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The Aversive Agent Lithium Chloride Suppresses Phasic Dopamine Release Through Central GLP-1 Receptors

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Unconditioned rewarding stimuli evoke phasic increases in dopamine concentration in the nucleus accumbens (NAc) while discrete aversive stimuli elicit pauses in dopamine neuron firing and reductions in NAc dopamine concentration. The unconditioned effects of more prolonged aversive states on dopamine release dynamics are not well understood and are investigated here using the malaise-inducing agent lithium chloride (LiCl). We used fast-scan cyclic voltammetry to measure phasic increases in NAc dopamine resulting from electrical stimulation of dopamine cell bodies in the ventral tegmental area (VTA). Systemic LiCl injection reduced electrically evoked dopamine release in the NAc of both anesthetized and awake rats. As some behavioral effects of LiCl appear to be mediated through glucagon-like peptide-1 receptor (GLP-1R) activation, we hypothesized that the suppression of phasic dopamine by LiCl is GLP-1R dependent. Indeed, peripheral pretreatment with the GLP-1R antagonist exendin-9 (Ex-9) potently attenuated the LiCl-induced suppression of dopamine. Pretreatment with Ex-9 did not, however, affect the suppression of phasic dopamine release by the kappa-opioid receptor agonist, salvinorin A, supporting a selective effect of GLP-1R stimulation in LiCl-induced dopamine suppression. By delivering Ex-9 to either the lateral or fourth ventricle, we highlight a population of central GLP-1 receptors rostral to the hindbrain that are involved in the LiCl-mediated suppression of NAc dopamine release.
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INTRODUCTION

Phasic increases in the firing of midbrain ventral tegmental (VTA) dopamine neurons and resulting phasic increases in extracellular nucleus accumbens (NAc) dopamine concentration occur both spontaneously and in response to either unconditioned primary rewards or conditioned predictors of reward (Cohen *et al*, 2012; Joshua *et al*, 2008; Matsumoto and Hikosaka, 2009; Owesson-White *et al*, 2012; Roitman *et al*, 2004; Schultz, 1998; Sombers *et al*, 2009; Zweifel *et al*, 2009). These phasic increases are both necessary and sufficient for positive reinforcement and associative learning (Steinberg *et al*, 2013; Tsai *et al*, 2009), supporting a mechanism by which rewarding stimuli reinforce approach behaviors necessary for survival (eg procuring food). While electrophysiological and electrochemical data consistently demonstrate increases in dopamine neuron firing and release evoked by reward and reward predictive cues, the encoding of aversive stimuli, a process equally important for survival, by the mesolimbic dopamine system remains controversial (see McCutcheon *et al*, 2012 for review).

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Discrete aversive stimuli evoke pauses in the firing rate of a clear majority of dopamine neurons (Cohen *et al*, 2012; Matsumoto and Hikosaka, 2009; Mirenowicz and Schultz, 1996) and suppress phasic dopamine release in the NAc (Badrinarayan *et al*, 2012; Oleson *et al*, 2012; Roitman *et al*, 2008; Wheeler *et al*, 2011; but also see Anstrom *et al*, 2009; Brischoux *et al*, 2009; Budygin *et al*, 2012; Park *et al*, 2015 for reported increases in phasic dopamine activity to aversive stimuli under some conditions). However, while discrete stimuli are commonly used to study phasic dopamine responses, the time domain of aversive stimuli can range from discrete to prolonged. Long lasting aversive states can be pharmacologically induced by drugs such as salvinorin A (SalvA), which increases immobility time in the forced swim test, decreases cocaine-induced locomotion and increases the threshold for brain stimulation reward (Carlezon *et al*, 2006; Chartoff *et al*, 2008). SalvA also decreases phasic dopamine release (Ebner *et al*, 2010). SalvA acts directly on dopamine neurons by binding to kappa-opioid receptors on dopamine terminals (Margolis *et al*, 2014). Additional agents induce prolonged aversive states but have no known direct action on dopamine neurons. The purpose of the studies herein is to determine whether dopamine neuron excitability and hence dopamine release evoked by electrical stimulation is reduced by the aversive agent lithium chloride (LiCl).

In animal models, systemically delivered LiCl gives rise to indices of nausea/malaise that include hypophagia (McCann *et al*, 1989), delayed gastric emptying (McCann *et al*, 1989),

lying-on belly behavior (Meachum and Bernstein, 1992) and pica (the ingestion of non-nutritive substances; Mitchell *et al*, 1976). Illness resulting from LiCl is also known to condition taste avoidance or aversion (CTA; Nachman and Ashe, 1973; Parker and Carvell, 1986; Spector *et al*, 1988). Systemic LiCl activates neurons in circumventricular organs (eg area postrema) as well as a discrete population of hindbrain neurons that make and release glucagon-like peptide-1 (GLP-1) (Rinaman, 1999a; Thiele *et al*, 1996). GLP-1 receptors (GLP-1R) likely mediate LiCl-induced aversion, as GLP-1R antagonism attenuates the hypophagia, pica, and CTA produced by LiCl (Rinaman, 1999b; Seeley *et al*, 2000). Here, we investigated GLP-1 dependent effects of aversive LiCl on phasic dopamine signaling. Similarly to a previous investigation (Ebner *et al*, 2010), we probe effects of a drug-induced aversive state on phasic dopamine signaling by periodic electrical stimulation of dopamine neurons while simultaneously sampling dopamine concentration at dopamine terminal regions with fast-scan cyclic voltammetry (FSCV).

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Charles River Laboratories, Chicago, IL) weighing 325–425 grams at testing were individually housed in plastic cages on a 12:12 light:dark cycle (lights on at 7 am). Rats were fed and watered *ad libitum*. Animal care and use was in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

Surgical Procedures

Rats were anesthetized with intraperitoneal (IP) ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) and prepared for voltammetric recording as described in detail elsewhere (Fortin *et al*, 2015). All implants were targeted relative to bregma using the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 2007). A FSCV guide cannula (Bioanalytical Systems, West Lafayette, IN) was implanted above the NAc core [+1.3 mm anteroposterior (AP), 1.5 mm mediolateral (ML) and − 2.5 mm dorsoventral (DV)]. A chlorinated silver reference electrode (Ag/AgCl) was placed in the contralateral cortex. Rats receiving intracerebroventricular (ICV) infusions were also implanted with a 22-gauge guide cannula (Plastics One, Roanoke, VA) targeting either the lateral [− 0.8 mm AP, 2.1 mm ML, − 3.7 mm DV, angled 10° away from midline (Experiment 4)] or fourth ventricle [− 11.5 mm AP, − 6.5 mm DV on midline (Experiment 5)]. A carbon fiber electrode was advanced into the NAc core using a custom micro-manipulator (UIC Machine Shop, Chicago, IL). A bipolar stimulating electrode (Plastics One, Roanoke, VA) was lowered dorsal to the rostral VTA (−5.2 mm AP, − 0.8 mm ML, − 7.0 mm DV). The DV position of the stimulating electrode was optimized for maximal electrically-evoked dopamine release by lowering it in 0.2-mm increments while concurrently using FSCV to measure NAc dopamine release following VTA stimulation (60 monophasic pulses, 60 Hz, 4 ms/pulse, 120 μA). All implants were secured with skull screws and dental cement. Following surgery, rats were removed from the stereotaxic frame for recordings (Experiments 1 and 2) or 5–7 days of postoperative recovery (eg return to preoperative body weight; Experiments 3–5).

FSCV

Both the FSCV recording and Ag/AgCl reference electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Crist Instruments, Hagerstown, MD) above a behavioral chamber (Med-Associates, Inc, St Albans City, VT). The FSCV recording electrode was lowered into the NAc core. A triangular voltage waveform was applied to the carbon-fiber [from − 0.4 to 1.3 to − 0.4 V relative to the Ag/AgCl reference electrode (400 V/s)]. The waveform was applied first at 60 Hz for 30 min to hasten the electrode equilibration process. The rate was then switched to 10 Hz for 15 min before data acquisition. Each application of the waveform resulted in a background current. Current resulting from the oxidation and reduction of dopamine was detected after background subtraction (Fortin *et al*, 2015). Waveform application, current measurements and electrical stimulation were all computer-controlled via software written in LabVIEW (National Instruments, Austin, TX).

Experimental Design

For all experiments, trains of current pulses were delivered to the VTA (24 monophasic pulses, 4 ms/pulse, 60 Hz, 120–170 μA) every 5 min. Each stimulation train evoked a sharp rise in NAc dopamine concentration that decayed exponentially. Drug injections occurred after peak dopamine concentration evoked by 3 successive electrical stimulations was stable (eg differed by $<10\%$; 'baseline'). Following all experiments, recording electrodes were calibrated to permit the conversion of detected current to concentration (Sinkala *et al*, 2012).

Experiment 1. Anesthetized rats ($n=18$) were removed from the stereotaxic frame, placed in a behavioral chamber and connected to a FSCV headstage. Stimulations occurred once every 5 min for the duration of the experiment. After baseline dopamine recordings, rats received an IP injection of either LiCl (0.15 M, 20 ml/kg; $n=5$) (Sigma-Aldrich, St Louis, MO) or vehicle (0.15 M NaCl, 20 ml/kg, $n=5$) (Sigma-Aldrich, St Louis, MO). A subset of rats ($n=8$) were pretreated (after baseline and − 20 min relative to LiCl or vehicle injection) with the GLP-1R antagonist exendin-(9–39) (Ex-9, 100 μg/ml in 0.9% saline, 1 ml/kg, IP; American Peptides, Sunnyvale, CA). The dose of LiCl was chosen based on its ability to: 1) induce signs of visceral malaise (McCann *et al*, 1989; Meachum and Bernstein, 1992; Mitchell *et al*, 1976) and 2) condition a taste aversion with a single IP injection (Bernstein *et al*, 1992), and is therefore considered aversive. The peripheral dose of Ex-9 is at or above those demonstrated to potentiate feeding in rats under certain conditions (Turton *et al*, 1996; Williams *et al*, 2009). Recordings were terminated 60 min after injection of LiCl or vehicle control.

Experiment 2. To examine the ability of Ex-9 to attenuate dopamine suppression by other prolonged aversive agents, we repeated Experiment 1 with the kappa opioid receptor agonist SalvA in place of LiCl. Anesthetized rats ($n=16$) were prepared as in Experiment 1. After baseline, all rats received peripheral pretreatment of either Ex-9 (100 μg/ml, 1 ml/kg, IP, $n=8$) or vehicle (0.9% saline, 1 ml/kg, IP, $n=8$). After an additional 20 min, half of the rats in each pretreatment group were injected with either SalvA (2.0 mg/ml, 1 ml/kg, IP, $n=8$) or vehicle (1 ml/kg, IP, $n=8$ see below). SalvA was provided by Dr Cécile Béguin (McLean Hospital, Belmont, MA) and dissolved in a vehicle of 75% dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO) in distilled water. The dose of SalvA was chosen based on previous studies demonstrating that 2.0 mg/kg induces depressive-like behaviors as measured by intracranial self-stimulation (ICSS) and increased immobility in the forced swim test (Béguin *et al*, 2008; Carlezon *et al*, 2006). Additionally, our lab has previously demonstrated that this dose decreases phasic dopamine release in the NAc (Ebner *et al*, 2010). Recordings were terminated 60 min after injection of SalvA or vehicle.

Experiment 3. Awake rats ($n=11$) were connected to the FSCV headstage in a behavioral chamber. After baseline, rats received an IP injection of LiCl (0.15 M, 20 ml/kg, IP, $n=6$) or vehicle (0.15 M NaCl, 20 ml/kg, IP, $n=5$). Recordings were terminated 60 min after injection.

Experiment 4. Awake rats ($n=20$) were connected to the FSCV headstage in a behavioral chamber. Lateral ICV directed injectors (1 mm projection) were connected to an infusion line loaded with Ex-9 (100 μg/μl) or vehicle (artificial cerebrospinal fluid, aCSF). After baseline dopamine recordings, a pump was used to deliver 1 μl/2 min of Ex-9 ($n=10$) or aCSF ($n=10$) to the lateral ventricle. After an additional 20 min, half of the rats in each pretreatment condition were injected with LiCl (0.15 M, 20 ml/kg, IP, $n=10$) or vehicle (0.15 M NaCl, 20 ml/kg, IP, $n=10$). This ICV dose of Ex-9 attenuates the food intake suppressive effects of systemically delivered GLP-1R agonists (Kanoski *et al*, 2011). Recordings were terminated 60 min after injection of LiCl or vehicle.

Experiment 5. Awake rats ($n=10$) were prepared similarly to Experiment 4. After baseline, rats were pretreated with an infusion of Ex-9 (100 μg/μl, 1μl, $n=5$) or aCSF vehicle ($n=5$) into the fourth ventricle. After an additional 20 min, all rats were injected with LiCl (0.15 M, 20 ml/kg, IP, $n=10$). Recordings were terminated 60 min after injection of LiCl or vehicle.

Histological Verification

Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg; Sigma-Aldrich, St Louis, MO). To verify the recording site, a polyimide-insulated stainless steel electrode (A-M Systems, Carlsborg, WA) was lowered to the DV depth of the carbon fiber during FSCV recording and current was passed to create an electrolytic lesion. When appropriate (Experiments 4 and 5), targeted ventricles were infused with 1 μl of India ink (AMTS, Inc, Lodi, CA) at a rate of 1 μl/2 min. Brains were removed, stored in formalin for 24 h and then transferred to 30% sucrose in 0.1 M phosphate buffer. Brains sections (40 μm) through the NAc and the lateral or fourth ventricle were made using a cryostat and mounted on slides. Lesion location was determined using light microscopy. Cannula placement within a ventricle was verified by presence of ink within the ventricle but not within the parenchyma. Data presented here represent recordings made in the NAc core and central infusions made to either the lateral or fourth ventricles where appropriate.

Data Analysis

Peak oxidative current of dopamine evoked by stimulation trains was measured (see Fortin *et al*, 2015 for details). Data was normalized to the average of the last three consecutive, stable (differing by $<10\%$) stimulations before treatment ('baseline') and expressed as both '% of baseline [DA]' and '% change [DA] from baseline'. In experiments without a pretreatment condition (eg Ex-9 or its vehicle alone; Experiments 3 and 5), a two-tailed student's t-test was used to investigate % change differences between treatment groups at the 60-minute post-LiCl or vehicle time point. For experiments with pretreatment (Experiments 1, 2 and 4), differences in % change between groups were investigated at the 60-minute post-LiCl, SalvA or vehicle control time point using a two-way [pretreatment (vehicle, Ex-9) × post-treatment (vehicle, aversive agent (LiCl or SalvA))] ANOVA. A two-way ANOVA was similarly used to investigate differences in baseline dopamine concentration between groups (Experiments 1–5). Significant ANOVA effects were further explored using Tukey's HSD *post hoc* test. Statistical analyses were performed using GraphPad 5.0 (Prism) and SAS 9.3.

RESULTS

Each train of current pulses to the VTA evoked a dopamine 'transient'—a rapid increase in dopamine concentration in the NAc core that returned to pre-stimulation levels along an exponential decay presumably due to reuptake by the dopamine transporter (2-3 s; Stamford *et al*, 1984). In all experiments, average baseline evoked dopamine concentration did not differ across groups (213.37 ± 12.9 nM; mean ± 1 standard error of the mean for all baseline transients). As such, data was expressed and analyzed as percent change from baseline.

Experiment 1

Figure 1 illustrates representative transients evoked before and after systemic treatment of LiCl or vehicle control. The peak dopamine concentration evoked by electrical stimulation in a representative vehicle treated rat before and 60 min after treatment remained consistent. However, LiCl treatment caused a substantial reduction in the magnitude of the dopamine transient 60 min after treatment relative to before (Figure 1a and b). In Experiment 1, anesthetized rats were pretreated with either nothing ($n=10$) or the GLP-1R antagonist Ex-9 ($n=8$). The average time course of

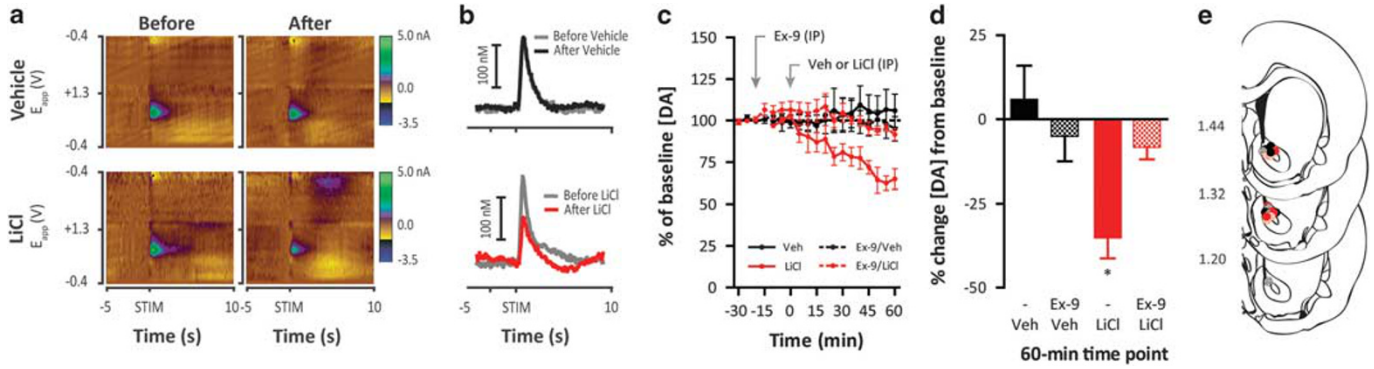

Figure 1 Systemic LiCl decreases phasic dopamine release evoked by electrical stimulation of the VTA via GLP-1 receptors in anesthetized rats. (a) Representative examples of electrochemical data acquired in response to electrical stimulation of the VTA. Colorplots depict changes in current (color) as a function of applied electrode potential (E_{app}; *y*-axis) and time (s; *x*-axis). STIM denotes the time at which a train of current pulses was delivered to the VTA. In all colorplots, dopamine can be observed based on its oxidation (green feature at ~ +0.65 V) and reduction (light yellow feature at ~ − 0.2 V) currents just after the onset of electrical stimulation. Examples were taken just before (left) and 60 min after (right) systemic vehicle (top) or LiCl (bottom) treatment in anesthetized rats. (b) Dopamine concentration over time extracted from the colorplots. Conversion of current from the oxidation of dopamine to concentration was made based on post-recording calibration of electrodes for the examples in 1a. (c) Time-dependent effects of treatments. A subset of rats (broken lines) were pretreated (IP) with Ex-9 after three baseline stimulations. Following an additional four stimulations, these rats were injected (IP) with either LiCl (red) or vehicle (veh; black). Rats without pretreatment (solid lines) were injected (IP) with either LiCl (red) or vehicle (black) after three baseline stimulations. Data are expressed as % of baseline dopamine concentration evoked by electrical stimulation of the VTA once every 5 min. Data points represent group means and error bars are ± 1 standard error of the mean (SEM). (d) Statistical comparison of the average % change in dopamine concentration from baseline at the 60-minute post-LiCl (red) or vehicle (black) time point demonstrates a significant ($p<0.05$) decrease in dopamine concentration in the animals that received LiCl treatment alone ($n=5$; solid red bar) relative to animals that were pretreated with Ex-9 followed by LiCl ($n=4$; stippled red bar), animals treated with vehicle alone ($n=5$; solid black bar) or those pretreated with Ex-9 before vehicle treatment ($n=4$; stippled black bar). Error bars indicate mean ± SEM. (e) NAc recording sites depicted as circles (black filled = vehicle, red filled = LiCl, black crosshatched = Ex-9/vehicle, red crosshatched = Ex-9/LiCl) on coronal sections modified from Paxinos and Watson (2007). Numbers on the left indicate approximate distance from bregma.

treatment effects on evoked dopamine in all four groups (nothing-vehicle, nothing-LiCl, Ex-9-vehicle, Ex-9-LiCl) is shown in Figure 1c. In this and all subsequent studies, we analyzed the 60-minute time point (Figure 1d) for statistical differences between treatment groups. There was a main effect of treatment [vehicle vs LiCl; $F(1,17)=8.64$; $p<0.05$] but no main effect of pretreatment [nothing vs Ex-9; $F(1,17)=1.09$; $p>0.05$]. These main effects were moderated by a significant treatment × pretreatment interaction [$F(1,17)=6.18$; $p<0.05$]. The significant interaction was further explored with Tukey's test, which revealed that the nothing-LiCl (−35.0 ± 7.1% baseline) condition was significantly different compared to all other groups (6.0 ± 7.1, − 5.0 ± 7.4, − 8.4 ± 8.0% baseline for nothing-vehicle, Ex-9-vehicle and Ex-9-LiCl, respectively).

Experiment 2

The kappa-opioid receptor agonist SalvA suppressed evoked dopamine concentration in the NAc core to a similar extent compared to LiCl (Figure 2a). At the 60-minute time point, there was a significant main effect of treatment [vehicle vs SalvA; $F(1,15)=22.2$, $p<0.001$] but no effect of pretreatment [vehicle vs Ex-9; $F(1,15)=0.01$, $p>0.05$] and no interaction [$F(1,15)=0.03$, $p>0.05$]. That is, as shown in Figure 2b, SalvA had a similar effect on evoked dopamine in vehicle (−37.7 ± 9.0%) versus Ex-9 (−38.4 ± 9.6%) pretreated rats. Tukey's test revealed that both SalvA treated groups exhibited significantly reduced evoked dopamine release relative to vehicle treated groups regardless of pretreatment (−2.1 ± 5.7% baseline for vehicle-vehicle and 0.04 ± 6.4% for Ex-9-vehicle).

Experiment 3

Similar to results from anesthetized rats (Figure 1), injection of LiCl in awake rats reduced electrically evoked dopamine release (Figure 3a). The LiCl-induced decrease in dopamine concentration from baseline was significantly different from vehicle treated animals at 60 min (−34.8 ± 10.5% compared to 6.6 ± 12.2% change for LiCl and vehicle treated animals, respectively; $t(9)=2.59$, $p<0.05$; Figure 3b).

Experiment 4

As systemic Ex-9 pretreatment blocked the ability of systemic LiCl to suppress evoked dopamine transients (Experiment 1), we asked whether central GLP-1 receptors contribute to this effect. Indeed, lateral ICV Ex-9 pretreatment attenuated the LiCl-induced suppression of evoked dopamine (Figure 4a). There was a main effect of treatment [vehicle vs LiCl; $F(1,19)=5.3$, $p<0.05$] but no main effect of pretreatment [vehicle versus Ex-9; $F(1,19)=2.3$, $p>0.05$]. Importantly, a significant pretreatment X treatment interaction moderated these main effects [$F(1,19)=7.1$, $p<0.05$]. The significant interaction was further explored with a Tukey's test, which revealed that vehicle-LiCl (−31.3 ± 5.3% baseline) reduced evoked release compared to all other groups (−0.1 ± 5.3, − 7.0 ± 4.0, − 4.3 ± 13.6% baseline for vehicle-vehicle, Ex-9-vehicle and Ex-9-LiCl, respectively; Figure 4b).

Experiment 5

While Ex-9 rescued the LiCl-induced dopamine suppression when delivered to the lateral ventricle, this was not the case when delivered to the fourth ventricle (Figure 5a). Both

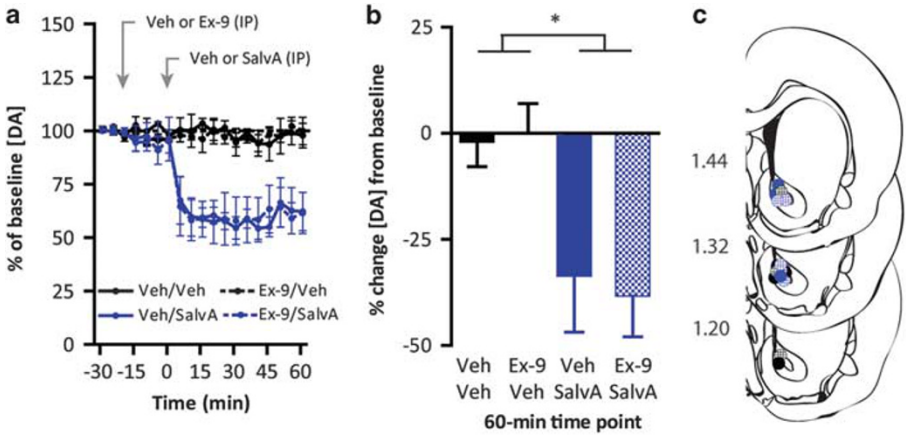

Figure 2 Systemic SalvA decreases electrically-evoked dopamine release independent of GLP-1 receptors in anesthetized rats. (a) Time-dependent effects of treatments. After three baseline stimulations, rats were either pretreated (IP) with Ex-9 (broken lines) or vehicle (veh; solid lines). Following an additional four stimulations, rats were injected (IP) with either SalvA (blue) or vehicle (black). Data are expressed as baseline dopamine concentration evoked by electrical stimulation of the VTA once every 5 min. Data points represent group means and error bars are ± 1 standard error of the mean (SEM). (b) Statistical comparison of the average % change in dopamine concentration from baseline at the 60-minute post-SalvA (blue) or vehicle (black) time point demonstrates a significant (*$p < 0.001$) decrease in dopamine concentration in the animals that received SalvA treatment after either vehicle ($n = 4$; solid blue bar) or Ex-9 ($n = 4$; stippled blue bar) pretreatment relative to animals that received vehicle treatment after either vehicle ($n = 4$; solid black bar) or Ex-9 ($n = 4$; stippled black bar) pretreatment. Error bars indicate mean ± SEM. (c) NAc recording sites depicted as circles (black filled = vehicle/vehicle, blue filled = vehicle/SalvA, black crosshatched = Ex-9/vehicle, blue crosshatched = Ex-9/SalvA) on coronal sections modified from Paxinos and Watson (2007). Numbers on the left indicate approximate distance from bregma.

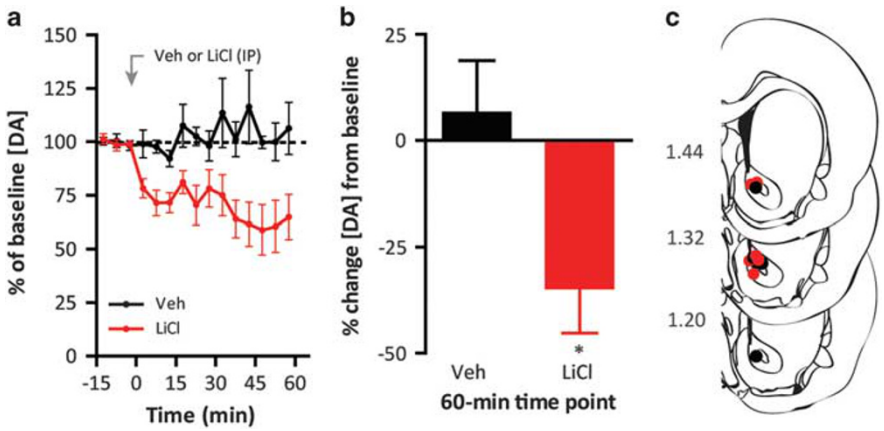

Figure 3 Systemic LiCl decreases electrically-evoked dopamine release in awake rats. (a) Time-dependent effects of treatments. After three baseline stimulations, rats were injected (IP) with either LiCl (red) or vehicle (veh; black). Data are expressed as % of baseline dopamine concentration evoked by electrical stimulation of the VTA once every 5 min. Data points represent group means and error bars are ± 1 standard error of the mean (SEM). (b) Statistical comparison of the average % change in dopamine concentration from baseline at the 60-minute post-LiCl (red) or vehicle (black) time point demonstrates a significant (*$p < 0.05$) decrease in dopamine concentration in the animals that received LiCl treatment ($n = 6$; red bar) relative to animals that received vehicle treatment ($n = 5$; black bar). Error bars indicate mean ± SEM. (c) NAc recording sites depicted as circles (black filled = vehicle, red filled = LiCl) on coronal sections modified from Paxinos and Watson (2007). Numbers on the left indicate approximate distance from bregma.

vehicle and Ex-9 fourth ICV pretreated animals demonstrated a similar decrease in evoked dopamine concentration 60 min after LiCl treatment (−35.0 ± 7.8% and − 35.6 ± 3.4% baseline for vehicle-LiCl and Ex-9-LiCl, respectively; $t(8) = -0.28$, $p > 0.05$; Figure 5b).

DISCUSSION

The present study addressed whether the aversive agent LiCl, which induces visceral malaise and supports aversive conditioning, alters phasic dopamine signaling. We found that systemic administration of LiCl suppressed the magnitude of electrically-evoked dopamine release in the NAc core of both anesthetized (Experiment 1) and awake (Experiment 3) rats. As many of the behavioral effects of LiCl are dependent on intact GLP-1R signaling (Rinaman, 1999b; Seeley *et al*, 2000), we investigated the necessity of GLP-1R availability for the dopamine-suppressive effects of LiCl. We found a role for forebrain (Experiment 4) but not hindbrain (Experiment 5) GLP-1 receptors in mediating the dopamine suppressive effects of LiCl.

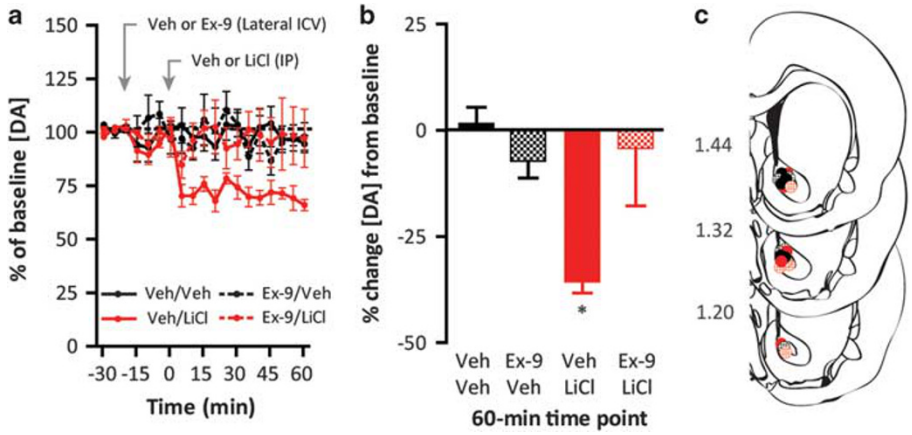

Figure 4 Systemic LiCl decreases electrically-evoked dopamine release via central GLP-1 receptors in awake rats. (a) Time-dependent effects of treatments. After three baseline stimulations, rats were either pretreated (lateral ICV) with Ex-9 (broken lines) or vehicle (veh; solid lines). Following an additional four stimulations, rats were injected (IP) with either LiCl (red) or vehicle (black). Data are expressed as % of baseline dopamine concentration evoked by electrical stimulation of the VTA once every 5 min. Data points represent group means and error bars are ± 1 standard error of the mean (SEM). (b) Statistical comparison of the average % change in dopamine concentration from baseline at the 60-minute post-LiCl (red) or vehicle (black) time point demonstrates a significant (*$p < 0.05$) decrease in dopamine concentration in the animals that received LiCl treatment after vehicle pretreatment ($n = 5$; solid red bar) relative to animals that received LiCl treatment after Ex-9 pretreatment ($n = 5$; stippled red bar) or vehicle treatment after either vehicle ($n = 5$; solid black bar) or Ex-9 ($n = 5$; stippled black bar) pretreatment. Error bars indicate mean ± SEM. (c) NAc recording sites depicted as circles (black filled = vehicle/vehicle, red filled = vehicle/LiCl, black crosshatched = Ex-9/vehicle, red crosshatched = Ex-9/LiCl) on coronal sections modified from Paxinos and Watson (2007). Numbers on the left indicate approximate distance from bregma.

In contrast to the well-established effects of rewarding stimuli on dopamine neurotransmission, the responses of dopamine neurons to aversive stimuli are less clear (McCutcheon *et al*, 2012). The present data strengthen a dopamine-suppressive action of aversive stimuli that is consistent with investigations of discrete stimuli in awake and behaving subjects. These studies utilize electrophysiological and electrochemical recordings to demonstrate pauses in dopamine neuron firing (Cohen *et al*, 2012; Matsumoto and Hikosaka, 2009; Mirenowicz and Schultz, 1996) and dopamine release (Badrinarayan *et al*, 2012; Roitman *et al*, 2008; Wheeler *et al*, 2011) in the NAc following discrete aversive stimuli. Our work extends these findings to include a dopamine-suppressive action of an agent, LiCl, which produces a long-lasting aversive state (Bernstein *et al*, 1992; Tomasiewicz *et al*, 2006). Dopamine neuron responses to stimuli can differ between anesthetized and awake subjects (Koulchitsky *et al*, 2012). We investigated the dopamine response to LiCl in both anesthetized and awake rats and consistently found that LiCl suppressed evoked dopamine release.

The involvement of the GLP-1 system in the behavioral manifestations of LiCl injection has been long supported. Both LiCl and GLP-1 produce similar physiological consequences, many of which are proxies of nausea/ malaise. These effects include a reduction in food intake (McCann *et al*, 1989; Tang-Christensen *et al*, 1996) and gastric emptying (McCann *et al*, 1989; Wettergren *et al*, 1993), generation of CTA (Nachman and Ashe, 1973; Thiele *et al*, 1997) and pica (Mitchell *et al*, 1976; Kanoski *et al*, 2012). GLP-1 antagonists have successfully been used to block the aversive-like behaviors (eg reduction in food intake, pica, CTA) induced by LiCl (Rinaman, 1999b; Seeley *et al*, 2000), indicating that these manifestations of LiCl are, at least in part, mediated through GLP-1R signaling. We found that LiCl-induced suppression of dopamine release in the NAc was dependent on GLP-1R availability. The GLP-1 antagonist Ex-9, when injected systemically or centrally via the lateral ventricle prevented LiCl-induced suppression of dopamine. Thus, this work extends the role of GLP-1 receptors in LiCl's actions to modulation of the mesolimbic dopamine system.

Here, systemic delivery of the GLP-1R antagonist Ex-9 blocked LiCl-induced phasic dopamine suppression. GLP-1 receptors are found in the periphery (Bullock *et al*, 1996; Campos *et al*, 1994) including on vagal afferents (Hayes *et al*, 2014 for review). Peripheral administration of LiCl activates peripheral nerves (eg vagus) that project centrally. Thus, it is possible that the LiCl effects observed here were due in part to GLP-1 release and action in the periphery. However, LiCl (Martin *et al*, 1978) and other emetic agents (Mansouri *et al*, 2008) alter behavior independent of the vagus nerve—suggesting a central locus of action. Indeed, we observed the same effect of GLP-1R blockade on LiCl-induced dopamine suppression when Ex-9 was given centrally (into the lateral ventricle; Experiment 4) compared to systemically (Experiment 1). GLP-1 receptors are expressed throughout the brain (Merchenthaler *et al*, 1999). Therefore, activation of central GLP-1 receptors by peripherally released GLP-1 remains a possible mediator of LiCl-induced dopamine suppression. However, peripherally released GLP-1 undergoes rapid degradation by the enzyme dipeptidyl peptidase IV (DPP-IV) before entering circulation (Hansen *et al*, 1999). Thus, a more plausible explanation for GLP-1R dependent effects of LiCl on dopamine signaling is both a central source and site of action for GLP-1.

In addition to an intestinal source (Eissele *et al*, 1992; Holst, 2007), GLP-1 is produced and released from a group of neurons in the nucleus of the solitary tract (NTS) of the hindbrain (Jin *et al*, 1988; Larsen *et al*, 1997). These hindbrain GLP-1 neurons are activated by peripherally administered LiCl (Rinaman, 1999a). Here, we demonstrate

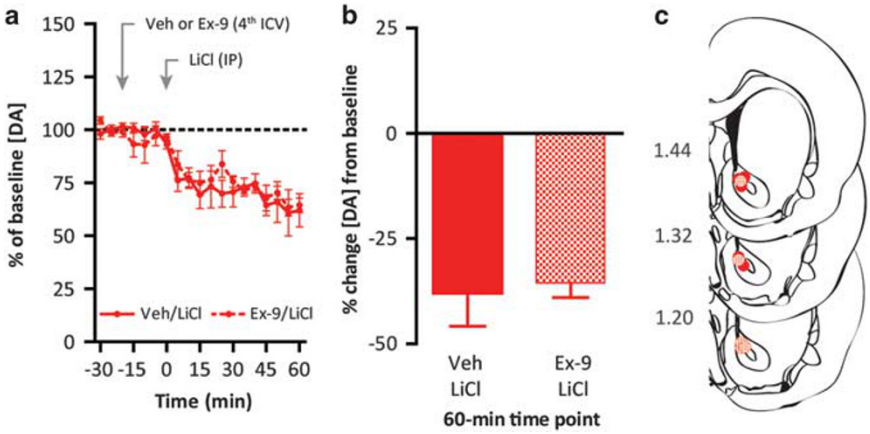

Figure 5 Systemic LiCl decreases electrically-evoked dopamine release via GLP-1 receptors that are rostral to the hindbrain in awake rats. (a) Time-dependent effects of treatments. After three baseline stimulations, rats were either pretreated (fourth ICV) with Ex-9 (red broken lines) or vehicle (veh; red solid lines). Following an additional four stimulations, all rats were injected (IP) with LiCl. Data are expressed as % of baseline dopamine concentration evoked by electrical stimulation of the VTA once every 5 min. Data points represent group means and error bars are ± 1 standard error of the mean (SEM). (b) Statistical comparison of the average % change in dopamine concentration from baseline at the 60-minute post-LiCl time point demonstrates a decrease in dopamine concentration in both ($p > 0.05$) the animals that received LiCl treatment after vehicle pretreatment ($n = 5$; solid red bar) and after Ex-9 pretreatment ($n = 5$; stippled red bar). Error bars indicate mean ± SEM. (c) NAc recording sites depicted as circles (red filled = vehicle/LiCl, red crosshatched = Ex-9/LiCl) on coronal sections modified from Paxinos and Watson (2007). Numbers on the left indicate approximate distance from bregma.

that central GLP-1 receptors are necessary for the LiCl-suppressive effects on phasic dopamine signaling (Experiment 4). Although NTS GLP-1R activation has been shown to suppress aspects of food reward and alter indices of dopamine function (Richard *et al*, 2015), our results suggest that NTS and other caudal brainstem GLP-1 receptors are not involved in LiCl-induced dopamine suppression. Restricting Ex-9 to the hindbrain (fourth ventricle) failed to block the dopamine-suppressive effects of LiCl (Experiment 5).

A more likely candidate for the site of LiCl-induced GLP-1 action observed here (Experiment 4) is the VTA, where GLP-1 receptors are expressed on nearly 50% of dopamine neurons (Toth *et al*, 2011). Furthermore, a subset of NTS GLP-1 producing neurons project directly to the VTA (Alhadeff *et al*, 2012; Dossat *et al*, 2011). Intra-VTA infusion of a GLP-1 agonist decreases palatable food consumption (Alhadeff *et al*, 2012; Dossat *et al*, 2011) and goal-directed behavior for food reward (Dickson *et al*, 2012). While the NAc also receives direct projections from GLP-1 producing neurons (Alhadeff *et al*, 2012; Dossat *et al*, 2011) and GLP-1 R manipulation in the NAc affects food-directed behavior (Dickson *et al*, 2012; Dossat *et al*, 2013), we have recently shown that bath application of the GLP-1R agonist Exendin-4 to NAc slices fails to alter phasic dopamine signaling (Mietlicki-Baase *et al*, 2014). It is therefore more plausible that GLP-1 modulation of dopamine neuron excitability is via direct action in the VTA.

Additional sites for LiCl-induced, GLP-1-mediated suppression of phasic dopamine signaling are possible. For example, the lateral parabrachial nucleus contains GLP-1 receptors that when activated, suppress food intake (Richard *et al*, 2014). Neurons in this region have recently been shown to play an essential role in LiCl-induced conditioned taste aversion learning (Carter *et al*, 2015). These neurons may indirectly influence dopamine signaling through their projection to the central nucleus of the amygdala (Carter *et al*, 2013). The locus of GLP-1 receptors critical in mediating the LiCl-induced suppression of dopamine will be the target of future studies.

While aversive stimuli and aversive agents appear to suppress dopamine, multiple pathways exist to mediate this effect. While we found an essential role for GLP-1 receptors in LiCl-induced suppression, GLP-1 receptors were not necessary for SalvA-induced suppression of dopamine release (Experiment 2). The observed decrease in dopamine signaling following SalvA administration is likely due to activation of kappa-opioid receptors on dopamine terminals in the NAc. Indeed, a kappa-opioid receptor agonist suppresses evoked dopamine release in the NAc in a brain slice preparation (Britt and McGehee, 2008). A behavior indicative of SalvA's aversive properties, conditioned place avoidance, is dependent on kappa-opioid receptors on dopaminergic neurons (Chefer *et al*, 2013). Thus, aversive agents are capable of suppressing dopamine release through multiple pathways.

Understanding the varied pathways by which aversive stimuli suppress dopamine neurotransmission can further elucidate the role of dopamine in associative learning (Schultz, 1998; Steinberg *et al*, 2013) and goal-directed action (Haber, 2014). The unconditioned dopamine-suppressive effects of LiCl observed here may influence the learning process that occurs during the development of a conditioned taste aversion. LiCl, when paired with a novel, palatable taste, like a sucrose solution, conditions voluntary avoidance (Nachman and Ashe, 1973) or active rejection (Parker and Carvell, 1986; Spector *et al*, 1988) of the taste upon subsequent exposure. We have previously shown that pairing of an intra-oral sucrose solution with LiCl can condition dopamine release patterns in addition to behavior. Intra-oral delivery of sucrose in LiCl-naïve animals evokes an increase in NAc dopamine concentration (Roitman *et al*, 2008). However, in rats that have had intra-oral sucrose paired with LiCl, sucrose now suppresses phasic dopamine

signaling (McCutcheon *et al*, 2012). It is possible that the unconditioned dopamine suppressive effects of LiCl observed here serve to alter mesolimbic dopamine neuronal plasticity and are responsible for the changes in dopamine responses to tastes following LiCl pairing. The unconditioned effects of LiCl to suppress phasic dopamine signaling in the NAc may be a critical component in switching behavior from approach to avoidance as a taste aversion develops. In circumstances where associations between aversive drugs and foods are maladaptive, such as in chemotherapy, understanding the processes by which aversive agents act to influence avoidance behavior is essential. Our results strongly implicate the GLP-1R in mediating the unconditioned suppression of dopamine signaling by the emetic agent LiCl and support the GLP-1R as a target in the treatment of maladaptive aversive associations.

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The authors declare no conflict of interest.

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