Days 1, 2, 4, 6, 7, 9, 12, 14, 15, 18, 23, 25 and 28. Hence, opportunities to binge were provided throughout the entire study duration and the interval between the binge sessions was gradually increased as the training progressed. Once the rats showed robust, reproducible binge-eating, the binge-eating behaviour could be maintained by giving the rats only one or two binge sessions per week. The jars were placed in the cages at ~10:30 h which was at the beginning of the dark phase for the rats. The weights of the jars were recorded before and after the two-hour test session ensuring that the binge-eating sessions took place in the dark phase when rats consume most of their food. The body weight of each rat and its food and water intake were measured on every morning of the study; readings were taken in the final stage of the light phase at ~08:45 h.

When modelling binge-eating, it is important to differentiate between this aberrant behaviour and the normal over-consumption of palatable food. We have developed a model of dietary-induced obesity in female, Wistar rats in which they are given access to a simplified cafeteria diet consisting of high fat chow, ground peanuts and ground milk chocolate (Dickinson et al., 2001; Heal and Jagger, 2005; Vickers et al., 2011). Rats identical to the ones used in the binge-eating model maintained on a cafeteria diet consumed 56 kJ of chocolate in an equivalent two-hour period at the start of the dark phase (unpublished, “in house” data). Rats were only classified as binge-eaters if they consumed >2.0× more food (>112 kJ) in the final three baseline, binge-eating sessions (Heal et al., 2016).

We selected female rats because eating disorders are more prevalent in women than men and female rats are relatively weight-stable when adult, whereas males continue to grow throughout adulthood, which is not physiologically relevant to man. It is possible that binge-eating could be affected by the oestrous cycle. However, we have not noted any day-to-day variance in the responses of female rats in the model. With rats having a four-day oestrous cycle, we have relied on using large group sizes to provide a cross-section of oestrous cycling across the cohorts of rats.

On Day 28, the various groups of binge-eating rats (Cohorts 1–3) were killed by rising CO₂ concentration and their brains removed according to a timed schedule one hour after the final binge session on chocolate. The respective cohorts of non-binge-eating control rats were killed by the same method and their brains removed one hour after presentation of the empty pots.

The body weights of the rats used in these experiments were not different across the three cohorts of animals (Day 28 body weight: Cohort 1 binge-eating rats=300.9±2.3 g (n=20), controls=300.2±1.8 g (n=20); Cohort 2 binge-eating rats=302.2±2.3 g (n=10), controls=306.8±3.1 g (n=10); Cohort 3 binge-eating rats=305.3±3.8 g (n=10), controls=308.1±2.1 g (n=10)).

Radioligand binding experiments

\(D_1\) and \(D_2\) receptor and DAT site binding in striatal homogenates. Whole brains were removed and striata were dissected before being frozen on dry ice. The tissues from each hemisphere were frozen separately and stored at −20°C until the day of the assay.

To perform an eight- or 10-concentration radioligand saturation binding analysis, which is the definitive method for measuring tissue receptor density (Bmax) and affinity (Kd), the whole striatum from each rat was required for each assay. Although this precluded an analysis of \(D_1\), \(D_2\), or DAT sites in sub-regions of the striatum or NAc, it circumvented any artefact potentially arising from pooling the tissue from different rats.

Left striata from 10 of the rats in Cohort 1 were used to measure \(D_1\) receptor density. The tissue was homogenised in 8 mL ice-cold assay buffer (50 mM Tris buffer, pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ (homogenisation and assay buffer)) using a tight fitting glass/Teflon homogeniser (12 strokes, 800 rpm) and the homogenate centrifuged at 40,000 g for 10 min. The membrane pellet was re-suspended in 8 mL ice-cold assay buffer and re-centrifuged at 40,000 g for 10 min. The resulting pellet was re-suspended in ice-cold assay buffer at a tissue concentration of 2.5 mg/mL (1.0 mg wet weight tissue/tube) and used immediately in the assay. All centrifugations were carried out at 4°C. Left striatal membranes (400 µL; equivalent to ~1 mg wet weight of tissue/tube) were incubated with 50 µL [³H]SCH 23390 (eight concentrations; ranging from 0.125–12 nM) and either 50 µL of buffer (total binding) or 50 µL of (-)butaclamol (1 µM; compound for the determination of non-specific binding) for 30 min at 37°C. There was one tube for the determination of total binding and one tube for the determination of non-specific binding, at each radioligand concentration for each animal.

Right striata from 10 of the rats in Cohort 1 were used to measure \(D_2\) receptor density. The tissue was homogenised in 8 mL ice-cold buffer containing 50 mM Tris buffer, pH 7.4 containing 200 mM NaCl, 5 mM KCl using a tight fitting glass/Teflon homogeniser (12 strokes, 800 rpm) and the homogenate centrifuged at 40,000 g for 10 min. The membrane pellet was re-suspended in 8 mL ice-cold buffer and re-centrifuged at 40,000 g for 10 min. This washing procedure was repeated once more. The pellet was re-suspended in 8 mL ice-cold Tris buffer and re-centrifuged at 20,500 g for 10 min. The resulting pellet was re-suspended in ice-cold assay buffer (50 mM Tris buffer, pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂) at a tissue concentration of 6.25 mg/mL (2.5 mg wet weight tissue/tube) and used immediately in the assay. All centrifugations were carried out at 4°C. Right striatal membranes (400 µL; equivalent to ~2 mg wet weight of tissue/tube) were incubated with 50 µL [³H]raclopride (eight concentrations ranging from 0.125–12 nM) and either 50 µL of buffer (total binding) or 50 µL of (-)sulpiride (1 µM; compound for the determination of non-specific binding) for 30 min at 23°C. There was one tube for the determination of total binding and one tube for the determination of non-specific binding, at each radioligand concentration for each animal.

The remaining left or right striata from the other 10 rats in Cohort 1 that had been used for neurochemical fingerprinting experiment were used to determine the density of striatal DAT.
sires. The tissues were homogenised in 8 mL ice-cold assay buffer (50 mM Tris buffer, pH 7.4 containing 200 mM NaCl, 5 mM KCl) using a tight fitting glass/Teflon homogeniser (12 strokes, 800 rpm) and the homogenate centrifuged at 40,000g for 10 min. The membrane pellet was re-suspended in 8 mL ice-cold assay buffer and re-centrifuged at 40,000g for 10 min. This washing procedure was repeated once more. The resulting pellet was re-suspended in ice-cold assay buffer at a tissue concentration of 2.5 mg/mL (1.0 mg wet weight tissue/tube) and used immediately in the assay. All centrifugations were carried out at 4°C. Striatal membranes (200 µL; equivalent to 0.5 mg wet weight of tissue/tube) were incubated with 25 µL of [3H]GBR-12935 at 10 concentrations (ranging from 0.125–20 nM) and either 25 µL of buffer (total binding) or 25 µL of mazindol (1 µM; non-specific binding) for 90 min at 4°C. There was one tube for the determination of total binding and one tube for the determination of non-specific binding at each radioligand concentration for each animal.

Membrane bound radioactivity was recovered by filtration under vacuum through Skatron 11731 filters, pre-soaked in 0.5% polyethyleneimine (PEI). Filters were rapidly washed with ice-cold buffer and radioactivity determined by liquid scintillation counting. Protein concentration (mg/mL) was determined using a Sigma Total Protein Kit - Micro Lowry, Onishi and Barr Modification.

D₁ receptor autoradiography. Since we observed changes in the density of striatal D₁ receptors, but not D₂ receptors or DAT sites, a second experiment was performed to look at D₁ receptors in smaller regions of the caudate-putamen, the NAc and cortical regions of rat brain.

Whole brains were collected from binge-eating rats and their control littermates (n=8/group) before being frozen on dry ice and stored at −20°C. D₁ receptor autoradiography was performed in the CP-M, CP-L, NAc, MPC and DFC. Coronal sections (10 µm) of these samples were cut in a cryostat at −20°C, mounted on gelatine-coated slides, and stored at −80°C for the receptor autoradiographic assay.

Brain sections were preincubated in assay buffer (50 mM Tris-HCl, pH 7.3, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂) for 60 min at room temperature. Brain sections were then incubated separately for 60 min at room temperature in 1 nM [3H]-Sch 23390 in the presence of 100 nM ketanserin to mask binding to 5-HT2A/C receptors. Non-specific binding was determined with 1 µM cis-flupenthixol. After incubation, slides were washed (2×5 min) in ice-cold assay buffer, dipped in ice-cold water, and air-dried (Tarazi et al., 2001, 2008).

Radiolabelled slides and calibrated [3H]-Sch 23390 standards were exposed to Kodak Biomax MR films for five weeks at 4°C. Films were developed and fixed in Kodak D-19 for 5 min at room temperature (Tarazi et al., 2001, 2008). Quantification of images was made using a MCID image analyser and M4 software. A calibration curve was generated from exposed [3H]-microscale standards. Brain regions of interest (MPC, DFC, NAc, CP-M and CP-L) were outlined and their optical density was measured from both brain hemispheres with two sections representing total binding, and two samples representing non-specific binding. Optical density of sampled regions was measured and the amount of ligand bound within each area was calculated as nCi/mg tissue. Mean values of non-specific binding in each region were subtracted from the mean total binding to determine specific binding, which was converted into fmol/mg of radioligand bound.

μ-Opioid receptor binding in homogenates of the striatum and prefrontal cortex. Whole brains were removed and left and right frontal cortices and left and right striata were dissected, pooled and weighed before being frozen on dry ice. The tissue was stored at −80°C until the day of the assay.

The pooled left and right frontal cortices or pooled left and right striata were homogenised in 10 mL ice-cold assay buffer (50 mM Tris buffer, pH 7.4) using a tight-fitting glass/Teflon homogeniser (12 strokes, 800 rpm) and the homogenate centrifuged at 20,500g for 10 min. The membrane pellet was re-suspended in 10 mL ice-cold assay buffer and re-centrifuged at 20,500g for 10 min. This washing procedure was repeated twice more. The resulting pellet was re-suspended in ice-cold assay buffer at a tissue concentration of 2.5 mg/mL (1.0 mg wet weight tissue/tube) and used immediately in the assay. All centrifugations were carried out at 4°C. Pooled striatal membranes (400 µL; equivalent to 4 mg wet weight of tissue/tube) were incubated with 50 µL [3H]-DAMGO (eight concentrations; ranging from 0.625–12 nM) and either 50 µL of buffer (total binding) or 50 µL of (-)naloxone (50 µM; compound for the determination of non-specific binding) for 90 min at 25°C. There was one tube for the determination of total binding and one tube for the determination of non-specific binding, at each radioligand concentration for each animal.

Membrane-bound radioactivity was recovered by filtration under vacuum through Skatron 11731 filters, pre-soaked in 0.5% PEI. Filters were rapidly washed with ice-cold buffer and radioactivity determined by liquid scintillation counting. Protein concentration (mg/mL) was determined using a Sigma Total Protein Kit - Micro Lowry, Onishi and Barr Modification.

Neurochemical analysis

Brains were removed onto ice and the striata (including both dorsal and ventral striatum), prefrontal cortex and hypothalamus rapidly dissected and snap frozen in liquid nitrogen. This procedure took no more than 120 s to prevent the effects of rapid post-mortem changes in monoamines and their metabolites, particularly 3-MT. The frozen tissues were stored in individual plastic microcentrifuge tubes at −80°C.

The dissected tissues were weighed and homogenised prior to determination by high performance liquid chromatography (HPLC) coupled to amperometric detection of monoamine neurotransmitters (noradrenaline, dopamine and 5-HT) and metabolites (5-HIAA, DOPAC and HVA) in prefrontal cortex, hypothalamus and striatum as described by Cheetham et al. (1996). Concentrations of these monoamines and metabolites were calculated by reference to an internal standard, dihydroxybenzylamine (DHB). The concentration of the dopamine metabolite, 3-MT, was measured in striatum by HPLC coupled with coulometric electrochemical detection as previously described by Heal et al. (1990).
Drugs
The radioligands, [3H]SCH 23390, [3H]raclopride, [3H]GBR-12935 and [3H]DAMGO, were obtained from Perkin Elmer. (-)-Naloxone and all other reagents were of analytical grade purity and were obtained from SLS (Nottingham, UK), Fisher (Loughborough, UK) or Sigma-Aldrich (Poole, UK).

Data and statistical analysis

Binge-eating. During the establishment of binge-eating behaviour, the binge-eating group was compared to the control group. Body weight was analysed by analysis of covariance with Day 1 body weight as a covariate. During each binge-eating session, the following three measures were analysed, i.e. (a) chocolate intake (kJ) during the two-hour session, (b) total food intake (kJ) during the two-hour session and (c) total food intake (kJ) and water intake (g) during the 24 h including this two-hour session. Comparisons were by Student’s t-test.

Radioligand binding. Values for the equilibrium dissociation constant (Kd) and the maximum density of binding sites (Bmax) were determined by non-linear regression analysis fitted to a one-site binding model. Kd data were log-transformed and Bmax data were square root-transformed. Statistical analysis was by Student’s t-test. Means and standard error of the mean (SEM) were calculated by back-transformation (anti-log) of the results of the statistical analysis of the log-transformed Kd and square root-transformed Bmax data. In all analyses, p<0.05 was considered to be statistically significant. The chance of a false positive is 5% for each compound for each variable. All tests were carried out as two-sided tests.

Neurochemical analysis

Analysis was by Student’s t-test of log transformed data. Means and SEM were calculated by back-transformation (anti-log) of the results of the statistical analysis of the log-transformed data. A value of p<0.05 was the level accepted for statistical difference. The chance of a false positive is 5% for each compound for each variable. All tests were carried out as two-sided tests.

Statistical powering

Previous experience with the binge-eating rat model has taught that n=8 rats/group is required to achieve statistical significance (p<0.05). The size of groups of naïve rats used to establish binge-eating was ≥10 rats because in our experience ~5% of rats do not develop binge-eating behaviour in this paradigm.

Results

Establishment of binge-eating behaviour

When freely-fed, lean, female Wistar rats were given unpredictable intermittent two-hour access to chocolate over a period of 28 days, they gradually developed a characteristic pattern of hyperphagia on the binge session days, with significantly greater food intake than control animals, followed by substantial reductions of food intake in the days immediately after these sessions. Results for Cohort 1 are shown in Figure 1(a). The cumulative food intake results (kJ/week) showed small increases in the weekly consumption of food in the binge-eating rats compared with the non-binge controls in the first and second weeks of training, but food intake returned to control levels in the third and fourth weeks. Results for Cohort 1 are shown in Figure 1(b). We have observed this effect previously in groups of binge-eating rats, e.g. Vickers et al. (2015). As shown in Figure 1(a), as the rats develop a taste for chocolate over the first one or two weeks of their consumption of this palatable food increases. However, at this time, they do not show the consistent, marked reductions in chow intake on the subsequent non-binge days. As a consequence, their overall calorie intake is higher than the non-binge controls in this early phase (p<0.05 for Week 1 and p<0.05 for Week 2). As binge-eating becomes established, the reductions in chow consumption on the days following a chocolate bingeing sessions normalises the binge-eating rats’ weekly calorie intake. Similar acquisition profiles of binge-eating were observed for the rats in Cohorts 2 and 3 (data not shown).

There were some minor fluctuations in the water intake of the rats but, overall, this remained fairly constant throughout the 28-day training period and was not significantly different from the control group (data not shown).

The body weights of the binge-eating rats showed a gradual increase over the 28-day binge-eating training period, but did not differ significantly from the control group (Figure 2).

The consumption of chocolate and normal chow in the binge-eating sessions on Day 28 for the three cohorts of rats used in the experiments is reported in Table 1. During the two-hour sessions, the binge-eating cohorts of rats consumed ~4× as much food as the non-bingeing controls. Moreover, chocolate accounted for >90% of the energy (kJ) consumed by the binge-eating rats in these sessions.

Radioligand-receptor binding in the brains of rats with acquired binge-eating behaviour

D1 and D2 receptor and DAT binding in the striatum. When brains taken from binge-eating rats were compared with those from the control group, the density of striatal D1 receptors labelled with [3H]SCH 23390 was significantly (p<0.01) reduced by 38.7% in the binge-eating group (Figure 3). There was no significant change in the affinity of the D1 receptors in the binge-eating group compared with controls (Table 2). On the other hand, binge-eating did not alter the density or affinity of D2 receptors in the striatum compared with controls (Figure 3 and Table 2).

Binge-eating did not alter either the density of striatal DAT sites (Figure 3) or the affinity of DAT sites for [3H]GBR-12935 (Table 2) in the brains of the rats when compared against the results from the non-binge control group.

Autoradiographic measurement of D1 receptor density in various brain regions. Dopamine D1 receptors were significantly (p<0.05) reduced by 22% and 23% respectively in the CP-M and CP-L in the binge-eating rats compared with the normal controls (Figure 4(b)). No significant difference in D1 receptor density between the binge-eating rats and the controls was found in the NAc, MPC or DFC (Figure 4(b)).
µ-Opioid receptor binding. When brains taken from the binge-eating cohort of rats were compared with those from the non-binge control group, the density of striatal µ-opioid receptors was significantly ($p<0.05$) increased by 29% in the binge-eating group (Figure 5). There was no significant change in the affinity of the µ-opioid receptors (Table 2).

In the frontal cortices taken from the brains of binge-eating rats, the density of µ-opioid receptors was not altered (Figure 5); neither was the affinity of µ-opioid receptors for [³H]DAMGO (Table 2).

**Neurochemical analysis**

When the binge-eating group was compared with the non-binge-eating control group, there were no significant differences between the concentrations of the monoamine neurotransmitters, dopamine, noradrenaline and 5-HT, in the striatum, frontal cortex or hypothalamus (Table 3). The concentrations of the three monoamine neurotransmitters in the different brain regions varied markedly. The dopamine concentration was ~12,000 ng/g wet...
weight in striatum, 500–600 ng/g wet weight in hypothalamus and
50–60 ng/g wet weight in the prefrontal cortex. The rank order for
the concentrations of noradrenaline was hypothalamus>prefrontal
cortex>striatum, and for 5-HT, it was hypothalamus>prefrontal
cortex>striatum. Dopamine showed the greatest variation across
brain regions and 5-HT the least.

Binge-eating behaviour did not significantly alter the concentra-
tions of dopamine’s major metabolites, 3-MT, DOPAC and
HVA, in the striatum, or DOPAC and HVA in the prefrontal cor-
tex and hypothalamus (Table 3).

The dopamine/DOPAC ratio, which is an index of dopamine
turnover, i.e. the net of dopamine synthesis, release and

Table 1. Summary of the food intake results on the final Day 28 binge-eating session one hour prior to termination of the rats.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Food available</th>
<th>Chow consumption in the 2 h binge-eating session (kJ)</th>
<th>Chocolate consumption in the 2 h binge-eating session (kJ)</th>
<th>Total food consumption in the 2 h binge-eating session (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neurochemistry (Cohort 1)</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Non-binge-eating control cohort</td>
<td>Normal chow</td>
<td>39.4±7.1</td>
<td>NA</td>
<td>39.4±7.1</td>
</tr>
<tr>
<td>(n=10)</td>
<td>Binge-eating cohort</td>
<td>12.5±2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>173.2±16.0</td>
<td>185.7±14.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>(n=10)</td>
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<td><strong>D1, D2 and DAT binding (Cohort 1)</strong></td>
<td></td>
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<tr>
<td>Non-binge-eating control cohort</td>
<td>Normal chow</td>
<td>40.2±4.2</td>
<td>NA</td>
<td>40.2±4.2</td>
</tr>
<tr>
<td>(n=10)</td>
<td>Binge-eating cohort</td>
<td>9.1±3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>155.4±20.9</td>
<td>164.5±20.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>(n=10)</td>
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<tr>
<td><strong>μ-Opioid receptor binding (Cohort 2)</strong></td>
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<tr>
<td>Non-binge-eating control cohort</td>
<td>Normal chow</td>
<td>47.3±7.4</td>
<td>NA</td>
<td>47.3±7.4</td>
</tr>
<tr>
<td>(n=10)</td>
<td>Binge-eating cohort</td>
<td>7.2±7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>193.4±18.3</td>
<td>200.5±18.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>(n=10)</td>
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<tr>
<td><strong>D1 receptor autoradiography (Cohort 3)</strong></td>
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<tr>
<td>Non-binge-eating control cohort</td>
<td>Normal chow</td>
<td>34.2±5.8</td>
<td>NA</td>
<td>34.2±5.8</td>
</tr>
<tr>
<td>(n=8)</td>
<td>Binge-eating cohort</td>
<td>5.3±1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>174.9±18.7</td>
<td>180.2±18.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n=8)</td>
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</table>

DAT: dopamine reuptake transporter; NA: not applicable.

Results are means±standard error of the mean (SEM) with n values in parentheses. Analysis was by unpaired t tests. Significant differences compared to non-binge-eating controls: <sup>a</sup>p<0.01, <sup>b</sup>p<0.001.
metabolism, was significantly reduced by 18.5% (p<0.05) in the hypothalamus, indicating that the rate of dopamine turnover was increased in the binge-eating group (Table 3). The dopamine/DOPAC ratios were not significantly altered in either the striatum or prefrontal cortex (Table 3).

Binge-eating had no effect on the concentration of 5-HT's major metabolite, 5-HIAA, in the striatum, prefrontal cortex or hypothalamus (Table 3). However, the 5-HT/5-HIAA ratio, which is an index of 5-HT turnover, was significantly decreased by 15.1% (p<0.01) in the hypothalamus, of the binge-eating rats indicating that 5-HT turnover was increased. The 5-HT/5-HIAA ratios were not significantly altered in either the striatum or prefrontal cortex (Table 3).

Discussion

We have recently reported that binge-eating rats exhibit compulsive and perseverative responding in a novel food reward/punished conflict model (Heal et al., 2016) and cognitive impulsivity and intolerance of deferred gratification in delay-discounting (Hutson et al., 2015; Vickers et al., 2017). Lisdexamfetamine, which is a clinically effective treatment for BED, significantly reduced both the compulsive/perseverative and impulsive behaviours of binge-eating rats (Heal et al., 2016; Hutson et al., 2015; Vickers et al., 2017). These results are consistent with the clinical findings that in adults with BED lisdexamfetamine attenuated the obsessive-compulsive and impulsive features of the disorder (McElroy et al., 2015b).

The aims of the present study were to investigate whether dopaminergic or µ-opioidergic systems were dysregulated in the brains of binge-eating rats and, if these systems were dysregulated, whether the pharmacological actions of drugs that are effective in treating BED have the potential to counteract these neurochemical abnormalities. Other groups have previously investigated possible changes in D1, D2 and µ-opioid receptors and DAT sites in various brain regions when rats have been
subjected to alternating bouts of food restriction followed by access to sucrose or glucose solutions, or sweetened/fat mixtures (Bello et al., 2002, 2003, 2011; Colantuoni et al., 2001), but because these models rely on food restriction, they are very different from the freely-fed/intermittent, limited chocolate access model that we have developed (Vickers et al., 2015). Moreover, it has not been established whether rats from these food-restriction/palatable food access models display the compulsive/perseverative and impulsive behaviours linked to BED that we have observed in our binge-eating model (Heal et al., 2016; Hutson et al., 2015; Vickers et al., 2015, 2017). Also, because drugs that are efficacious or ineffective in treating BED have not been studied in these food-restriction/palatable food access models, their translational validity for identifying mechanisms relevant to pharmacological treatment is unknown.

The neurochemical changes produced by binge-eating are summarised in Table 4. The neurotransmitters, dopamine and 5-HT, were selected because the former is an important mediator in cognition, motivation and reward (Arnsten, 2001, 2011; Baik, 2013; Robbins and Arnsten, 2009), and both monoamines are important signals in the hypothalamic control of food intake (Halford et al., 2007; Harrold et al., 2012; Meguid et al., 2000). There is also evidence to show that central dopaminergic systems are dysregulated in human eating disorders (Wang et al., 2001, 2009, 2011) and in animal models of eating disorders (Bello et al., 2002; Geiger et al., 2008, 2009; Johnson and Kenny, 2010; Pothos et al., 1995).

Binge-eating reduced the density of D1 receptors in the caudate putamen (including the medial and lateral regions), but not NAc. Striatal DAT sites, which terminate dopaminergic neuronal signalling thereby regulating synaptic dopamine concentrations, and the dopamine/DOPAC ratio, an index of dopamine turnover rate (Cheng and Wooten, 1982; Hallman and Jonsson, 1984), were both unchanged. When these results are taken together, they indicate that striatal dopaminergic signalling in the caudate putamen via D1 receptors may be attenuated. Palatable foods are highly rewarding and access to them increases dopamine efflux in the NAc (Geiger et al., 2009; Hajnal et al., 2004; Rada et al.,

Figure 4. (a) Diagram of the brain regions of binge-eating rats used for D1 receptor autoradiography. Coronal sections (10 µm) of these samples were cut in a cryostat at −20°C, mounted on gelatine-coated, and stored at −80°C for receptor autoradiographic assay. The brain regions illustrated are medial prefrontal cortex (MPC), dorsolateral frontal cortex (DFC), nucleus accumbens core (NAc), medial caudate putamen (CP-M) and lateral caudate putamen (CP-L). (b) D1 receptor autoradiography in sub-regions of CP-M and CP-L, MPC and DFC, and NAc of brains taken from groups of binge-eating rats and non-binge-eating controls. Data are means+standard error of the mean (SEM) (n=8/group). Statistical analysis was by Student’s t-test. Significantly different from controls: *p<0.05.

Figure 5. Radioligand-receptor binding in the brains of rats with established binge-eating behaviour: density (Bmax) of µ-opioid receptors in the striatum and frontal cortex. Values were determined by non-linear regression analysis fitted to a one-site binding model and data were square root-transformed. Data are means+standard error of the mean (SEM) (n=10/group) and were calculated by back-transformation (anti-log) of the results of the statistical analysis of the square root-transformed data. Statistical analysis was by Student’s t-test. Significantly different from non-binge-eating controls: *p<0.05.
and it has been reported that obesity is linked to a decrement in the rewarding property of food (Geiger et al., 2009). Therefore, our observation of reduced striatal D1 signalling in binge-eating rats is in agreement with this hypothesis. No deficit in D1 receptor density was found in the NAc, MPC or DFC, indicating the regional specificity of the change in these receptors produced by binge-eating.

In contrast, the density and affinity of striatal D2 receptors were unaltered in the brains of the binge-eating rats. Johnson and Kenny (2010) investigated the effect of exposure to a palatable diet on the development of compulsive eating behaviour and obesity. The rats with extended access to a cafeteria diet had a reduced density of D2 receptors in the striatum irrespective of whether or not they were obese. The normal weight rats with restricted access to a cafeteria diet, which is the group with the closest similarity to our binge-eating rats, had no reduction in striatal D2 receptor density. The latter finding is in agreement with our results. In this study, we have only investigated the effects of binge-eating on D2 receptors in the striatum and it must be emphasised our results do not exclude the possibility that D2 receptors may be altered in other brain regions.

The neuroanatomy of the prefrontal cortex is very different from the striatum with most neuronally released dopamine being cleared from the extracellular space by reuptake into noradrenergic neurones via noradrenaline reuptake transporter sites (see Heal et al., 2012). The lack of change in D1 receptors in the prefrontal cortex in the binge-eating rats was accompanied by no changes in dopamine turnover rate or the concentrations of DOPAC and HVA. Together these results indicate that binge-eating does not alter D1 receptor-mediated signalling in the prefrontal cortex.

The effect of binge-eating on µ-opioid receptor binding was investigated because it has been proposed that endogenous opioid systems in the CNS are highly important in the motivation to eat (DiFeliceantonio et al., 2012; Giuliano et al., 2012; Mena et al., 2011, 2013; Nathan et al., 2012; Peciña et al., 2006) and in
the rewarding aspects of the consumption of highly palatable foods (Colantuoni et al., 2001; DiFeliceantonio et al., 2012; Giuliano et al., 2012; Katsura and Taha, 2014; Mena et al., 2011; Nathan et al., 2012; Pecía et al., 2006). Furthermore, it has been hypothesised that central opioid receptor-mediated neurotransmission may become dysregulated in eating disorders including BED (Cambridge et al., 2013; Davis et al., 2009). Additionally, and consistent with these observations, binge-eating behaviour in the current animal model was attenuated by the opioid receptor antagonist nalmefene (Vickers et al., 2015).

When compared with the non-binge-eating group, the binge-eating rats had a highly significant, ~29% increase in the density of striatal µ-opioid receptors with no change in ligand affinity. These findings suggest that binge-eating may be associated with a substantially increased reward (“liking”) response to chocolate consumption mediated through increased µ-opioid receptor signalling in the striatum which is in agreement with work by Davis et al. (2009) and DiFeliceantonio et al. (2012). Up-regulation of striatal µ-opioid receptors in binge-eating rats is also consistent with reports that feeding in satiated rats can be elicited by microinjections of µ-opioid agonists into the NAc (Pecía et al., 2006), dorsal striatum (DiFeliceantonio et al., 2012; Pecía et al., 2006), and ventromedial prefrontal cortex (Mena et al., 2011, 2013). In contrast, binge-eating did not alter the density or affinity of µ-opioid receptors in the prefrontal cortex demonstrating that binge-eating does not produce a generalised increase in µ-opioid receptors in the CNS.

Although the concentrations of dopamine, DOPAC and HVA were unchanged in the hypothalamus, the dopamine turnover rate was increased by a ~20%. This effect was accompanied by a ~15% increase in 5-HT turnover (5-HT/5-HIAA ratio). Dopamine and 5-HT are important signals in the complex matrix of pathways in the hypothalamus that regulate physiological food intake (see Halford et al., 2007; Meguid et al., 2000). It is well established that drugs which increase hypothalamic dopamine or 5-HT efflux suppress feeding behaviour (Halford et al., 2007; Parada et al., 1988a,b). Since the binge-eating rats were euthanised one hour after they had been given a two-hour chocolate binge, the observation that dopamine and 5-HT turnover in the hypothalamus were increased in this overfed state would be predicted to have a suppressant action on appetite and food intake and, therefore, this mechanism may in part be responsible for the decreased consumption of normal chow in the day immediately following the binge session. Simansky et al. (1985) subjected rats to a four-hour schedule of standard chow intake. After rats had been given access to food, they found that hypothalamic dopamine turnover was increased, which is in agreement with our findings. Also consistent with our results, these researchers found no change in dopamine turnover in striatum, olfactory tubercle, amygdala-pyramidal lobe or NAc albeit in non-bingeing animals (Simansky et al., 1985).

The results reveal that the psychopathology of binge-eating may at least in part be due to hypoactive D1 receptor and hyperactive µ-opioid receptor signalling in the striatum although a potential role for other receptors, e.g. α1-adrenoceptors, cannot be excluded. Currently, we are exploring possible dysregulation of brain GABAergic systems in binge-eating that may have a role in modulating the function of all of these other neurotransmitters.

When considering the cause of the hypoactive D1 receptor and hyperactive µ-opioid receptor signalling in the striatum, there are two interconnected factors which are potential drivers of these neurochemical changes, i.e. binge-eating behaviour and the repeated consumption of palatable foods. While there is evidence that underactive reward systems contribute to the overconsumption of palatable foods and obesity, our view is it is not the predominant driver of changes on D1 and µ-opioid receptors. First, because our rats exhibit binge-eating behaviour, but are normal weight, we can discount obesity as being a causal factor in the neurochemical changes that we have found. Second, in the delay-discounting experiment (Hutson et al., 2015; Vickers et al., 2017), the non-binge controls were freely-fed, but were given chocolate/sucrose pellets as food rewards in the delay-discounting training and test sessions. The non-binge controls consumed 50 pellets/session/day (2.25 g/session/day) during delay-discounting training and a mean of 60 pellets in the test sessions (2.7 g/session/day). Although chocolate/sucrose pellets were regularly consumed by the binge-eating and control groups of rats over a prolonged period and comprised a substantial portion of the rats' daily food intake, it was the binge-eating rats that showed marked impulsivity in the delay-discounting test compared with the non-binge controls. Furthermore, administration of lisdexamfetamine prevented the impulsive responding of the binge-eating rats in the test, but did not reduce the number of chocolate/sucrose pellets consumed by them showing that its effect was directed at impulsivity and not the drive to consume palatable food. Our hypothesis that binge-eating is causal in the striatal dysregulation of D1 receptor and µ-opioid receptor signalling could be investigated by studying these systems in freely-fed, normal weight, binge-eating rats with intermittent access to chocolate and comparing the results to freely-fed, dietary-induced obese rats with unlimited access to this palatable food.

Lisdexamfetamine, which is a d-amphetamine prodrug, has been shown to be effective in reducing binge-eating in human BED (Citrome, 2015; McElroy et al., 2015a,b, 2016) and in rats (Vickers et al., 2015). Furthermore, this prodrug reduces the core symptoms of impulsivity and compulsivity in adults with BED (McElroy et al., 2015b) and in our rat model (Heal et al., 2016; Hutson et al., 2015; Vickers et al., 2017). Lisdexamfetamine produces large and prolonged increases in dopamine efflux in the striata of rats in vivo (Rowley et al., 2012, 2014) and this pharmacological action would counteract the deficit in striatal D1 receptor-mediated signalling. Furthermore, Vickers et al. (2015) observed that the prevention of chocolate bingeing by lisdexamfetamine was partially reversed by D1 receptor antagonist pretreatment. It must be emphasised that this is not the only pharmacological mechanism because lisdexamfetamine’s effect on chocolate bingeing was also partially prevented by the α1-adrenoceptor agonist, prazocin (Vickers et al., 2015).

Nalmefene is an opioid receptor antagonist that produces prolonged blockade of µ-opioid receptors in the human brain (Ingman et al., 2005) and blockade of µ-opioid receptors by various antagonists including nalmefene has been reported to attenuate binge-eating behaviour in preclinical studies (Cottone et al., 2008; Giuliano et al., 2012; Nathan and Bullmore, 2009; Vickers et al., 2015). Nalmefene has also been reported to decrease food intake with its greatest effect on the consumption of highly palatable foods (Yeomans et al., 1990). Furthermore, results of a multicentre, double-blind, placebo-controlled, phase II trial of the µ-opioid receptor antagonist, GSK1521498, in subjects with BED showed that GSK1521498 significantly reduced the desirability response...
of BED subjects to high fat and high sugar dairy products and it decreased their consumption in a buffet meal setting (Ziauddeen et al., 2013). Since µ-opioid antagonists would be predicted to reduce excessive striatal µ-opioid receptor signalling, the preclinical and clinical findings are consistent with a dysregulation of this system having a role in the pathology of binge-eating.

Sibutramine, which is a noradrenaline and 5-HT reuptake inhibitor, non-selectively reduced the consumption of chocolate and chow by binge-eating rats (Vickers et al., 2015). This result is consistent not only with its profile as an anti-obesity drug rather than a treatment for BED, but also fits with our finding that this pharmacology would be unlikely to counteract either the underactive D1 receptor signalling or the overactive µ-opioid receptor signalling present in binge-eating.

In summary, we have identified specific dysregulation of D1 and µ-opioid receptor systems in the striatum in a rat model of binge-eating. These neurochemical changes are consistent with a maladaptive µ-opioid receptor system in the striatum in a rat model of binge-eating. Furthermore, preclinical and clinical experience with drugs to treat binge-eating are consistent with efficacy being linked at least in part to their actions to increase D1 receptor signalling or decrease µ-opioid receptor signalling in the striatum.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: DJ Heal and SC Cheetham are fulltime employees and major shareholders in RenaSci Ltd. J Gosden M Hallam and MR Prow are fulltime employees of RenaSci Ltd. P Hutson is an employee of Shire and holds stock/stock options in Shire. F Tarazi and YK Choi declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References


Baik JH (2013) Dopamine signaling in reward-related behaviors. Front Neural Circuits 7: 152.


Clare JG (2004) Binge-type eating induced by limited access in rats does not require energy restriction on the previous day. Appetite 42: 139–142.


