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The galanin receptor agonist, galnon, attenuates cocaine-induced reinstatement and dopamine overflow in the frontal cortex

Yvonne E. Ogbonmwan[1]*, Natale R. Sciolino[2]*, Jessica L. Groves-Chapman[2], Kimberly G. Freeman[3], Jason P. Schroeder[1], Gaylen L. Edwards[3], Philip V. Holmes[2,4] & David Weinshenker[1]

Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA[1], Neuroscience Program, Biomedical and Health Sciences Institute, University of Georgia, Athens, GA, USA[2], Department of Physiology and Pharmacology, University of Georgia, Athens, GA, USA[3] and Department of Psychology, University of Georgia, Athens, GA, USA[4]

ABSTRACT

Relapse represents one of the most significant problems in the long-term treatment of drug addiction. Cocaine blocks plasma membrane monoamine transporters and increases dopamine (DA) overflow in the brain, and DA is critical for the motivational and primary reinforcing effects of the drug as well as cocaine-primed reinstatement of cocaine seeking in rats, a model of relapse. Thus, modulators of the DA system may be effective for the treatment of cocaine dependence. The endogenous neuropeptide galanin inhibits DA transmission, and both galanin and the synthetic galanin receptor agonist, galnon, interfere with some rewarding properties of cocaine. The purpose of this study was to further assess the effects of galnon on cocaine-induced behaviors and neurochemistry in rats. We found that galnon attenuated cocaine-induced motor activity, reinstatement and DA overflow in the frontal cortex at a dose that did not reduce baseline motor activity, stable self-administration of cocaine, baseline extracellular DA levels or cocaine-induced DA overflow in the nucleus accumbens (NAc). Similar to cocaine, galnon had no effect on stable food self-administration but reduced food-primed reinstatement. These results indicate that galnon can diminish cocaine-induced hyperactivity and relapse-like behavior, possibly in part by modulating DA transmission in the frontal cortex.

Keywords Cocaine, cortex, dopamine, galanin, galnon, reinstatement.

Correspondence to: David Weinshenker, Department of Human Genetics, Emory University School of Medicine, Whitehead 301, 615 Michael St, Atlanta, GA 30322, USA. E-mail: dweinshenker@genetics.emory.edu

INTRODUCTION

Cocaine addiction, defined as intense craving and compulsive use of cocaine despite negative consequences, is a major problem in our society. Current treatment options (e.g. behavioral therapy) are not very effective, and there are currently no Food and Drug Administration-approved or generally accepted pharmacotherapies for cocaine dependence. Because relapse is a major obstacle in the treatment of drug addiction, one promising strategy is to develop therapies that block the ability of triggers such as the drug itself, drug-associated cues or stress to precipitate relapse (Sinha 2009; Bossert *et al.* 2013). Cocaine blocks plasma membrane monoamine transporters, which in turn increases extracellular levels of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) in the brain. It is well established that DA is critical for mediating the motivational and reinforcing effects of cocaine, and blocking its transmission attenuates drug-seeking behavior during reinstatement, a model of relapse (Schmidt *et al.* 2005; Bossert *et al.* 2013). NE and 5-HT play modulatory roles and are also implicated in reinstatement.

One intriguing molecule that modulates DA transmission and behavioral responses to addictive drugs is the neuropeptide galanin. Galanin and its G protein-coupled receptors (GPCR; GalR1-3) are expressed within the mesocorticolimbic circuit implicated in drug addiction

*These authors contributed equally to the work.

(Melander *et al.* 1986; Hawes & Picciotto 2004). Galanin receptors can also be activated by galnon, a synthetic non-peptide agonist that crosses the blood-brain barrier and binds to GalR1 and GalR2 (Saar *et al.* 2002). In general, galanin reduces DA release (Melander *et al.* 1987; Nordstrom *et al.* 1987; Jansson *et al.* 1989; Tsuda *et al.* 1998), and both galanin and galnon attenuate responses to drugs of abuse (Picciotto 2008). For example, intracerebroventricular administration of galanin attenuates morphine conditioned place preference (Zachariou, Parikh & Picciotto 1999), and opiate withdrawal is decreased by galanin overexpression or galnon and exacerbated by genetic knockout of galanin or GalR1 (Zachariou *et al.* 2003; Holmes *et al.* 2012). Moreover, galanin knockout mice are hypersensitive to morphine and cocaine conditioned place preference, and these phenotypes are abolished by galnon administration (Narasimhaiah, Kamens & Picciotto 2009). By contrast, complete knockout of galanin has minimal effect on cocaine self-administration in mice using several doses and schedules of reinforcement (Narasimhaiah *et al.* 2009; Brabant, Kuschpel & Picciotto 2010). However, several important aspects of drug responses have not been examined after galanin receptor activation, including relapse-like behavior and DA transmission. In this study, we examined the consequences of galnon administration on drug seeking during the maintenance and reinstatement phases of operant cocaine self-administration, as well as on cocaine-induced changes in DA overflow in the frontal cortex and the nucleus accumbens (NAc).

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats (151–175 g) were used for all experiments. Self-administration experiments were conducted at Emory University ($n = 54$) rats purchased from Charles River, Wilmington, MA, USA) and motor activity and microdialysis experiments were conducted at the University of Georgia ($n = 107$, rats purchased from Harlan, Prattville, AL, USA). Rats were individually housed in clear polycarbonate cages (50 × 30 × 30 cm) and given *ad libitum* access to food and water unless otherwise specified in a temperature- and humidity-controlled animal facility and maintained on a 12-hour reverse light/dark cycle. Testing occurred during the dark phase with background noise emitted by a white noise generator. Animals were allowed to acclimate to the vivarium for 1 week prior to surgery. Rats were treated in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Experiments were approved by Emory and University of Georgia Institutional Animal Care and Use Committees.

Drugs

Cocaine was obtained from the National Institute of Drug Abuse (NIDA) and dissolved in 0.9% physiological saline. Galnon (Bachem, Torrance, CA, USA) was sonicated in 1% dimethyl sulfoxide. Galnon is a non-selective galanin receptor agonist ($K_d = 1$ μM) with a half-life of approximately 60 minutes (Bartfai & Wang 2013). Galnon was injected 20–30 minutes before cocaine based on effective pre-treatment schedules in rodents (Lu *et al.* 2005a; Rajarao *et al.* 2007; Narasimhaiah *et al.* 2009; Jackson *et al.* 2011). All injections were performed in a volume of 1 ml/kg.

Stereotaxic cannulation surgery

Rats were anesthetized with isoflurane administered by vaporizer with oxygen delivered through a nose cone, and the surgical site was shaved and cleaned with Betadine. Rats were positioned in a stereotaxic apparatus, a longitudinal incision was made along the scalp, and three screws were anchored to the skull. Unilateral guide cannulae (MAB 6.6.IC; SciPro, Sanborn, NY, USA) were implanted targeting the frontal cortex (3.2 mm anterior, 2.2 mm lateral, −1.5 mm ventral, relative to bregma) or NAc shell (1.7 mm anterior, 0.6 mm lateral, −6.5 mm ventral) according to the atlas of Paxinos & Watson (1998). Cannulae and a plastic guard were fixed to the skull using fast-drying epoxy. Rats received banamine (2.5 mg/kg, s.c.) immediately and 24 hours after surgery. At the end of experiments, 4 μl of dye (2 mg/ml India ink) was injected to verify cannulae placement, determined by inspection of dye and termination of the cannulae track in 12 μm Nissl-stained coronal cryosections.

Motor activity

Rats were placed in the center of an open arena (44.5 × 44 × 30 cm; ENV 515–16, Med Associates, St. Albans, VT, USA) that contained fresh bedding as described (Sciolino, Dishman & Holmes 2012). Infrared beam breaks were used to track the coordinate position and movement of the rat (ENV-520, Med Associates) every 50 milliseconds using default settings (SOF-810). Behavior was recorded continuously during the 140-minute test, pausing in 20-minute intervals for all rats to collect a dialysis sample or to inject drug. Behavior was sampled for 40 minutes before treatment with vehicle or galnon (2, 5 or 10 mg/kg i.p.; phase I, 'habituation'), collected for another 20 minutes before cocaine (10 mg/kg i.p.; phase II, 'pre-treatment'), and recorded for 60 minutes post-cocaine (phase III, 'cocaine'). Ambulation was initiated after three beam breaks and measured as ambulatory distance traveled in continuous (< 500 milliseconds without rest) movement outside a 2 × 2 area of x–y beams. Non-ambulatory movement was defined as

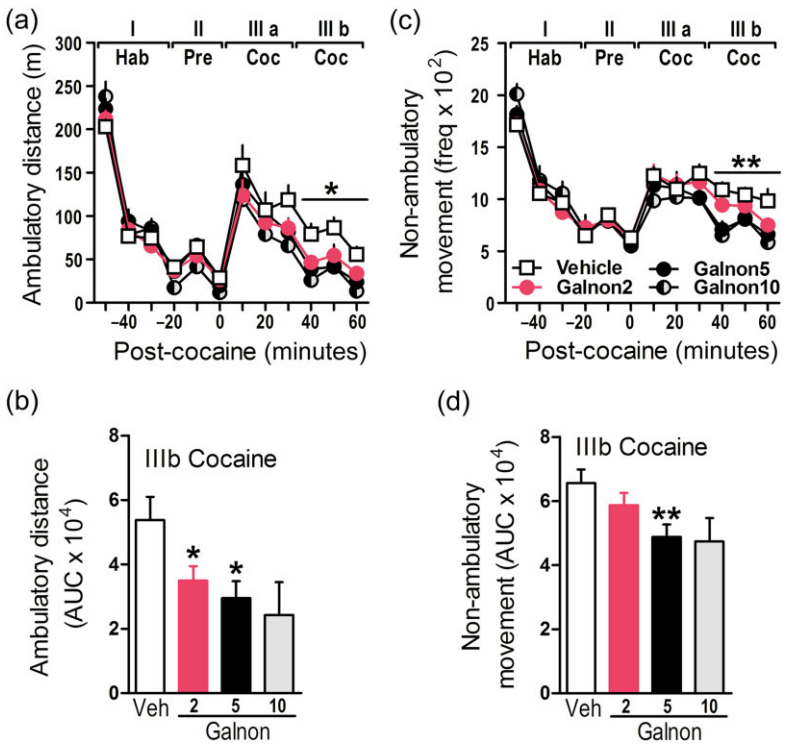

Figure 1 Galnon reduces cocaine-induced hyperactivity. Rats were placed in an open field for 40 minutes, administered vehicle or galnon (2, 5 or 10 mg/kg, i.p.), and injected with cocaine (10 mg/kg, i.p.) 20 minutes later. Shown are the mean ± SEM for (a) ambulatory distance across all time points, (b) AUC for ambulatory distance during the second half of post-cocaine time, (c) frequency of non-ambulatory movement across all time points and (d) AUC for non-ambulatory movement during the second half of post-cocaine time. $n = 46$ (vehicle), 32 (galnon 2 mg/kg), 23 (galnon 5 mg/kg) and 5 (galnon 10 mg/kg). $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle. Hab = habituation (phase I); Pre = pretreatment (phase II); Coc = cocaine (phase III); Veh = vehicle

movements that did not achieve criteria for ambulation, and thus was the continuous movement within a 2 × 2 area of x–y beams. Rats were not habituated to the open field before testing because pilot experiments showed that novelty-induced increases in ambulation diminished steadily across baseline testing, after which time locomotor exploration was nearly zero (i.e. see Fig. 1a).

In vivo microdialysis

Microdialysis was performed in all rats during the motor activity test (see above), as we described previously (Soares *et al.* 1999). A microdialysis probe was inserted into cannulae targeting the frontal cortex (MAB 9.6.2, 0.6 mm OD, 6 kDa cut-off PES membrane; SciPro, Sanborn, NY, USA) or NAc (S-8020, 0.36 mm OD, 20 kDA cut-off PAN membrane; Synaptech, Marquette, MI, USA) the night before testing and extended 2 mm beyond the guide cannulae. Probes were connected to tubing (PE50, VWR, Westchester, PA, USA) before experimentation. Rats were allowed to freely explore an open field while artificial cerebrospinal fluid (aCSF); (pH 7.4; 0.13 M NaCl, 3.1 mM $KHCO_3$, 1 mM $MgCl_2$, 2 mM $NaHCO_3$, 2.5 mM dextrose, 1.2 mM $CaCl_2$; Sigma Chemicals, St. Louis, MO, USA) was delivered through the probe at 2 µl/min. Dialysate was collected every 20 minutes during the 140 minutes open field test, including before galnon (2, 5, or 10 mg/kg i.p.) or vehicle injection at time –40, before cocaine at time 0, and for 60 minutes post-cocaine. These procedures were employed to allow DA levels to equilibrate and become Ca^{++}-dependent (i.e. probes inserted 24 hours before testing, baseline sampling lasted 40 minutes before drug manipulation), as shown previously (Santiago & Westerink 1990). Dialysate was transferred to sterile microcentrifuge vials (Fischer Scientific, Suwanee, GA, USA) pre-filled with 0.1% phosphoric acid and dihydroxybenzoic acid (10% sample volume, 0.08 ng/ul) or 0.1 M perchloric acid in ethylenediaminetetraacetic acid (EDTA; 1 mM of final solution volume). Different preservatives were used in a counterbalanced manner to determine if analyte detection could be improved, but preservatives did not alter DA content in our study (data not shown). Samples were transported in an opaque container on ice and stored at –80°C. Tubing was flushed with 70% EtOH, distilled H_20, air and aCSF between rats.

High-performance liquid chromatography (HPLC)

Samples were thawed and injected into the HPLC system that consisted of two ESA 584 pumps (ESA, Chelmsfor, MA, USA) with a pre-column filter (Synergi Max-RP 4u Security Guard, 150 × 4.6 mm, Phenomenex Inc., Torrance, CA, USA) and Max-RP cartridges (Phenomenex). Mobile phase, containing 100 mM sodium phosphate monobasic (Fisher), 0.1 mM EDTA (Sigma), 0.25 mM octanesulfonic acid (Sigma), and 5% acetonitrile (JT Baker, Center Valley, PA, USA) was delivered at 1 ml/min. Samples and standards were housed and sampled using an ESA 542 autosampler maintained at 4°C. Dialysate injection was 20 µl, and a duplicate set of standards was

run every 12 samples. Peaks were detected over 30 minutes using an ESA CoulArray electrochemical detector (−150 mV on initial electrode, 200 mV on a subsequent electrode). The position and height of peaks for DA were compared with reference standard solutions (Sigma; diluted in aCSF). Peak areas from chromatograms were integrated and analyzed by CoulArray Data Station Software 3.05.

Food training

To facilitate acquisition of drug self-administration, rats were first trained to lever press for food (45-mg pellets) in an operant chamber (Med Associates) prior to surgery. Each chamber was equipped with a house light and two retractable levers with a stimulus light above each lever. Animals were trained on a fixed ratio 1 (FR1) schedule with a 20-second time out. One lever press on the active lever resulted in the delivery of one food pellet. Presses on the inactive lever had no programmed consequences. Food training sessions lasted 8 hours, or until the animal met criteria, defined as at least 70% active lever selection and at least 100 food pellets obtained. Most rats met criteria on the first day of food training, but a few required 2–3 days.

Jugular catheter surgery

All instruments and implants were sterilized prior to surgery. Rats were surgically implanted with a catheter into the right jugular vein after food training, as described (Schroeder *et al.* 2010, 2013). Rats were anesthetized with isoflurane administered by vaporizer with oxygen delivered through a nose cone, and the surgical site was shaved and cleaned with Betadine. Catheter tubing was threaded subcutaneously from the back and guided over the shoulder into the right jugular vein, and tubing was sutured down. Rats received meloxicam (1 mg/kg, s.c.) immediately following surgery, and allowed to recover for 1 week prior to cocaine self-administration. Catheters were flushed daily with 0.05 ml gentamicin (3 mg/ml) and 0.1 ml heparinized saline (30 ml in sterile saline) to help maintain patency. Catheter patency was verified prior to cocaine self-administration by administering 0.08–0.12 ml of the short-acting barbiturate methohexital sodium (10 mg/ml, IV; Eli Lilly, Indianapolis, IN, USA), which rapidly produces moderate sedation.

Cocaine self-administration

Daily cocaine self-administration sessions were run for 2 hours on a FR1 schedule. At the start of each session, both active and inactive levers were extended, and rats received a non-contingent infusion of cocaine (0.5 mg/kg). During training, each press of the active lever resulted in a cocaine infusion (0.5 mg/kg, 167 μl/kg) accompanied by a discrete flashing light above the lever. Following a 20-second timeout period (during which time active lever presses were recorded but did not result in drug infusion), the stimulus light was extinguished and responses were again reinforced. Responses on the inactive lever had no programmed consequences. To prevent overdose, the session was terminated early if the number of cocaine infusions exceeded 40. The effects of galnon were assessed once rats reached a stable level of responding (number of drug infusions varied by < 20% of the mean and preference for the active lever was at least 75% for 3 consecutive days, with a minimum of 5 total days of cocaine self-administration). Rats received an injection of vehicle or galnon (2 mg/kg, i.p.) 30 minutes prior to the self-administration session. Each rat received both pre-treatments in a counterbalanced fashion.

Extinction

Following completion of the maintenance phase of cocaine self-administration, lever pressing was extinguished in daily 2-hour sessions during which presses on the previously active lever no longer resulted in delivery of cocaine or presentation of cocaine-paired cues. Behavior was considered extinguished when active lever presses over 3 consecutive days was < 30% of the average number of active lever presses during the last 3 days of maintenance.

Reinstatement

Rats were pre-treated with vehicle or galnon (2 mg/kg, i.p.) the day after extinction criteria were met. Thirty minutes later, they were given a non-contingent priming injection of saline or cocaine (10 mg/kg, i.p.) and placed in operant chambers under extinction conditions (i.e. presses on the 'active' lever had no programmed consequences) for 2 hours. Each rat received both pre-treatments in a counterbalanced fashion, separated by extinction sessions until they met criteria (described above).

Food self-administration

Separate groups of rats were used for the food self-administration and reinstatement experiments. Rats were maintained on a restricted diet of 16 g of normal rat chow per day, given in the evening at least 1 hour after self-administration sessions ended. Parameters of food self-administration were identical to the cocaine self-administration experiments, except that rats received a food pellet instead of a cocaine infusion for each active lever press, and sessions lasted 1 hour and were terminated if the number of reinforcers obtained exceeded 60. Once rats reached maintenance criteria, rats received an injection of vehicle or galnon (2 mg/kg, i.p.) 30 minutes

prior to the self-administration session. Each rat received both pre-treatments in a counterbalanced fashion.

Extinction and food-primed reinstatement

After extinction training was completed (extinction criteria were identical to those used for cocaine-primed reinstatement), one group of rats was pre-treated with vehicle or galnon (2 mg/kg, i.p.). Thirty minutes later, they were placed in the operant chambers for either another extinction session or a food-primed reinstatement session. For the 'food-primed reinstatement' group of rats, three food pellets were delivered non-contingently in the first 10 seconds of the session and levers were presented. Responses on either of the levers had no programmed consequence. Throughout the 60-minute food reinstatement session, a food pellet was delivered every 3 minutes non-contingently, and responses upon the formerly active and inactive levers were recorded. Each rat received both pre-treatments in a counterbalanced fashion, separated by extinction sessions as described above.

Statistics

Self-administration data were analyzed by ANOVA followed by Newman–Keuls *post hoc* tests or by chi-square (for the fraction of rats that obtained the maximum number of rewards). Motor activity was combined from rats implanted with cannulae in the cortex and NAc because there were no differences between these groups. Motor activity were analyzed using repeated measures ANOVA, and area under the curve (AUC) was performed as follow-up tests for significant interaction effects to detect the source of group differences during relevant experimental phases, specifically during habituation (phase I: −60 to −20 minutes), pre-treatment (phase II: −20 to 0 minutes) and cocaine phases (phase III: 0 to 60 minutes). Analyte levels are reported in nmol/ml for descriptive purposes. Repeated measures ANOVA was used to analyze percentage of DA overflow (post-cocaine analyte at 0, 20, 40 or 60 minutes/lowest baseline analyte at −40 or −20 minutes × 100) to reduce within-subject variability. To test a priori hypotheses and minimize a Type II error, AUC for % DA was performed to assess the effects of galnon during the cocaine phase and *t*-tests were performed to assess the effects of galnon at baseline (time 0). Based on standard criteria (2 standard deviations ± mean), occasional outliers (e.g. < 1% of values) were removed and missing values (e.g. ~6% of values due to loss of the microdialysis sample during transfer or collection) were replaced by the group mean to prevent loss of statistical power. In addition, one rat in the galnon 10 mg/kg group was removed entirely from locomotor activity analyses because this subject achieved outlier criteria. The number of subjects per group differed in the behavioral and dialysis studies because HPLC data were not available for all subjects and we focused our analysis on the vehicle and 2 mg/kg galnon dose. Data were analyzed using SPSS (IBM PAWS Statistical Software, Chicago, IL, USA) and graphed using Prism 5.0 (GraphPad, La Jolla, CA, USA).

RESULTS

Galnon reduces cocaine-induced motor activity

The effect of galnon (2, 5 or 10 mg/kg) on baseline and cocaine-induced motor activity were assessed in an open field. Ambulatory distance changed across time in a cubic fashion ($F_{1,100} = 130.97$, $P < 0.01$; cubic; Fig. 1a). Distance traveled diminished across time compared with initial exploration (−40 versus 0 minutes; $P < 0.01$), was stimulated immediately after cocaine administration (10 minutes; $P < 0.01$), and then steadily declined at 40 minutes ($P < 0.01$), 50 minutes ($P < 0.01$) and 60 minutes post-cocaine ($P < 0.01$; Fig. 1a) compared with the initial effects of cocaine at 10 minutes. Although the main effect of galnon was not significant for ambulatory distance ($F_{3, 102} = 1.08$, $P = 0.36$), there was a significant drug × time interaction ($F_{3, 102} = 3.94$, $P < 0.01$; Fig. 1a). Follow-up AUC analyses reveled that ambulatory distance was no different across groups during habituation in phase I (−60 to −20 minutes; $F_{3, 102} = 0.39$, $P = 0.76$), after pre-treatment with vehicle or galnon in phase II (−20 to 0 minutes; $F_{3, 102} = 0.92$, $P = 0.44$), overall post-cocaine in phase III (1 to 60 minutes; $F_{3, 102} = 1.56$, $P = 0.21$) or initially after cocaine administration in phase IIIa (1 to 30 minutes; $F_{3, 102} = 0.43$, $P = 0.73$). However, galnon reduced cocaine-induced ambulatory distance later in phase IIIb compared with vehicle (31 to 60 minutes; $F_{3, 102} = 3.08$, $P < 0.05$). *Post hoc* tests revealed a significant effect of the 2 mg/kg ($P < 0.05$) and 5 mg/kg ($P < 0.05$) doses. The 10 mg/kg dose also tended to reduce the locomotor effects of cocaine during phase IIIb, but it was not significant ($P = 0.13$; Fig. 1b).

Non-ambulatory movement was also different across time ($F_{1,102} = 150.30$, $P < 0.01$; cubic; Fig. 1c). Non-ambulation was less frequent across time compared with initial ambulation (−40 versus 0 minutes; $P < 0.01$), more frequent immediately after cocaine administration (10 versus 0 minutes; $P < 0.01$) and then became less frequent at 40 minutes ($P < 0.01$), 50 minutes ($P = 0.01$) and 60 minutes post-cocaine ($P < 0.01$; Fig. 1c) compared with initial effects of cocaine at 10 minutes. Although the main effect of galnon was not significant for non-ambulatory movement ($F_{3, 102} = 0.74$, $P = 0.53$), there was a significant drug × time interaction ($F_{3, 102} = 4.02$, $P < 0.01$; Fig. 1c). Follow-up AUC analyses revealed that non-ambulatory movement was no

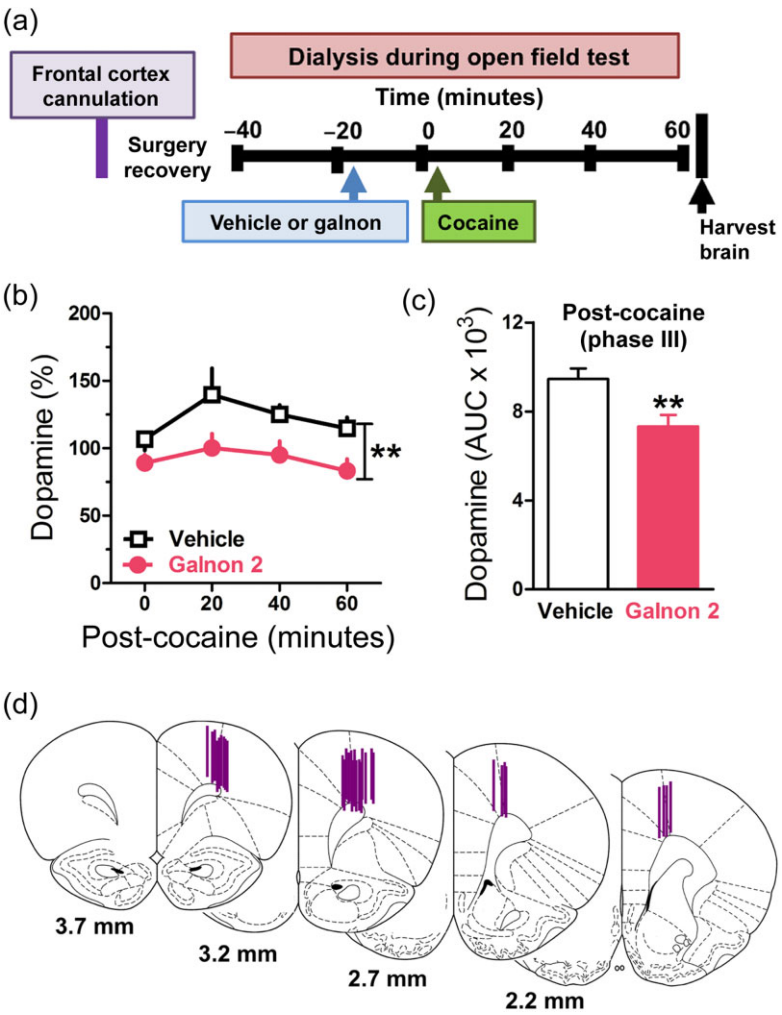

Figure 2 Galnon attenuates cocaine-induced increases in dopamine overflow in the frontal cortex. Microdialysis samples were collected through probes targeting the frontal cortex from rats administered vehicle or galnon (2, 5 or 10 mg/kg, i.p.), and injected with cocaine (10 mg/kg, i.p.) during behavioral testing (see Fig. 1). Shown are (a) experimental timeline, (b) mean ± SEM extracellular DA levels (% baseline) for vehicle ($n = 14$) and galnon 2 mg/kg ($n = 9$) across all time points, (c) mean ± SEM AUC for total post-cocaine extracellular DA levels and (d) probe placements. $^{**}P < 0.01$ compared with vehicle

different across groups during habituation in phase I (−60 to −20 minutes; $F_{3,102} = 0.94$, $P = 0.43$), after pre-treatment with vehicle or galnon in phase II (−20 to 0 minutes; $F_{3,102} = 0.07$, $P = 0.97$), overall post-cocaine in phase III (1 to 60 minutes; $F_{3,102} = 1.36$, $P = 0.26$), or initially after cocaine administration in phase IIIa (1 to 30 minutes; $F_{3,102} = 0.34$, $P = 0.80$). However, galnon reduced cocaine-induced non-ambulatory movement later in phase IIIb compared with vehicle (31 to 60 minutes; $F_{3,102} = 2.90$, $P < 0.05$). *Post hoc* tests showed a significant effect of the 5 mg/kg dose ($P < 0.01$), but not at the 2 mg/kg ($P = 0.26$) or 10 mg/kg doses ($P = 0.15$; Fig. 1d). Based on these results and other data showing galnon suppresses general motor activity and consummatory behavior at higher doses (Abramov *et al.* 2004; our unpublished data), we chose the 2 mg/kg dose for the remainder of the experiments.

Galnon attenuates cocaine-induced DA overflow in the frontal cortex but not the NAc

DA in the frontal cortex was measured in rats treated with vehicle and galnon (2 mg/kg) during motor activity testing, as shown in the timeline (Fig. 2a). Extracellular DA levels were not different between groups at baseline (vehicle 77.81 ± 18.87 nmol/ml, galnon 73.39 ± 22.33 nmol/ml, $P > 0.05$) or following galnon pre-treatment relative to vehicle (time 0; $t_{22} = 1.59$, $P = 0.11$; Fig. 2b). DA overflow increased following cocaine administration, and was attenuated by galnon (Fig. 2b). Two-way repeated measures ANOVA revealed a significant effect of time ($F_{1,22} = 5.09$, $P < 0.05$; quadratic) and galnon ($F_{1,22} = 7.27$, $P = 0.01$), but not a galnon × time interaction ($F_{1,22} = 0.76$, $P = 0.39$; Fig. 2b). AUC analyses revealed that galnon significantly reduced post-cocaine DA overflow relative to vehicle ($t_{22} = 3.03$, $P < 0.01$; Fig. 2c).

DA in the NAc was measured in a separate group of rats treated with vehicle or galnon (2 mg/kg) during motor activity testing (Fig. 3a). Extracellular DA levels were not different between groups at baseline (vehicle 30.60 ± 3.31 nm/ml, galnon 28.12 ± 3.54 nmol/ml, $P > 0.05$) or following galnon pre-treatment relative to vehicle (time 0; $t_{13} = -1.13$, $P = 0.28$; Fig. 3b). Cocaine increased DA overflow ($F_{1,13} = 15.26$, $P < 0.01$;

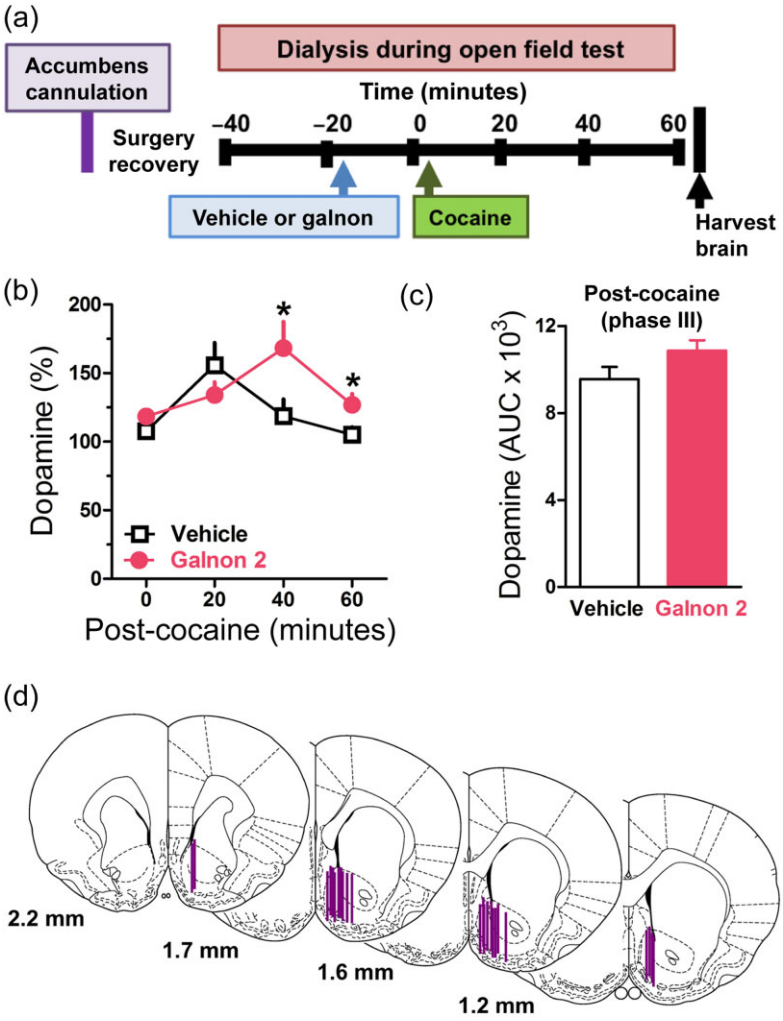

Figure 3 Galnon does not alter cocaine-induced increases in dopamine overflow in the nucleus accumbens (NAc). Microdialysis samples were collected through probes targeting the NAc shell from rats administered vehicle or galnon (2, 5 or 10 mg/kg, i.p.), and injected with cocaine (10 mg/kg, i.p.) during behavioral testing (see Fig. 1). Shown are (a) experimental timeline, (b) mean ± SEM extracellular DA levels (% baseline) for vehicle ($n = 8$) and galnon 2 mg/kg ($n = 7$) across all time points, (c) mean ± SEM AUC for total post-cocaine extracellular DA levels and (d) probe placements. *$P < 0.05$ compared to vehicle

quadratic), but there was no main effect of galnon ($F_{1,13} = 1.85$, $P = 0.20$). There was a significant galnon × time interaction ($F_{1,13} = 10.36$, $P < 0.01$; cubic; Fig. 3b). *Post hoc* tests revealed that extracellular DA levels were not different 20 minutes post-cocaine ($P = 0.29$), but were significantly different 40 ($P < 0.05$) and 60 minutes post-cocaine ($P < 0.05$; Fig. 3b). Total post-cocaine DA overflow, as assessed by AUC, was not significantly different in the vehicle and galnon groups ($t_{13} = -1.65$, $P = 0.12$; Fig. 3c).

Galnon has minimal effect on cocaine self-administration but blocks cocaine-primed reinstatement of cocaine seeking

After reaching maintenance criteria for cocaine self-administration, rats were pre-treated with vehicle or galnon (2 mg/kg, i.p.) 30 minutes prior to a self-administration session, and we found that galnon had no effect on operant responding for drug over the 2-hour session (one-way repeated measures ANOVA: active lever, $F_{2,14} = 0.24$, $P = 0.79$; reinforcers earned, $F_{2,14} = 0.01$, $P = 0.99$; inactive lever, $F_{2,14} = 0.03$, $P = 0.97$; chi-square test for fraction of rats that obtained the maximum number of reinforcers = 1.07, $P = 0.59$; Fig. 4a & b). However, breaking down the 2-hour session into 30-minute bins revealed a modest but significant difference in the pattern of cocaine infusions. Vehicle-treated rats tended to obtain most of their infusions during the first 30 minutes and then tapered off, while infusions were more stable in galnon-treated rats, resulting in fewer infusions during the first 30 minutes and more infusions during the final 30 minutes compared with vehicle (Supporting Information Fig. S1). A two-way repeated measures ANOVA showed a main effect of time ($F_{3,21} = 10.08$, $P < 0.001$) and a time × treatment interaction ($F_{3,21} = 3.38$, $P < 0.05$), although *post hoc* tests did not reveal significant pairwise differences for any individual time bin.

Rats were next subjected to non-reinforced sessions until meeting extinction criteria, and were then pre-treated with vehicle or galnon (2 mg/kg, i.p.) 30 minutes

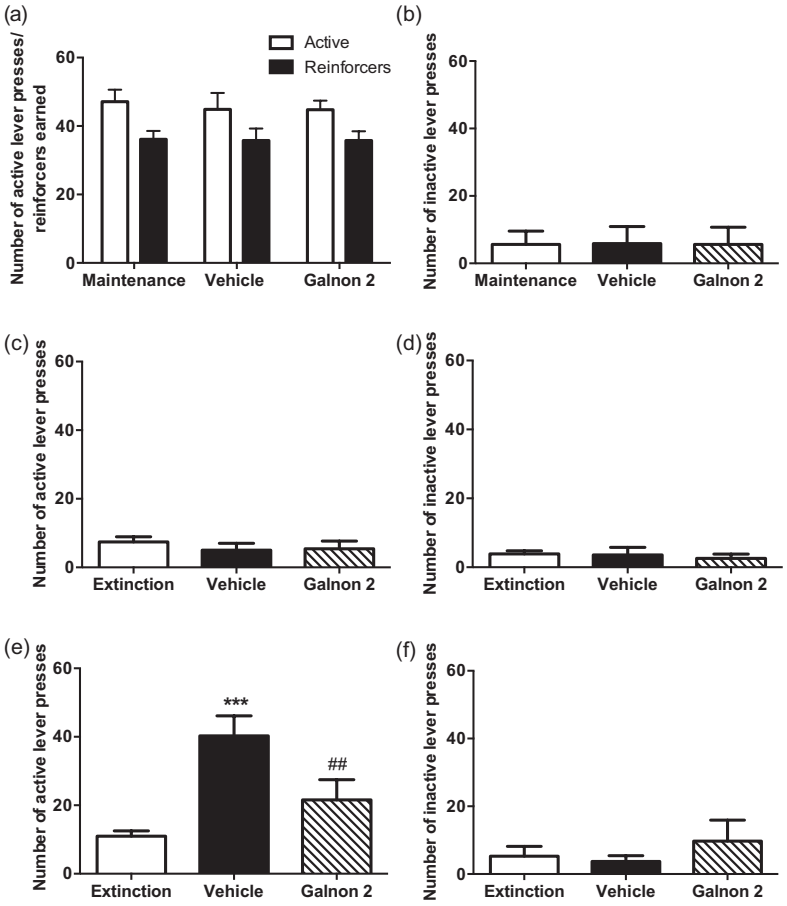

Figure 4 Galnon has no effect on cocaine self-administration but attenuates cocaine-primed reinstatement. Following the establishment of stable maintenance responding for cocaine on a FR1 schedule (maintenance values reflect an average of the last 3 days of maintenance sessions), subjects ($n = 8$) were treated with vehicle or galnon (2 mg/kg, i.p.) 30 minutes prior to a self-administration session. Shown are the mean ± SEM of (a) active lever presses and reinforcers earned and (b) inactive lever presses during the 2-hour test session. Lever pressing was then extinguished (extinction values reflect an average of the last 3 days of extinction), and rats were pre-treated with vehicle or galnon (2 mg/kg, i.p.) 30 minutes prior to a cocaine-primed (10 mg/kg, i.p., $n = 7$) or saline-primed ($n = 5$) reinstatement test. Shown are the mean ± SEM active and inactive lever responses during the 2-hour saline-primed (c and d) and cocaine-primed (e and f) sessions. $^{***}P < 0.001$ compared with extinction; $^{\#\#}P < 0.01$ compared with vehicle

prior to a reinstatement test following a non-contingent priming injection of saline or cocaine (10 mg/kg, i.p.). A saline prime did not reinstate cocaine-seeking behavior, and no differences were seen between pre-treatment groups (active lever, $F_{2,8} = 1.77$, $P = 0.23$; inactive lever, $F_{2,8} = 0.31$, $P = 0.74$; Fig. 4c & d). In contrast to its inability to alter cocaine self-administration, we found that galnon attenuated cocaine-primed reinstatement of cocaine seeking (Fig. 4e). One-way repeated measures ANOVA showed a main effect of treatment on active lever presses ($F_{2,12} = 16.07$, $P < 0.001$). *Post hoc* tests revealed that vehicle-pre-treated rats robustly reinstated following cocaine prime compared with extinction ($P < 0.001$), while galnon-pre-treated rats did not significantly reinstate ($P > 0.05$). Galnon-pre-treated rats also displayed significantly fewer active lever presses than vehicle-pre-treated animals ($P < 0.01$). Inactive lever presses were low and did not differ between groups ($F_{2,12} = 1.35$, $P = 0.30$; Fig. 4f).

Galnon attenuates food-primed reinstatement of food seeking

To determine whether the effects of galnon on cocaine-primed reinstatement were specific to drug-induced relapse-like behavior, we also evaluated the consequences of galnon on food self-administration and reinstatement. Similar to what we found with cocaine, galnon (2 mg/kg, i.p.) did not significantly affect reinforced food self-administration (one-way repeated measures ANOVA: active lever, $F_{2,18} = 0.12$, $P = 0.88$; all rats obtained the maximum number of reinforcers except for one animal on one day that obtained 57 instead of the 61 possible; inactive lever, $F_{2,18} = 3.45$, $P = 0.054$; Fig. 5a & b). In contrast to our cocaine self-administration data, breaking down the 1-hour session into 15-minute bins did not reveal a difference in the pattern of food reinforcement; all animals obtained a majority of their food rewards during the first 15 minutes, and all rats except for one obtained the maximum number of food rewards in the first 30 minutes (Supporting Information Fig. S2). Galnon also had no effect on extinction responding (active lever, $F_{2,10} = 0.1$, $P = 0.91$; inactive lever, $F_{2,10} = 4.28$, $P = 0.05$; Fig. 5c & d). However, galnon did partially attenuate food-primed reinstatement of food seeking (active lever: $F_{2,18} = 12$, $P < 0.001$; Fig. 5e). *Post hoc* tests revealed that, while both vehicle- ($P < 0.001$) and galnon- ($P < 0.05$) pre-treated rats significantly reinstated following a food prime compared with extinction,

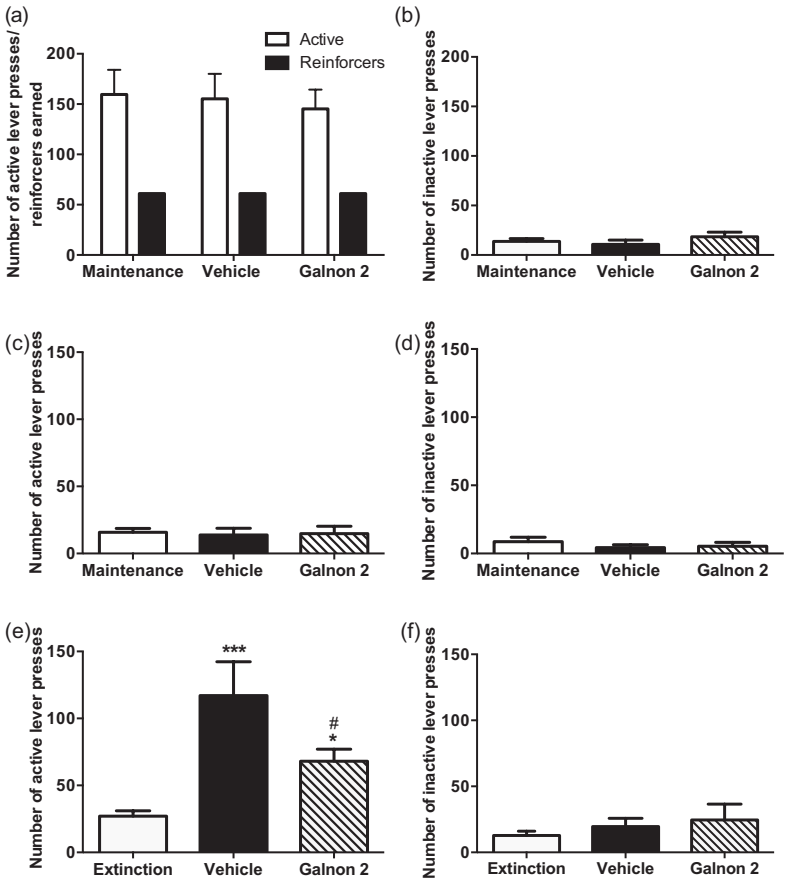

Figure 5 Galnon has no effect on food self-administration but attenuates food-primed reinstatement. Following the establishment of stable maintenance responding for food pellets on a FR1 schedule (maintenance values reflect an average of the last 3 days of maintenance sessions), subjects ($n = 10$) were treated with vehicle or galnon (2 mg/kg, i.p.) 30 minutes prior to a self-administration session. Shown are the mean ± SEM of (a) active lever presses and reinforcers earned and (b) inactive lever presses during the 1-hour test session. Lever pressing was then extinguished (extinction values reflect an average of the last 3 days of extinction), and rats were pre-treated with vehicle or galnon (2 mg/kg, i.p.) 30 minutes prior to another extinction session ($n = 6$) or a food-primed reinstatement test ($n = 10$). Shown are the mean ± SEM active and inactive lever responses during the 1-hour extinction (c and d) and food-primed reinstatement (e and f) sessions. $^{*}P < 0.05$ compared with extinction; $^{***}P < 0.001$ compared with extinction; $^{\#}P < 0.05$ compared with vehicle

galnon-pre-treated animals displayed significantly less active lever pressing than vehicle-pre-treated animals ($P < 0.05$). Inactive lever presses were low and did not differ between groups ($F_{2,18} = 0.93$, $P = 0.41$; Fig. 5f).

DISCUSSION

Our study is the first to report that galanin receptor activation decreases relapse-like behavior at a dose that had no impact on locomotion following habituation to a novel environment or reinforced cocaine/food self-administration. The behavioral effects of galnon were accompanied by a complete suppression of cocaine-evoked DA overflow in the frontal cortex, but not the NAc. These results are consistent with, and extend, previous reports showing that galanin receptor signaling diminishes the rewarding properties of cocaine and other drugs of abuse, and support targeting the galanin system as a potential treatment for addiction.

The effects of galnon on motor activity

Galanin signaling has little impact on basal motor activity and attenuates drug-induced ambulation under some conditions. For instance, galnon fails to alter general locomotion at doses below 5 mg/kg (Abramov *et al.* 2004; Hawes *et al.* 2008; Brabant *et al.* 2010), but at high doses (e.g. 10 mg/kg and above), it impairs motor activity and food consumption under some conditions (Abramov *et al.* 2004; our unpublished data). Consistent with these data, we found that doses of galnon ranging from 2 to 10 mg/kg had no effect on motor activity before cocaine administration. We also found that galnon decreased cocaine-induced motor activity during the second half of the time course examined, consistent its ability to suppress morphine-induced locomotion in galanin knockout mice (Hawes *et al.* 2008). In contrast to our present findings in rats, locomotor activity following cocaine was not altered in mice pre-treated with galnon (1–4 mg/kg) or in galanin knockout mice (Brabant *et al.* 2010), suggesting the existence of species differences. Combined, these data indicate that, at low doses that do not reliably alter baseline motor activity, galnon modestly attenuates hyperlocomotion following acute administration of cocaine or morphine. To avoid a general locomotor effect of galnon, we chose the lowest dose (2 mg/kg) for the remaining experiments in this study, which aimed to examine the effects of galnon on reward-related behaviors and neurochemistry.

The effects of galnon on cocaine and food self-administration and reinstatement

In general, galanin receptor signaling opposes the rewarding properties of cocaine and other drugs of abuse. For example, conditioned place preference to cocaine and morphine is facilitated in galanin knockout mice, while galanin or galnon suppresses the rewarding effects of these drugs (Zachariou *et al.* 1999; Hawes *et al.* 2008; Picciotto 2008; Narasimhaiah *et al.* 2009; Brabant *et al.* 2010). However, nicotine conditioned place preference is attenuated in galanin knockout mice (Neugebauer *et al.* 2011), suggesting the nature of the drug influences the valence of galanin on reward. The effect of galnon on conditioned place preference likely does not extend to the reinforcing properties of cocaine as measured by operant self-administration; neither galanin knockout nor galnon altered cocaine self-administration in mice (Narasimhaiah *et al.* 2009; Brabant *et al.* 2010). Our data showing that galnon did not profoundly alter stable operant responding for cocaine during the maintenance phase are consistent with these results, although we only examined FR1 responding, and it has been reported that galanin or a GalR1 agonist can alter operant reward responding under higher contingency schedules of reinforcement (McNamara & Robinson 2010; Anderson *et al.* 2013). There was modest change in the pattern of cocaine infusions between groups. The significance of this result is not clear, but it may be related to the delay in peak accumbal DA overflow in response to cocaine we observed in galnon-treated rats.

The effect of galanin receptor activation on relapse-like behavior has not been studied, and we employed the reinstatement paradigm to address this gap in the literature. It is important to note that reinstatement responding differs from the maintenance phase of self-administration sessions because it is run under extinction conditions (i.e. active lever presses have no programmed consequences), and represents non-reinforced drug-seeking behavior thought to model aspects of relapse (Bossert *et al.* 2013). We found that galnon abolished cocaine-primed reinstatement. There was no significant difference between extinction and reinstatement responding following galnon pre-treatment, while robust reinstatement was observed following vehicle pre-treatment. This reduction in drug seeking appears to be specific for reinstatement as opposed to a general suppression of motor activity or operant behavior because the dose of galnon used had no effect on baseline motor activity, cocaine self-administration, food self-administration or inactive lever presses during any phase. Moreover, galnon only modestly attenuated cocaine-induced motor activity, which unlikely accounts for the robust loss of reinstatement behavior because reinstatement was also reduced for a non-drug reinforcer (food) that does not produce hyperactivity.

The effects of galnon on cocaine-induced changes in DA overflow

Cocaine-primed reinstatement of cocaine seeking requires DA transmission in the prefrontal cortex, but not the NAc (Cornish & Kalivas 2000; McFarland & Kalivas 2001; McFarland, Lapish & Kalivas 2003; Schmidt *et al.* 2005; Sun & Rebec 2005). Galanin can reduce DA release in some brain regions (Melander *et al.* 1987; Nordstrom *et al.* 1987; Jansson *et al.* 1989; Tsuda *et al.* 1998), but the consequences of galanin receptor activation on cocaine-induced DA overflow have not been investigated. We employed *in vivo* microdialysis in the frontal cortex and NAc to determine whether changes in extracellular DA accompanied galnon's ability to attenuate the behavioral effects of cocaine. The region of frontal cortex selected for microdialysis sampling primarily represents motor cortex. This area receives dense dopaminergic innervation from ventral tegmental area neurons (Lindvall, Bjorklund & Divac 1978) and was selected because it is a reliable target for catecholamine recovery based on our previous microdialysis experiments (Soares *et al.* 1999). The region is also highly sensitive to acute cocaine challenge as indicated by magnetic resonance imaging of regional cerebral blood volume in rats (Chen *et al.* 2011). In these studies, cocaine elicited equivalent increases in peak blood volume in motor cortex, dorsal striatum and NAc. DA overflow in the motor cortex thus serves as a proxy for cocaine-induced activation of the mesocortical system. We found that galnon had no immediate impact (e.g. 20 minutes later) on baseline DA levels following habituation to a novel environment. We also found that galnon prevented cocaine-induced increases in locomotor activity and DA overflow in the frontal cortex. These data suggest that attenuated cocaine-induced DA overflow in the frontal cortex may contribute to the ability of galnon to reduce cocaine-induced locomotor activity and possibly aspects of relapse-like behavior, although the latter hypothesis needs to be directly evaluated by collecting dialysate in the prefrontal cortex during reinstatement testing. Our results are consistent with data from slice preparations showing that galanin reduces DA release (Melander *et al.* 1987; Nordstrom *et al.* 1987; Tsuda *et al.* 1998), although one study found increased DA utilization in the striatum following galanin administration (Jansson *et al.* 1989).

By contrast, we show that galnon does not reduce DA overflow in the NAc following cocaine administration. A simple explanation is that mesocortical DA neurons are more sensitive to inhibition by galanin receptor activation than mesolimbic DA neurons. Relative to other areas (e.g. NAc, ventral tegmental area), all three galanin receptors

are highly expressed in the prefrontal cortex (Hawes & Picciotto 2004), and this region is changed substantially in both structure and function by cocaine administration (Kalivas 2007; Morales & Pickel 2012; Tritsch & Sabatini 2012). Alternatively, the failure of galnon to alter DA overflow in the NAc may result from complex interactions within mesolimbic circuitry. For example, because cortical DA transmission opposes subcortical DA transmission (Pycock, Carter & Kerwin 1980; Doherty & Gratton 1996; King, Zigmond & Finlay 1997; Ventura *et al.* 2004), suppression of cortical DA overflow by galnon could help preserve normal accumbal DA levels. In support of this idea, our finding that the peak increase in cocaine-induced DA overflow in the NAc following galnon pre-treatment is delayed (~40 minutes post-cocaine) compared with vehicle-pre-treated animals (~20 minutes post-cocaine) implicates a secondary circuit rather than a direct excitatory influence of galnon on mesolimbic DA neurons.

The exact mechanisms underlying the changes in cortical DA transmission in the present study are not clear. One possibility is that galnon modulates cocaine-induced DA release by suppressing the activity of ventral tegmental area DA neurons that project to the frontal cortex or by acting directly on mesocortical DA terminals. Indeed, galanin typically inhibits neuronal activity (Xu, Zheng & Hokfelt 2005), and galanin receptors are present in both brain regions (Hawes & Picciotto 2004). Alternatively, galnon may act indirectly by altering the activity of other brain nuclei (e.g. locus coeruleus) that, in turn, project to and control the activity of mesocortical DA neurons (Picciotto 2008). In support of this hypothesis, galnon suppresses morphine-induced neuronal activity in the locus coeruleus, and transgenic overexpression of galanin in noradrenergic neurons reduces behavior associated with morphine withdrawal (Zachariou *et al.* 2003). Although future experiments are needed to identify the loci and mechanisms of action that mediate the benefit of galnon on cocaine-induced behavior, these data collectively suggest that galnon is well positioned to alter catecholamine transmission across circuits targeted by drugs of abuse and support a model in which galnon primarily affects mesocoritcal DA neurons that drive cocaine-primed reinstatement.

CONCLUSIONS

In summary, we report that galnon reduces reward-seeking behavior during reinstatement and cocaine-induced DA overflow in the frontal cortex. However, the results should be interpreted with caution, and further studies are required to define the underlying mechanisms. Galnon is a synthetic non-peptide galanin receptor agonist that crosses the blood–brain barrier and has equal binding affinity for GalR1 and GalR2 (Saar *et al.* 2002; Lu *et al.* 2005b). It is important to note that high concentrations of galnon (e.g. 10 uM) *in vitro* also produce agonist activity at other GPCRs and activate intracellular G-proteins independent of receptor activation (Floren *et al.* 2005). However, at doses comparable with those used in the present study, the behavioral effects of galnon are similar to galanin (Sollenberg, Bartfai & Langel 2005) and blocked by co-administration of a galanin receptor antagonist (Saar *et al.* 2002; Wu *et al.* 2003; Abramov *et al.* 2004). Thus, the effects of galnon in the present study are in all likelihood mediated, at least in part, by galanin receptor signaling. The modulatory action of galanin on other drug-induced behaviors appears to involve GalR1 and GalR2 receptor subtypes (Holmes *et al.* 2012; Einstein *et al.* 2013). Future experiments using selective antagonists for GalR1 and GalR2 will be necessary to assess the contribution of each receptor subtype. In addition, although all existing evidence points to a central effect of galanin and galnon (Zachariou *et al.* 1999, 2003), peripheral galanin receptors cannot be ruled out because of our systemic route of galnon administration. Although further experiments are required to identify the galanin receptor subtype and neuroanatomical substrates involved, the data presented here suggest that the galaninergic system is a candidate target for anti-relapse therapies.

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Disclosure/Conflict of Interest

DW is a co-inventor on a patent concerning the use of selective dopamine β-hydroxylase inhibitors for the treatment of cocaine dependence (US-2010-0105748-A1; 'Methods and Compositions for Treatment of Drug Addiction'). The other authors declare no conflicts of interest.

Authors Contribution

YEO, NRS, PVH and DW participated in the research design, YEO conducted the reinstatement experiments, NRS and JLGC conducted the motor activity and microdialysis experiments, KGF conducted the HPLC experiments, GLE contributed analytic tools, and YEO, NRS, PVH and DW wrote the manuscript.

References

Abramov U, Floren A, Echevarria DJ, Brewer A, Manuzon H, Robinson JK, Bartfai T, Vasar E, Langel U (2004) Regulation of feeding by galnon. Neuropeptides 38:55–61.

Anderson ME, Runesson J, Saar I, Langel U, Robinson JK (2013) Galanin, through GalR1 but not GalR2 receptors, decreases motivation at times of high appetitive behavior. Behav Brain Res 239:90–93.

Bartfai T, Wang MW (2013) Positive allosteric modulators to peptide GPCRs: a promising class of drugs. Acta Pharmacol Sin 34:880–885.

Bossert JM, Marchant NJ, Calu DJ, Shaham Y (2013) The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl) 229:453–476.

Brabant C, Kuschpel AS, Picciotto MR (2010) Locomotion and self-administration induced by cocaine in 129/OlaHsd mice lacking galanin. Behav Neurosci 124:828–838.

Chen YI, Famous K, Xu H, Choi JK, Mandeville JB, Schmidt HD, Pierce RC, Jenkins BG (2011) Cocaine self-administration leads to alterations in temporal responses to cocaine challenge in limbic and motor circuitry. Eur J Neurosci 34:800–815.

Cornish JL, Kalivas PW (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. J Neurosci 20:RC89.

Doherty MD, Gratton A (1996) Medial prefrontal cortical D1 receptor modulation of the meso-accumbens dopamine response to stress: an electrochemical study in freely-behaving rats. Brain Res 715:86–97.

Einstein EB, Asaka Y, Yeckel MF, Higley MJ, Picciotto MR (2013) Galanin-induced decreases in nucleus accumbens/striatum excitatory postsynaptic potentials and morphine conditioned place preference require both galanin receptor 1 and galanin receptor 2. Eur J Neurosci 37:1541–1549.

Floren A, Sollenberg U, Lundstrom L, Zorko M, Stojan J, Budihna M, Wheatley M, Martin NP, Kilk K, Mazarati A, Bartfai T, Lindgren M, Langel U (2005) Multiple interaction sites of galnon trigger its biological effects. Neuropeptides 39:547–558.

Hawes JJ, Picciotto MR (2004) Characterization of GalR1, GalR2, and GalR3 immunoreactivity in catecholaminergic nuclei of the mouse brain. J Comp Neurol 479:410–423.

Hawes JJ, Brunzell DH, Narasimhaiah R, Langel U, Wynick D, Picciotto MR (2008) Galanin protects against behavioral and neurochemical correlates of opiate reward. Neuropsychopharmacology 33:1864–1873.

Holmes FE, Armenaki A, Iismaa TP, Einstein EB, Shine J, Picciotto MR, Wynick D, Zachariou V (2012) Galanin negatively modulates opiate withdrawal via galanin receptor 1. Psychopharmacology (Berl) 220:619–625.

Jackson KJ, Chen X, Miles MF, Harenza J, Damaj MI (2011) The neuropeptide galanin and variants in the GalR1 gene are associated with nicotine dependence. Neuropsychopharmacology 36:2339–2348.

Jansson A, Fuxe K, Eneroth P, Agnati LF (1989) Centrally administered galanin reduces dopamine utilization in the median eminence and increases dopamine utilization in the medial neostriatum of the male rat. Acta Physiol Scand 135:199–200.

Kalivas PW (2007) Cocaine and amphetamine-like psychostimulants: neurocircuitry and glutamate neuroplasticity. Dialogues Clin Neurosci 9:389–397.

King D, Zigmond MJ, Finlay JM (1997) Effects of dopamine depletion in the medial prefrontal cortex on the stress-induced increase in extracellular dopamine in the nucleus accumbens core and shell. Neuroscience 77:141–153.

Lindvall O, Bjorklund A, Divac I (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. Brain Res 142:1–24.

Lu X, Barr AM, Kinney JW, Sanna P, Conti B, Behrens MM, Bartfai T (2005a) A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus. Proc Natl Acad Sci U S A 102:874–879.

Lu X, Lundstrom L, Langel U, Bartfai T (2005b) Galanin receptor ligands. Neuropeptides 39:143–146.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531–3537.

McNamara IM, Robinson JK (2010) Conditional stimulation by galanin of saccharin and ethanol consumption under free and response contingent access. Neuropeptides 44:445–451.

Melander T, Hokfelt T, Rokaeus A, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J Neurosci 6:3640–3654.

Melander T, Fuxe K, Harfstrand A, Eneroth P, Hokfelt T (1987) Effects of intraventricular injections of galanin on neuroendocrine functions in the male rat. Possible involvement of hypothalamic catecholamine neuronal systems. Acta Physiol Scand 131:25–32.

Morales M, Pickel VM (2012) Insights to drug addiction derived from ultrastructural views of the mesocorticolimbic system. Ann N Y Acad Sci 1248:71–88.

Narasimhaiah R, Kamens HM, Picciotto MR (2009) Effects of galanin on cocaine-mediated conditioned place preference and ERK signaling in mice. Psychopharmacology (Berl) 204:95–102.

Neugebauer NM, Henehan RM, Hales CA, Picciotto MR (2011) Mice lacking the galanin gene show decreased sensitivity to nicotine conditioned place preference. Pharmacol Biochem Behav 98:87–93.

Nordstrom O, Melander T, Hokfelt T, Bartfai T, Goldstein M (1987) Evidence for an inhibitory effect of the peptide galanin on dopamine release from the rat median eminence. Neurosci Lett 73:21–26.

Paxinos G, Watson C (1998) The Rat Brain: in Stereotaxic Coordinates, 4th edn. San Diego: Academic Press.

Picciotto MR (2008) Galanin and addiction. Cell Mol Life Sci 65:1872–1879.

Pycock CJ, Carter CJ, Kerwin RW (1980) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J Neurochem 34:91–99.

Rajarao SJ, Platt B, Sukoff SJ, Lin Q, Bender CN, Nieuwenhuijsen BW, Ring RH, Schechter LE, Rosenzweig-Lipson S, Beyer CE (2007) Anxiolytic-like activity of the non-selective galanin receptor agonist, galnon. Neuropeptides 41:307–320.

Saar K, Mazarati AM, Mahlapuu R, Hallnemo G, Soomets U, Kilk K, Hellberg S, Pooga M, Tolf BR, Shi TS, Hokfelt T, Wasterlain C, Bartfai T, Langel U (2002) Anticonvulsant activity of a nonpeptide galanin receptor agonist. Proc Natl Acad Sci U S A 99:7136–7141.

Santiago M, Westerink BH (1990) Characterization of the in vivo release of dopamine as recorded by different types of

intracerebral microdialysis probes. Naunyn Schmiedebergs Arch Pharmacol 342:407–414.

Schmidt HD, Anderson SM, Famous KR, Kumaresan V, Pierce RC (2005) Anatomy and pharmacology of cocaine priming-induced reinstatement of drug seeking. Eur J Pharmacol 526:65–76.

Schroeder JP, Cooper DA, Schank JR, Lyle MA, Gaval-Cruz M, Ogbonmwan YE, Pozdeyev N, Freeman KG, Iuvone PM, Edwards GL, Holmes PV, Weinshenker D (2010) Disulfiram attenuates drug-primed reinstatement of cocaine seeking via inhibition of dopamine beta-hydroxylase. Neuropsychopharmacology 35:2440–2449.

Schroeder JP, Epps SA, Grice TW, Weinshenker D (2013) The selective dopamine beta-hydroxylase inhibitor nepicastat attenuates multiple aspects of cocaine-seeking behavior. Neuropsychopharmacology 38:1032–1038.

Sciolino NR, Dishman RK, Holmes PV (2012) Voluntary exercise offers anxiolytic potential and amplifies galanin gene expression in the locus coeruleus of the rat. Behav Brain Res 233:191–200.

Sinha R (2009) Modeling stress and drug craving in the laboratory: implications for addiction treatment development. Addict Biol 14:84–98.

Soares J, Holmes PV, Renner KJ, Edwards GL, Bunnell BN, Dishman RK (1999) Brain noradrenergic responses to footshock after chronic activity-wheel running. Behav Neurosci 113:558–566.

Sollenberg U, Bartfai T, Langel U (2005) Galnon—a low-molecular weight ligand of the galanin receptors. Neuropeptides 39:161–163.

Sun W, Rebec GV (2005) The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. Psychopharmacology (Berl) 177:315–323.

Tritsch NX, Sabatini BL (2012) Dopaminergic modulation of synaptic transmission in cortex and striatum. Neuron 76:33–50.

Tsuda K, Tsuda S, Nishio I, Masuyama Y, Goldstein M (1998) Effects of galanin on dopamine release in the central nervous system of normotensive and spontaneously hypertensive rats. Am J Hypertens 11:1475–1479.

Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S (2004) Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. Neuropsychopharmacology 29:72–80.

Wu WP, Hao JX, Lundstrom L, Wiesenfeld-Hallin Z, Langel U, Bartfai T, Xu XJ (2003) Systemic galnon, a low-molecular weight galanin receptor agonist, reduces heat hyperalgesia in rats with nerve injury. Eur J Pharmacol 482:133–137.

Xu ZQ, Zheng K, Hokfelt T (2005) Electrophysiological studies on galanin effects in brain—progress during the last six years. Neuropeptides 39:269–275.

Zachariou V, Parikh K, Picciotto MR (1999) Centrally administered galanin blocks morphine place preference in the mouse. Brain Res 831:33–42.

Zachariou V, Brunzell DH, Hawes J, Stedman DR, Bartfai T, Steiner RA, Wynick D, Langel U, Picciotto MR (2003) The neuropeptide galanin modulates behavioral and neurochemical signs of opiate withdrawal. Proc Natl Acad Sci U S A 100:9028–9033.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Effects of galnon on the pattern of cocaine self-administration. Shown are the mean ± SEM of cocaine reinforcers earned during the 2-hour test session (see Fig. 4a) broken down into 30-minute bins

Figure S2 Effects of galnon on the pattern of food self-administration. Shown are the mean ± SEM of food reinforcers earned during the 1-hour test session (see Fig. 5a) broken down into 15-minute bins