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

Yvonne E. Ogbonmwan1*, Natale R. Sciolino2*, Jessica L. Groves-Chapman2, Kimberly G. Freeman3, Jason P. Schroeder1, Gaylen L. Edwards3, Philip V. Holmes2,4 & David Weinshenker1

Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA1, Neuroscience Program, Biomedical and Health Sciences Institute, University of Georgia, Athens, GA, USA2, Department of Physiology and Pharmacology, University of Georgia, Athens, GA, USA3 and Department of Psychology, University of Georgia, Athens, GA, USA4

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.

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.
(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 opioid 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, Pratville, 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 (Kd = 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 μl/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 canulae (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 canulla 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
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 canulae 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 canulae. 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 KHCO3, 1 mM MgCl2, 2 mM NaHCO3, 2.5 mM dextrose, 1.2 mM CaCl2; Sigma Chemicals, St. Louis, MO, USA) was delivered at 2 μl/min. 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 H2O, 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 methohexitol 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_{1,102} = 1.08, P = 0.36), there was a significant drug × time interaction (F_{1,102} = 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_{1,102} = 0.39, P = 0.76), after pre-treatment with vehicle or galnon in phase II (−20 to 0 minutes; F_{1,102} = 0.92, P = 0.44), overall post-cocaine in phase III (1 to 60 minutes; F_{1,102} = 1.56, P = 0.21) or initially after cocaine administration in phase III (1 to 30 minutes; F_{1,102} = 0.43, P = 0.73). However, galnon reduced cocaine-induced ambulatory distance later in phase IIIb compared with vehicle (31 to 60 minutes; F_{1,102} = 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
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 consum-
matory 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 act-

ivity testing, as shown in the timeline (Fig. 2a). Extracellu-
lar 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 ($F_{1,22} = 5.09, P < 0.05$; quad-
ratic) 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 nmol/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 ($F_{1,13} = 15.26, P < 0.01$;
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...
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 reinnstate cocaine-seeking behavior, and no differences were seen between pre-treatment groups (active lever, \( F_{1,8} = 1.77, P = 0.23 \); inactive lever, \( F_{1,8} = 0.31, P = 0.74 \); 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 reinnstate following cocaine prime compared with extinction (\( P < 0.001 \)), while galnon-pre-treated rats did not significantly reinnstate (\( P > 0.05 \)). 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 reinnstate following a food prime compared with extinction,
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 \( (F_{2,18} = 0.93, P = 0.41; \text{Fig. 5f}) \).

**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, Zigmund & 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, galanin 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, galanin 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 reduces reward-associated with morphine withdrawal (Zachariou et al. 2003). Although future experiments are needed 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.

Acknowledgements

These studies were funded by the National Institutes of Health (NIDA Grants DA027535 to DW and PVH, and DA033091 and DA015040 to YEO).

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


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

SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Figure S1 Effects of galanin 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 galanin 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