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When the party is over: depressive-like states in rats following termination of cortical D1 receptor over-expression

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Abstract

Rationale—Increased activity of prefrontal D1 dopamine receptors (D1R) is involved in reward-related behavior found in bipolar disorder and drug addiction. While the effects of elevated D1R are known, depressive-like behaviors also occur in these disorders after reward-seeking ends.

Objectives—The goal is to characterize how termination of D1R over-expression influences depressive-like behaviors.

Methods—An inducible (Tet.On), lentiviral vector was used to manipulate the expression of the *DRD1* gene in glutamate neurons within the prefrontal cortex in male, adult rats. Sexual activity and sucrose preference were studied in both D1R elevated ‘ON’ and relatively reduced ‘OFF’ states. Following termination of the D1R ‘ON’ state, depressive-like behavior was determined in the ‘OFF’ state. Expression of the transcriptional regulator, cyclic AMP-responsive element binding protein (CREB), was used as an indication of down-stream effects in the NA.

Results—‘ON’ D1R expression increased sexual activity that returned to baseline in the ‘OFF’ state. Sucrose preferences increased ~ 6% in ‘ON’ state but fell 11% below control levels when ‘OFF’. Consistent with a depressive-like phenotype, D1R ‘OFF’ decreased activity by 40%, impaired the ability to control (43%) and motivation to escape shock (27% more impaired) relative to dsRed ‘OFF’. CREB increased 29% in the NA in the D1R ‘OFF’ state relative to the ‘ON’ state.

Conclusions—This novel approach demonstrates that elevated D1R expression increased hedonic behavior, whereas the termination of D1R over-expression often resulted in depressive-like behavior. These observations support a role for D1R expression cycling in bipolar-associated behaviors and addiction.

Keywords

addiction; bipolar disorder; hedonia; lentivirus; prefrontal cortex; rat

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Introduction

Dopamine dysfunction has been observed in a number of disorders, but the course of these disorders often wax and wane. Elevations in prefrontal (PFC) dopamine D1 receptors (D1R) are observed in schizophrenia (Paspalas et al. 2013), bipolar disorder (Suhara et al. 1992; Gonul et al. 2009; Yao et al. 2013), and addiction (Kalivas et al. 2005). Too much or too little PFC D1R is also associated with working memory deficits (Goldman-Rakic 1999; Floresco and Phillips 2001; Paspalas et al. 2013). Increased PFC D1Rs on pyramidal neurons, some of which project to the nucleus accumbens (NA), are evident in reinstatement processes associated with cocaine addiction (Kalivas and McFarland 2003; Sonntag et al. 2014), impulsivity (Loos et al. 2010; Sonntag et al. 2014), sucrose preferences (Sonntag et al. 2014), and rewarding social interactions (Tanda et al. 2009). While the role of increased D1R in a number of behaviors is known, we raise the question of what happens to behavior when D1R over-expression on glutamate neurons is terminated.

Bidirectional changes in dopamine function have been studied with sensitizing doses of amphetamine or cocaine followed by their withdrawal (Barr et al. 1999; Stoker and Markou 2011). While studies show that reward-related behaviors are associated with increased D1R (see above), it is possible that relative reduction of D1R may result in decreased reward or anhedonia. Studies have primarily assessed changes in either hedonia or anhedonia with sucrose preference tests (Powell et al. 2011; Overstreet 2012), and these results are often in good agreement with shifts in the threshold in the intracranial self-stimulation paradigm (Der-Avakian and Markou 2012; Donahue et al. 2014). However, these behavioral tests of anhedonia do not fully capture the broader spectrum of behavioral symptoms observed in the human condition of depression (reviewed by Andersen 2015). Other behavioral changes associated with depression include decreased sexual activity (Kennedy and Rizvi 2009), activity, and reduced motivation (avolition). These are rarely studied in animals as a constellation of behaviors, but typically studied independently.

Dopamine-related neural mechanisms and structures associated with depressive-like states are also involved in reward processing, motivation, attention, and decision-making. These brain systems include ventral tegmental projections to the NA, amygdala, anterior cingulate cortex, PFC, and the hypothalamus (Koob and Kreek 2007; Der-Avakian and Markou 2012). Drug withdrawal-precipitated anhedonia reduces general dopamine activity within the PFC (Goldstein and Volkow 2002; Bolla et al. 2004; Kroener and Lavin 2010) and the NA (Salamone et al. 1994; Correa et al. 2002; Der-Avakian and Markou 2012). In contrast, elevated D1R in the PFC reduced NA dopamine D2 receptor expression (Sonntag et al. 2014), further demonstrating a functional link between these two regions.

Currently, we have limited mechanistic insight into the underlying neurobiological processes involved in anhedonia (Kato et al. 2007; Nestler and Hyman 2010). Increases in the transcription-regulating factor CREB (cAMP response element-binding protein) in the NA play a role in depressive- and anxiety-like states (Carlezon et al. 2005) in general and following drug-withdrawal (Muschamp and Carlezon 2013). For example, increased accumbens CREB levels are associated with increased immobility in the forced swim test (Pliakas et al. 2001) and following cocaine withdrawal (Chandra et al., 2015). However,

reduced levels of CREB in the NA shell impair the initiation of sexual behavior, with no effect on ejaculation (Barrot et al. 2005; Wallace et al. 2009).

Viral-mediated gene expression selectively targets specific neuronal populations within a single region. Here, the use of a lentiviral vector allowed us to study the role of D1R in motivated behaviors related to hedonia. Through this approach, we previously found that D1R over-expression on glutamate neurons in the medial PFC (mPFC; although more specifically the prelimbic region) increased impulsivity, sucrose preferences, and cocaine intake (Sonntag et al. 2014). Since D1R is sufficient to elevate reward-related salience that increases hedonic activities, we hypothesized that the absence of D1R elevation is associated with the loss of salience and hedonic activity.

In the present study, increased prefrontal dopaminergic signaling was produced by an *inducible*, cell-specific viral over-expression of D1R in the mPFC. Changes in behavior following changes in D1R expression were examined with an inducible virus system that allowed for over-expression of D1R ('ON' state) and its termination ('OFF' state); animals were tested in both states for sexual behavior, sucrose preferences, and locomotor activity, which were not contaminated by the previous state. Depressive-like behavior was determined in the 'OFF' state following 'ON'. Depressive-like behaviors were studied with active avoidance, which is sensitive to the initial stressor environment (Banasr and Duman 2008), and the triadic model of learned helplessness and is not context-dependent. Both tests involve the mPFC (Amat et al. 2005; Moscarello and LeDoux 2013).

2.0 Experimental procedures

2.1 Animals

Adult, male Sprague Dawley rats (350-375g) were obtained from Charles River Laboratories (Boston, MA). Rats were housed with food and water available *ad libitum* in constant temperature and humidity conditions ($22 \pm 2^\circ\text{C}$ and $55 \pm 25\%$) on a 12-hr light/dark cycle (light period 07:00–19:00). Different sets of rats were used for each experiment (including Western blot analysis), except the animals that were evaluated for sexual behavior who were also assessed for locomotor activity. Only males were used in this study to avoid an interference of estrous cycle-dependent hormonal changes in females on our induced high/low D1R state cycle. The experiments were conducted in accordance with the principles of animal care as set forth in the Guide for the Care and Use of Laboratory Animals (NIH), and were approved by the Institutional Animal Care and Use Committee at McLean Hospital.

2.2 Lentiviral vector and associated procedures

2.2.1 Production—A third generation Tetracycline-On inducible lentiviral vector system (Tet.On) was used. The system expressed the rat D1R (or the control, red fluorescent protein dsRed) driven by a CamKII α promoter in the presence of the tetracycline derivative, DOX. Virus production, concentration by ultracentrifugation, qRT-PCR-based titer determination was performed at the Massachusetts General Hospital Viral Core according to published protocols (Sonntag et al. 2014). Viral expression is shown in cultured cells (Figure

1a), as *in vivo* 'OFF' expression is not possible to detect. Briefly, cells were stained for D1R with rat anti-D1 DAR IgG (1:250; Sigma; secondary: anti-rat TRITC coupled IgG [1:200; Molecular Probes]), or CamKII α in mouse CamKII α IgG (1:250; Chemicon; secondary: anti-mouse Alexa 488-coupled IgG [1:200; Molecular Probes]) in the presence or absence of doxycycline to demonstrate 'ON' or 'OFF'.

2.2.2 Surgery—Rats were anesthetized with a ketamine/xylazine mixture (80/12 mg/kg) and received 1 μ l of virus (2×10^7 transducing units per μ l) bilaterally into the mPFC at stereotaxic coordinates (AP +2.7, ML: 0.4; DV: -2.8) of (Paxinos et al. 1980).

2.2.3 Virus placement and expression—Virus placement was confirmed histologically after the behavioral testing by inducing expression with DOX (Sigma-Aldrich, St. Louis, MO; see below). Subjects were perfused with 4% paraformaldehyde, the brains cryoprotected and cut into 40 μ m sections. After blocking in 10% donkey serum, sections were exposed to 1:250 antibody to the rat D1R IgG (Sigma-Aldrich), amplified with anti-rat IgG secondary, and visualized using standard diaminobenzidine or fluorochrome procedures (Brenhouse et al. 2008). Figure 1b shows where viral placement occurred. Subjects where the majority of placement was not within the mPFC were excluded from all analyses (n=3). D1R expression in the D1R 'ON' condition was increased \sim 183% of the dsRed 'OFF' group — about half of what we previously reported with the constitutive virus (Sonntag et al., 2014), but within physiological levels.

2.3.4 Doxycycline (DOX) treatment—Virus expression was controlled by 0.5 g/l DOX hyclate (Sigma-Aldrich) in the drinking water to produce an 'ON' state. This dose was based on preliminary studies conducted in the elevated plus maze, as we have shown constitutive D1R over-expression increases time spent in the open arms (Sonntag et al. 2014; data not shown). Full viral expression occurred within seven days after surgery/DOX exposure was initiated ('ON') and over-expression was reduced after three days of DOX removal ('OFF' state). D1R expression was confirmed for each subject in the 'ON' state prior to sacrifice.

2.4 Behavioral Assessments

Behaviors were selected to show a contrast between hedonic and anhedonic behavior. However, sexual behavior and sucrose preferences demonstrated state changes (e.g., 'ON' and 'OFF' states of D1R modulation) that did not appear contaminated by prior D1R expression states. Depressive-like behavior was demonstrated with active avoidance and the triadic model, where controllability in the escapable group is linked to the PFC (Amat et al. 2005) and avolition has been associated with lack of escape in the no shock group (Pryce et al., 2011). The depressive behaviors were not amenable to repeated testing due to carryover effects of the prior shock exposure.

2.4.1 Sexual behavior—Sexual behavior was tested for two days in the 'ON' state and 'OFF' state across throughout three cycles (timeline in Figure 2c). Testing occurred during the dark cycle. Each animal was put in a 26.5 \times 50.8 \times 32.4 cm glass observation chamber and allowed to habituate for 5 min. An ovariectomized female was treated with estradiol

benzoate (20 µg, subcutaneous 48h before testing) and progesterone (500 µg, subcutaneous 4h before testing) to induce sexual receptivity and was placed in the chamber with the male. Females were randomized between subjects and testing days. Mounts, intromissions and ejaculations were recorded for 25 min (Ågmo 1997; Barr et al. 1999). These same animals were then removed from DOX water for seven days and then re-tested in the 'OFF' state. This 'ON'/'OFF' cycle was repeated 3 times (timeline in Figure 2b).

2.4.2 Sucrose preference test—A two-bottle preference test (Willner et al. 1987; John et al. 2012) with three different sucrose concentrations was conducted. Rats were singly housed, and two water bottles in their home cage were weighed daily. Both bottles were filled with 0.5g/l DOX in the water and baseline drinking was assessed; no pre-existing side preferences were detected ($44.1 \pm 3\%$ consumed from one bottle). After four days of baseline, sucrose concentration was increased every two days from 0.25% to 0.5% to 1%. The amount of sucrose consumed was expressed as total liquid consumed each day. Animals were then placed in the 'OFF' condition for three days and the procedure was repeated (Figure 2d).

2.4.3 Locomotor activity—As we have previously shown that D1R over-expression does not produce changes in activity (Sonntag et al. 2014), subjects were initially tested in the 'OFF' stage after seven days in the 'ON' state and then tested again in the 'ON' state after seven days of DOX. Rats were placed in a $24 \times 18 \times 33$ cm dimly lit box (MedAssociates Inc, St Albans, VT) and the number of beam breaks was recorded for 1 hour during the light cycle.

2.4.4 Active avoidance—Active avoidance measures depressive-like behavior within the same context as the initial shock exposure. Subjects were tested in the 'OFF' state, three days after the termination of seven days in the 'ON' state. On Day 1, subjects received 60 0.65mA shocks (inter-trial interval of 40 ± 10 sec for both days) that were signaled by a tone and light and not allowed to escape from one side of a two-sided shuttlebox (Med Associates Inc, St Albans, VT). On Day 2, the tone and light signaled a 0.85mA shock, but subjects could avoid the shock by escaping to a second chamber. Latency to escape shock was measured.

2.4.5 Learned helplessness—The triadic model of learned helplessness allowed us to parse different aspects of depressive-like behavior (Amat et al. 2005; Pryce et al. 2011; Freund et al. 2013). Three conditions were tested. 1) escapable shock (ES), where the subject controls shock termination by turning a wheel on Day 1; this condition reflects activity within the PFC (Amat et al. 2005). 2) Inescapable shock (IS), where subjects were yoked to ES animals and likely reflects hippocampal activity. 3) No shock (NS), where subjects were restrained in a wheel-turn box and received no shock until Day 2; NS reflects motivation (Pryce et al., 2011). This helplessness paradigm tests in a second context than training, unlike active avoidance. Animals were tested after three days in the 'OFF' state, which followed the 'ON' state. On Day 1, subjects underwent 100 trials of a tail shock delivered on a variable interval (45 s) schedule that had escalated shock intensity to prevent habituation (1.0 mA for trials 1-30, 1.3 mA for trials 31-60, and 1.6 mA for trials 61-100).

On Day 2, subjects were placed into a shuttlebox (Med Associates Inc, St Albans, VT) and could terminate a 1 mA foot shock (signaled by a 3s warning tone) by shuttling to the other side for trials 1-5 (FR1), or by shuttling to the other side and back again for trials 6-30 (FR2). The shock was terminated after 30 s if the animal failed to shuttle as required. Depressive-like behavior was measured as the mean latency to escape in the last 25 trials.

2.5 Western immunoblot for CREB expression

Subjects were either sacrificed in the 'ON' state (9 days of DOX exposure) or sacrificed in the 'OFF' state (seven days on DOX followed by three days of DOX termination to assure that the contrast between 'ON' and 'OFF' would manifest). The mPFC was dissected for confirmation of D1R expression in all groups, which was used as a covariate in the analyses. The NA was also dissected, although subregions were not assessed, to determine CREB levels and stored at -80 °C until processing. Tissue was then homogenized in 1% sodium dodecylsulfate solution (SDS) containing a protease inhibitor cocktail (Pierce, Rockford, IL). Protein concentration was determined by the Bradford method (BioRad Laboratories, Hercules, CA; Bradford, 1976) and 40 µg of protein was mixed in 6 × SDS, centrifuged, and boiled for 3 min prior to separation by 15% SDS-PAGE. Following electrophoresis, proteins were transferred to a nitrocellulose membrane (BioRad Laboratories). The membranes were blocked with Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) in phosphate-buffered saline for 60 minutes at RT and incubated with primary polyclonal antibodies to CREB (43kDa; 1:400; rabbit; Cell Signaling Technologies, Danvers, MA), and D₁ receptors (52kDa; 1:400; rat; Sigma, St. Louis, MO) in Odyssey blocking buffer (LI-COR Biosciences) in PBS containing 0.1-0.2% Tween overnight 4 °C. The membranes were rinsed four times for five minutes at RT in PBS-T. After the rinsing procedure, the membranes were incubated for one hour at room temperature in IRDye 800-conjugated anti-rabbit (LI-COR Biosciences) or anti-rat (LI-COR Biosciences) 1:20,000 in Odyssey blocking buffer in 0.1% PBS-T. Protein loading was assessed by β-tubulin (55 kDa; 1:10,000, Covance Laboratories, Dedham, MA; secondary: anti-mouse β-tubulin for IRDye 700-conjugated anti-IgG [H&L; LI-COR Biosciences]) in PBS-T. An Odyssey infrared imaging system (LI-COR Biosciences) with excitation/emission filters at 780 nm/820 nm range was used to detect fluorescence. Protein values were normalized to β-tubulin expression.

2.6 Statistical analysis

Data were analyzed by 3-way ANOVA with Virus (D1R or dsRed), State ('ON' or 'OFF'), and Session (1-3; for sexual behavioral data) or Concentration (0.25%, 0.5%, 1%: for sucrose preference data) as variables using SPSS v.20 (IBM Corp). Virus was a between-subject variable and State, Session, or Concentration were within-subjects variables. A one-way ANOVA was used to analyze the active avoidance paradigm. Data for the learned helplessness paradigm were analyzed with a 2-way ANOVA with Virus and Group (ES, IS or NS) as independent variables. Bonferroni correction was used for any post-hoc comparisons between individual Group conditions to determine the effects of Virus.

CREB data were corrected for tubulin amounts, and analyzed with a Virus × State (in this case it was a between-subjects variable) ANCOVA and covaried for levels of mPFC D1R

from each subject (included both endogenous and virally-transduced expression levels). Bonferroni correction was used for post-hoc comparison in the D1R condition alone, consistent with our *a priori* hypothesis that change would only occur in the D1R group. Significance was set at $p < 0.05$.

3.0 Results

3.1 Sexual behavior

Animals transduced with either the D1R or dsRed were tested for sexual behavior across three 'ON'/'OFF' cycles. An 'ON'/'OFF' X Virus interaction for the frequency of sexual mounts was found ($F_{1,14} = 4.658$; $P = 0.049$). When in the 'ON' state, D1R animals showed more sexual mounts compared to dsRed animals, but in the 'OFF' state, D1R animals returned to control levels. Overall, D1R subjects had more mounts than dsRed subjects (Virus main effect: $F_{1,14} = 10.847$; $P = 0.005$; Figure 2a). Post-hoc comparisons shows that increased mounting behavior occurred during the first two 'ON' cycles (Bonferroni correction, $P < 0.05$). The same pattern was found for successful intromissions (i.e., percentage of intromissions that resulted in ejaculation; not shown) with D1R 'ON' animals having more ejaculations per intromission. When D1R was turned 'OFF', D1R subjects did not differ from dsRed animals ('ON'/'OFF' X Virus interaction: $F_{1,15} = 10.727$; $P = 0.005$). Taken together, sexual activity increased following the over-expression of D1R and returned to baseline when the over-expression was terminated. This cycling of sexual activity was observed throughout multiple 'ON'/'OFF' cycles.

3.2 Sucrose preference test

This second test of hedonic activity showed cycling between hedonia and anhedonia. In the two-bottle test of sucrose preference where animals could choose between water or 0.25, 0.5, or 1.0% sucrose, both D1R and dsRed subjects demonstrated concentration-dependent preferences for sucrose (Concentration main effect: $F_{1,20} = 156.74$; $P < 0.0001$). A Virus X 'ON'/'OFF' interaction ($F_{2,20} = 3.43$; $P = 0.05$; Figure 2b) shows that the sensitivity to sucrose concentrations depended on 'ON' or 'OFF' states indicating a 95% chance of correctly rejecting the null hypothesis. Sucrose preference was noticeably increased at the 0.25% sucrose solution in the D1R 'ON' animals compared to dsRed animals (post-hoc comparison with Bonferroni correction, $P < 0.05$). In the D1R 'OFF' state, however, sucrose preferences not only returned to baseline, but decreased compared to controls (for the high concentrations; Bonferroni correction, $P < 0.05$).

3.3 Locomotor activity

A 60 min open field test was used to determine the relationship of D1R to general locomotor activity (Figure 3). As this behavior is subject to novelty-induced activity when initially assessed, transduced subjects were tested in the 'OFF' state first (after experiencing seven days in the 'ON' state followed by three days 'OFF'), and then re-tested in the 'ON' state after seven days on DOX. D1R animals show a trend to be less active compared to dsRed animals (Virus main effect: $F_{1,15} = 3.571$, $P = 0.079$; Figure 3) that is driven by changes within the 'OFF' state (Bonferroni correction, $P < 0.05$).

3.4 Active avoidance

Depressive-like behavior was assessed in the 'OFF' phase following prior 'ON' state exposure. The results from this paradigm indicated no difference between Virus groups (Figure 4a; One-way ANOVA: $F_{1,18} = 0.01$, $P=0.9$).

3.5 Learned helplessness

D1R 'OFF' animals showed increased helplessness relative to dsRed controls that depended on the helplessness condition (latency to escape the shock; Virus X Group interaction: $F_{2,35} = 3.3$, $P=0.05$, indicating a 95% chance of correctly rejecting the null hypothesis). To determine which helplessness condition was different between virus group, post-hoc directed comparisons revealed that D1R 'OFF' subjects in the ES ($P=0.006$) and NS ($P=0.03$) group demonstrated significantly longer latencies to escape relative to dsRed controls; no differences were found between D1R 'OFF' subjects and controls in the IS group (Figure 4b).

3.6 CREB Western immunoblot in the NA

A Virus \times 'ON'/'OFF' ANCOVA was conducted, where 'ON'/'OFF' was a between-subjects variable and data co-varied for individual mPFC D1R expression (both endogenous- and virally-mediated expression), which was assessed in separate blots. While the overall interaction was not significant ($F_{1,19}=1.49$, $P=0.2$), the *a priori* prediction was that only a difference in CREB expression would be evident in the D1 animals. As such, an additional t-test with Bonferroni correction between D1R 'ON' and D1R 'OFF' was significant ($t_{10} = 2.58$, $P=0.03$).

4.0 Discussion

We show for the first time that manipulation of D1R within a single animal induces different states of behavior. Similar mechanisms may be found in bipolar disorder (Suhara et al. 1992; Yao et al. 2013), addiction (Ponce et al. 2015), and schizophrenia (Paspalas et al. 2013). Over-expression of D1R increases sexual activity and sucrose preferences, which fell below control values when D1R over-expression is reduced. Importantly, the data show that a reduction in D1R is sufficient to increase learned helplessness that is mediated by changes in the mPFC in the ES controllable shock condition (Amat et al. 2005) or PFC modulation of the NA in the case of the NS condition (Pryce et al. 2011). The use of the CK.D1 virus provides strong evidence that mPFC D1R on glutamatergic outputs mediates these behaviors.

The present data maybe most appropriately applied to the cycling between mania and depression (DeBello et al. 2008). Moreover, these data focus the broad dopamine hypothesis of bipolar disorder (Berk et al. 2007) on D1R in the mPFC by demonstrating its role in many of these behavioral states. Previous studies have linked mania to increased dopaminergic signaling (Murphy et al. 1971; Young et al. 2011) and depression with decreased dopaminergic signaling (Xing et al. 2006; Tye et al. 2013). Postmortem studies indicate a role for D1R in bipolar disorder (Pantazopoulos et al. 2004; Yao et al. 2013; Suhara et al. 1992). Here, D1R 'ON' increases sexual activity, which is often found

patients with mania (Basco and Celis-de Hoyos 2012; Dvorak et al. 2013), and induces hedonia (indicated by increased sucrose preference). Furthermore, we have previously demonstrated that elevated D1R on glutamatergic neurons in the mPFC increases impulsivity, novelty seeking, drug-seeking and taking while it decreases anxiety (Sonntag et al. 2014). All these behaviors are evident in the D1R 'ON' animals and consistent with a mania-like state.

The neural circuitry that underlies the effects of D1R 'OFF' on depressive-like behavior may further help define the role of the mPFC in the different symptoms of depression (Andersen 2015). The PFC modulates multiple regions in the brain that have been implicated in depression, including the NA, the dorsal raphe nucleus, the hippocampus, and the amygdala – the first three of which are affected by D1R changes.

Behaviors associated with hedonia/anhedonia are typically associated with changes in the NA. Relative to dsRed subjects, the increased sexual activity (e.g., the number of intromissions) in the D1R 'ON' subjects returns to a low baseline when D1R is 'OFF.' Reduced locomotion, also associated with depression (Pryce et al., 2011), is also evident in the 'OFF' state. While reduced activity could impair sexual behavior, the same pattern is observed for intromissions that result in an ejaculation, which requires little locomotion. A relative increase in CREB expression in the D1R 'OFF' condition was observed in the NA. Increased levels of accumbens CREB are associated with depressive-like states in rats with the forced swim test (Pliakas et al., 2001) or following drug withdrawal (Muschamp et al., 2011; Chandra et al., 2015). We observed an increase in CREB in the 'OFF' D1R condition, but not during the 'ON' state when sexual behavior was most effected; no change in CREB was observed in the dsRed subjects in either state. Our findings of a return to baseline of sexual activity in the D1 'OFF' seem to be in contrast to a report of reduced CREB expression (by a dominant negative mutant CREB virus) in the NA shell that is associated with increased latency to mount a receptive female and time to intromission during the first experience; mutant CREB was not associated with the number of mounts needed for ejaculation (Barrot et al. 2005). These findings were observed during the first encounter (D1R 'ON' state). Our CREB changes could possibly reflect downstream effects of D1R overexpression on different populations of neurons than those targeted by the mutant CREB virus. To this end, both core and shell of the NA were included in our sample.

Depressive behavior that is relevant to PFC D1R expression likely involves neural circuits that include the dorsal raphe nucleus and the NA. Under circumstances where helplessness is induced and context is associated with a predictive cue that signals shock (e.g., the tone in the active avoidance paradigm), the D1R 'OFF' animals show no apparent deficits. This finding is in line with Kram et al. (2002) who showed no correlation between mPFC D1R and helplessness when the context between training and testing did not change. However, the context change between training and testing in the triadic model reveals escape deficits in ES and NS animals. ES behavior involves mPFC modulation of the dorsal raphe nucleus (Amat et al. 2005). Consistent with reduced mPFC output during depressive states (Robbins 2005), D1R 'OFF' animals are impaired in the ES condition as they fail to transfer the ability control shock to a new environment. This behavioral observation is similar to previous

observations in rats that underwent maternal separation and have reduced D1R on glutamate output neurons in the mPFC (Leussis et al. 2012; Brenhouse et al. 2013).

Avolition and hedonia/anhedonia are associated with the NA (Muschamp et al. 2011; Berridge and Kringelbach 2013) and the mPFC 'OFF' state may have reduced modulation of the NA. The observed increased latency to escape in the NS group is probably related to motivational changes associated with the NA (Pryce et al. 2011). D1R expression in the mPFC projections to the accumbens plays a role in motivated behaviors (Richard and Berridge 2013; McFarland et al. 2003; Sonntag et al. 2014). While it is possible that the animals may have difficulty learning the contingency to escape or that reduced activity contributed to a "depressive-like" phenotype, the data in Figures 4 c and d argue against this hypothesis. D1R animals show no differences from dsRed animals for trials 1-5 (FR1 to escape) or trials 6-10 (FR2). Rather, a progressive impairment in escape latencies is observed across sessions. The constellation of reduced sexual behavior, stressor controllability (the ES group), motivation (the NS group), and sucrose-drinking in the D1R 'OFF' state are all suggestive of an anhedonic/depressive-like state. These behaviors may be due to either the contrast of the D1R 'ON' experience or a compensatory mechanism; the title "When the party is over..." describes the contrast that appears during drug withdrawal or post-mania.

This hypothesis of reduced dopamine signaling leading to depressive-like behavior is further supported by clinical data showing that dopamine agonists are helpful in bipolar depression (Dell'Osso et al. 2013). Extensive PFC D1R stimulation can result in a functional disconnection (Hains and Arnsten 2008). We hypothesize that the D1R over-expression induces autoregulatory mechanisms leading to a hypo-expression in the 'OFF' state. The result is decreased dopaminergic output signaling of the mPFC that leads to anhedonia and depressive-like behavior. Parkinson's patients who are treated with dopamine agonists can exhibit mania (Weintraub 2008), while tapering off of a dopamine agonist increases risk to develop depressions (Nirenberg 2013). These observations may be mediated by the D1R.

Here, we focused on accumbens CREB as a downstream target of reduced PFC modulation. In line with our findings, others have shown that CREB over-expression in accumbens reduces sucrose preference (Barrot et al. 2002), induces depressive-like behavior (Pliakas et al., 2001), and is associated with aversive states of drug withdrawal (Muschamp and Carlezon, 2013). Our earlier study on mPFC D1R overexpression demonstrated a reduction in D2 receptors in the NA (Sonntag et al., 2014). By isolating D1- and D2-expressing medium spiny neurons (MSNs) in the NA, a recent study demonstrated that repeated cocaine followed by withdrawal increased and decreased CREB in D1- or D2-expressing MSNs, respectively (Chandra et al., 2015). The data by Chandra and colleagues offer insight into the complexity of CREB signaling. Medial PFC D1R overexpression may affect the D2 MSNs more directly, whereas the compensatory actions of either D1R 'OFF' or cocaine withdrawal might be reflected in the D1 MSNs. Bi-functionality of CREB activities within the NA can facilitate reward-associated behaviors while simultaneously modulating dysphoria (Dineri et al., 2009). CREB regulation of reward behaviors is complex and is region- (even sub-region) and form- (phosphorylated or not) dependent (Zhong et al., 2014; Olson et al., 2005). The main focus of the current study is the induction of cycling behavior

by manipulating a single receptor in the mPFC. Inclusion of the CREB Western blots was to simply show downstream pathways are also affected by our manipulation.

Future studies will examine other regions downstream of the mPFC that are involved in depressive behaviors. Decreased mPFC modulation of the raphe in the 'OFF' state may underlie increased escape latency of the ES group in the triadic paradigm (Amat et al., 2005). Reduced activity in the ventral tegmental area (Eisch et al. 2003; Minton et al. 2009) could also induce depressive-like behaviors. Taken together, elevated mPFC D1R increased hedonic-like behaviors (sex, sucrose preferences), but more importantly, termination of D1R over-expression induced anhedonic- and depressive-like behaviors (decreased sucrose preferences, locomotor retardation, helplessness). The D1R expression pattern of 'ON'/ 'OFF' may therefore play an important role in the switch from mania/hedonia to depression/anhedonia that is clearly evident in bipolar patients, but also in individuals with schizophrenia or addiction. An inducible virus provides a new tool to study specific circuits underlying multiple behaviors.

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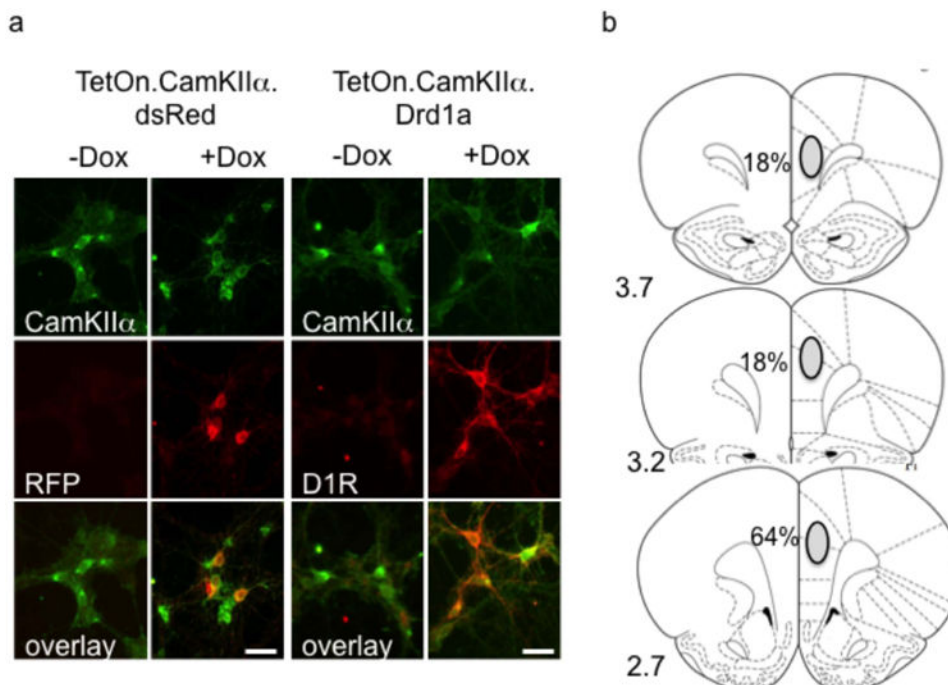


Fig. 1. The inducible CamKII α dependent lentiviral system in vitro and histological confirmation in vivo. (a) Virus-transduced rat E18 cortical primary cultures showing that D1R and dsRed (red fluorescent protein; RFP) are expressed in CamKII α positive cells depending on the presence of doxycycline (here in a concentration of 200 ng/ml). The size bar represents 20 μ m. (b) Location of D1R virus injections in the mPFC by histological confirmation and the percentage of subjects where the main portion of the bolus was found. Site location referenced to bregma coordinates

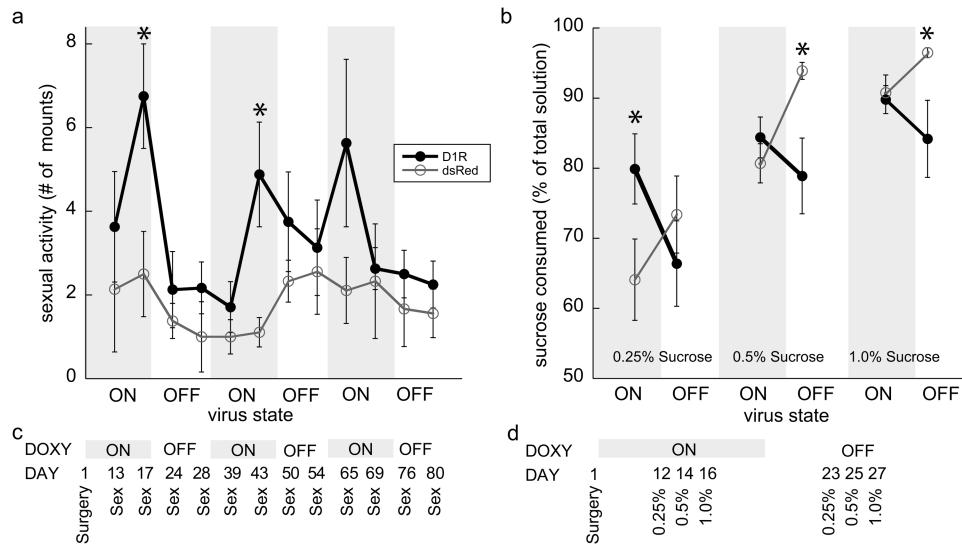


Fig. 2. Cycling of behaviors. (a) Number of sexual mounts following over-expression of D1R (black circles) following doxycycline exposure (ON' [grey shaded area]) compared to dsRed controls (white circles). Over-expression is terminated ('OFF') when doxycycline is removed. n=8 D1R and n=9 dsRed subjects (b) Sucrose preferences in the ON' (grey shaded area) and 'OFF' states. (c) + (d) Timelines of the testing schedule for sexual behavior and sucrose preferences. Means \pm SEM are presented for n=10 D1R and n=9 dsRed subjects * p<0.05, Bonferroni correction indicating significant differences within each state and/or concentration

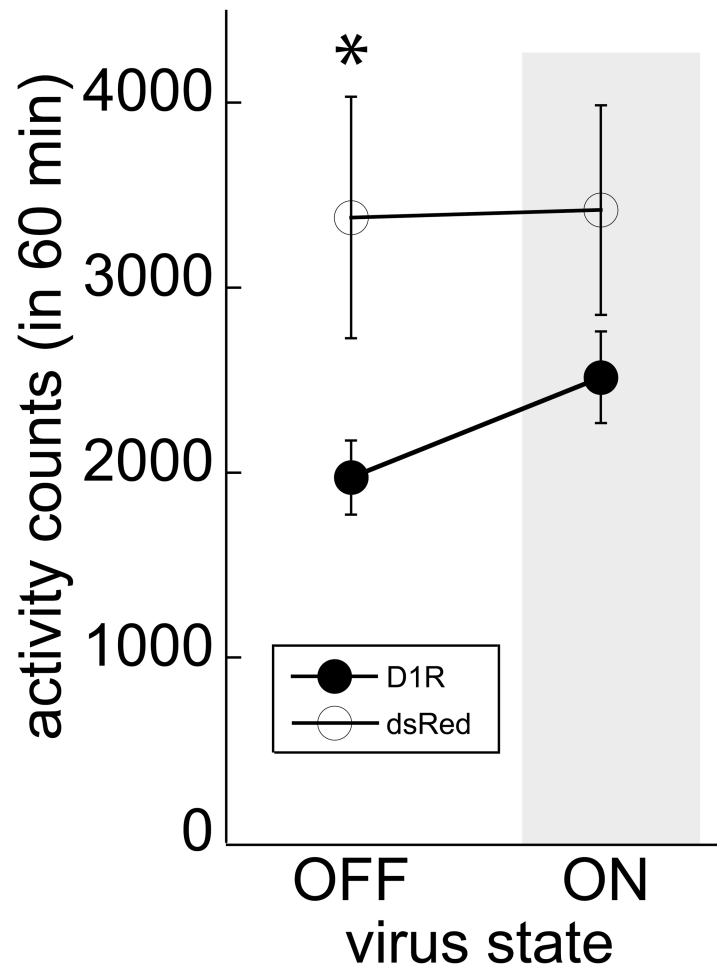


Fig. 3. Locomotor activity in the ‘OFF’ and ‘ON’ state. D1R animals (black circles) in the ‘OFF’ state were less active but did not show activity differences in the ‘ON’ (grey shaded area) state compared to dsRed animals (white circles). Means \pm SEM are presented for $n=8$ D1R and $n=9$ dsRed; * $p<0.05$

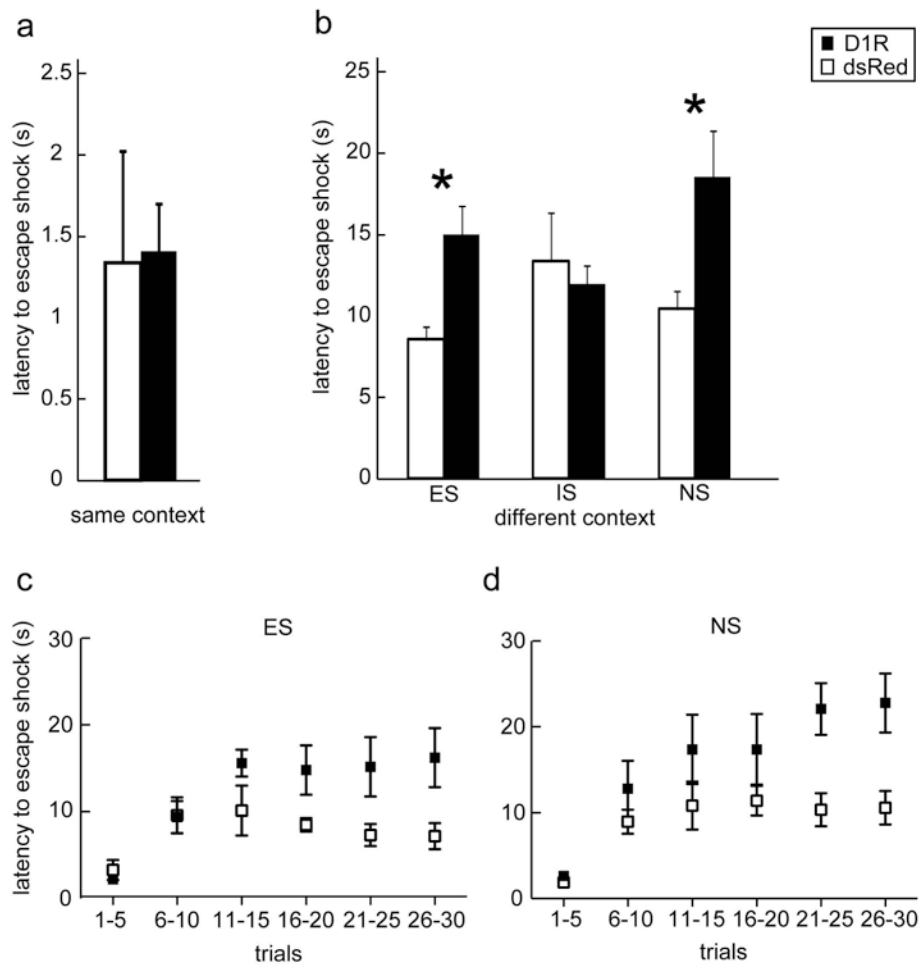


Fig. 4. Depressive-like behavior in the ‘OFF’ state. (a) In an active avoidance paradigm where training and testing occur in the same context; D1R (black bars; $n=11$) and dsRed (white bars; $n=8$) do not differ. (b) In the triadic learned helplessness paradigm, behavior was trained and tested in different contexts; during testing, shock was escapable (ES group), inescapable (IS group), or no shock (NS group). $n=5-7$ per virus condition and group. (c) and (d) illustrate how escape behavior changes across trials; D1R (black squares) and dsRed (white squares) do not differ in the first block of trials, suggesting no impairment in learning to escape initially. Bars represent means \pm SEM; * $p<0.05$

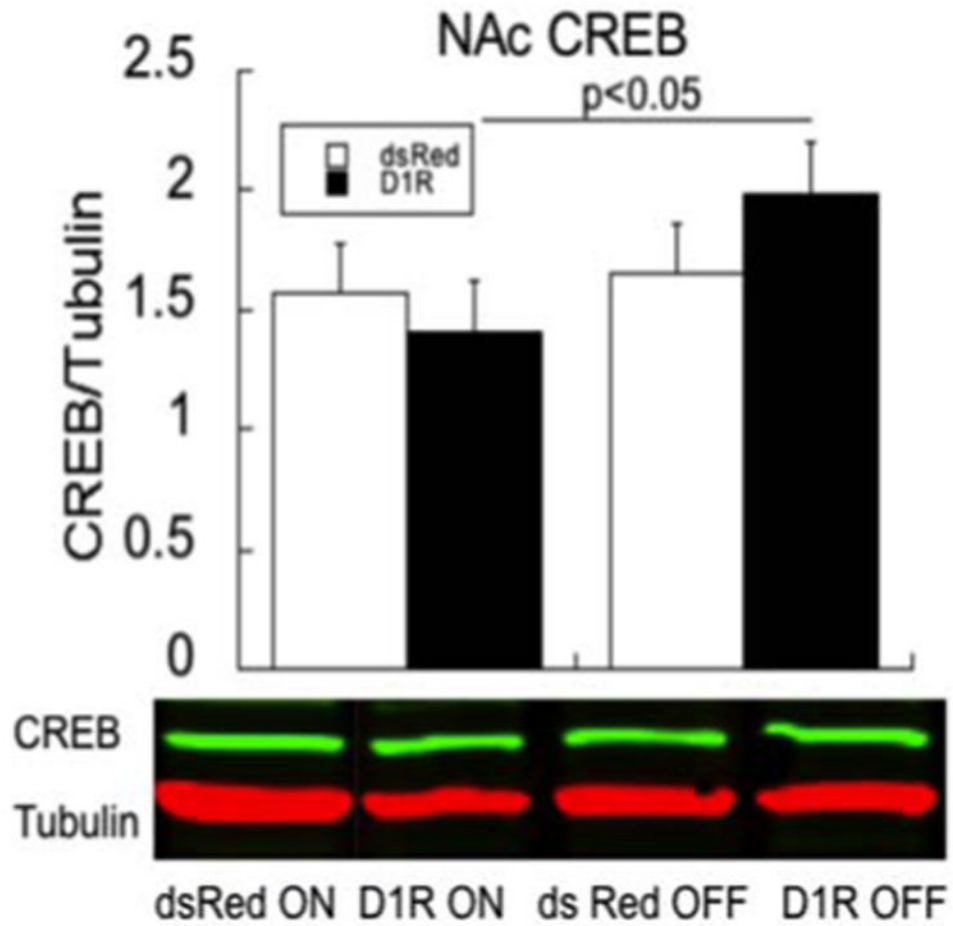


Fig. 5. CREB changes in the 'ON' and 'OFF' states in the NA in dsRed (white bars) and D1R (black bars) subjects; n=6 for each of the four groups. Data were co-varied for pLPFC D1R expression. Bars represent means \pm SEM; * p<0.05. Shown below are representative bands of CREB and the control protein, tubulin. Bars represent means \pm SEM