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Opiate exposure state controls dopamine D3 receptor and cdk5/calcineurin signaling in the basolateral amygdala during reward and withdrawal aversion memory formation

Laura G. Rosen[a,b], Walter J. Rushlow[a,b,c], Steven R. Laviolette[a,b,c,d,*]

[a] Addiction Research Group, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada
[b] Department of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada
[c] Dept. of Psychiatry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada
[d] Dept. of Psychology, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada

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ABSTRACT

The dopamine (DA) D3 receptor (D3R) is highly expressed in the basolateral nucleus of the amygdala (BLA), a neural region critical for processing opiate-related reward and withdrawal aversion-related memories. Functionally, D3R transmission is linked to downstream Cdk5 and calcineurin signaling, both of which regulate D3R activity states and play critical roles in memory-related synaptic plasticity. Previous evidence links D3R transmission to opiate-related memory processing, however little is known regarding how chronic opiate exposure may alter D3R-dependent memory mechanisms. Using conditioned place preference (CPP) and withdrawal aversion (conditioned place aversion; CPA) procedures in rats, combined with molecular analyses of BLA protein expression, we examined the effects of chronic opiate exposure on the functional role of intra-BLA D3R transmission during the acquisition of opiate reward or withdrawal aversion memories. Remarkably, we report that the state of opiate exposure during behavioural conditioning (opiate-naïve/non-dependent vs. chronically exposed and in withdrawal) controlled the functional role of intra-BLA D3R transmission during the acquisition of both opiate reward memories and withdrawal-aversion associative memories. Thus, whereas intra-BLA D3R blockade had no effect on opiate reward memory formation in the non-dependent state, blockade of intra-BLA D3R transmission prevented the formation of opiate reward and withdrawal aversion memory in the chronically exposed state. This switch in the functional role of D3R transmission corresponded to significant increases in Cdk5 phosphorylation and total expression levels of calcineurin, and a corresponding decrease in intra-BLA D3R expression. Inhibition of either intra-BLA Cdk5 or calcineurin reversed these effects, switching intra-BLA associative memory formation back to a D3R-independent mechanism.

1. Introduction

Chronic exposure to opiates can induce plastic alterations in dopamine (DA) receptor function in brain regions involved in associative memory formation (Lintas et al., 2011, 2012). One such area, the basolateral nucleus of the amygdala (BLA), contains high levels of the dopamine (DA) D3 receptor (D3R; Gurevich and Joyce, 1999; Vorel et al., 2002; Beaulieu and Gainetdinov, 2011; Avalos-Fuentes et al., 2013), a member of the D2 receptor family that is critically involved in addiction-related memory processing (Yan et al., 2013). Indeed, D3R transmission in the amygdala influences the acquisition and expression of appetitive Pavlovian behaviors and opiate-related associative memory cues (Hitchcott and Phillips, 1998; Galaj et al., 2015). Chronic exposure to drugs of abuse has been shown to induce plastic adaptations in the D3R system as demonstrated in post-mortem tissue from cocaine (Staley and Mash, 1996; Segal et al., 1997) and opiate-dependent individuals (Cosgrove, 2010). Furthermore, D3R-related genetic polymorphisms have been observed in individuals expressing early-onset heroin dependence (Kuo et al., 2014) and in those with a history of heroin addiction (Levran et al., 2014). Nevertheless, the precise functional role of intra-BLA D3R transmission in the processing of opiate-related learning and memory is not well understood.

Associative memory processing in the BLA involving select DA receptor subtypes and their downstream signaling cascades have been identified as critical players in opiate-related learning and memory (Lintas et al., 2011). For example, previous evidence has identified an

* Corresponding author at: 468 Medical Science Building, Department of Anatomy & Cell Biology, University of Western Ontario, London, Ontario N6C 3N1, Canada.
E-mail address: steven.laviolette@schulich.uwo.ca (S.R. Laviolette).

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intra-BLA, dopamine (DA) receptor-mediated, switching mechanism controlling the acquisition of opiate reward memories as a function of previous opiate exposure history and withdrawal state (Lyons et al., 2013). Specifically, intra-BLA D1 receptor (D1R) transmission and downstream modulation of the extracellular signal-related kinase (ERK) pathway, is critical for opiate reward memory formation during the early, non-dependent phase of opiate-related memory processing. However, following chronic opiate exposure and dependence, intra-BLA opiate-memory formation functionally switches to a D2 receptor (D2R) substrate associated with the calcium/calmodulin-dependent kinase II (CaMKII) memory signaling pathway (Lintas et al., 2011). Thus, functional and molecular alterations in the D2R family represent important amygdala adaptations during the opiate addiction process.

Given its relatively high concentration in the BLA and its established role in drug-related learning and memory, the D3R is a potential candidate for processing opiate-related associative memory in the BLA. The D3R is linked both to the cyclin-dependent kinase 5 (Cdk5) pathway and calcineurin downstream molecular pathways (Chergui et al., 2004; Chen et al., 2009; Liu et al., 2009; Avalos-Fuentes et al., 2013), both of which play important roles in synaptic plasticity and the formation of conditioned reward memory (Mansuy et al., 1998; Biala et al., 2005; Baumgärtel and Mansuy, 2012; Lyons et al., 2013). However, how chronic opiate exposure may alter the functional activity state of the D3R within the BLA and its downstream molecular pathways is not currently understood, especially in the context of opiate-related memory formation.

The aim of the present study was to examine how intra-BLA D3R transmission may regulate the formation of both opiate-related reward and withdrawal aversion-related associative memory formation. In addition, we examined how chronic opiate exposure may alter the functional role and expression levels of intra-BLA D3R and its downstream effects on the Cdk5/calcineurin signaling pathways. Using both reward and withdrawal aversion place conditioning procedures combined with intra-BLA molecular protein analyses in rats, we report that chronic opiate exposure and withdrawal causes profound changes in intra-BLA D3R expression and function during the formation of both opiate reward and withdrawal aversion associative memories. Furthermore, these opiate-related alterations are dependent upon concomitant alterations in the function and expression of the D3R-linked signaling pathways, calcineurin and Cdk5.

2. Materials and methods

2.1. Surgical procedures

All procedures were performed in accordance with the Canadian Council on Animal Care and approved by the Western University Council on Animal Care. Male Sprague-Dawley rats (350–400 g; Charles River) were anesthetized with ketamine/xylazine (80 mg and 6 mg/kg, respectively), given 1 mg/kg of meloxicam (non-steroidal anti-inflammatory analgesic) and placed into a stereotaxic device. Stainless steel guide cannulae (22 gauge; PlasticsOne) were bilaterally implanted into the BLA based on anatomical boundaries defined by Paxinos and Watson (2005) as follows: (no angle), from bregma, AP −2.6, ML +5.0, DV −7.2.

2.2. Drug treatments

The selective D3R antagonist, PG01037 dihydrochloride (PG01037; 133-fold selective over D2R; Tocris Bioscience), Calcineurin autoinhibitory peptide (calAIP; Tocris Bioscience), morphine (morphine hydrochloride, MacFarlane Smith) and heroin (diacetyl-morphine, MacFarlane Smith) were dissolved in physiological vehicle (0.9% NaCl; saline), pH adjusted to 7.4. The competitive Cdk5 antagonist Roscovitine (Sigma-Aldrich) was dissolved in a 50% DMSO and physiological vehicle solution. All intra-BLA drug or vehicle microinjections (0.5 μL per infusion) were delivered via tubing connected to a 1 μL Hamilton microsyringe over 1 min. Injectors were left in situ for an additional 1 min to ensure diffusion from the injector tip. For all groups, micro-infusions were performed immediately prior to injections of morphine or vehicle (i.p.) and subsequent placement in the conditioning environment. The dose of morphine used for CPP (5 mg/kg, i.p.) is a supra-reward threshold conditioning dose, and produces robust behavioural CPP (Sun et al., 2011; Lyons et al., 2013).

2.3. Comparison of opiate exposure states

Experimental groups consisted of non-dependent (i.e. rats that were previously naïve to opiates at the beginning of behavioural or molecular experiments) or chronically exposed and in withdrawal (i.e. rats that were chronically opiate exposed and in a state of withdrawal at the time of behavioural or molecular experiments), as previously described (Lintas et al., 2011; De Jaeger et al., 2013; Lyons et al., 2013; Rosen et al., 2015). For chronic opiate exposure/withdrawal groups, rats were pre-treated with once-daily injections of 0.5 mg/kg heroin (s.c.) in their home cage starting 7 days prior to behavioural conditioning. The first conditioning session began 21 h following the last heroin injection. During the conditioning procedure, maintenance doses of heroin were administered 2.5 to 3 h after the end of each conditioning session, for a total of 15 injections over the course of an experiment. Non-dependent rats received vehicle injections over the same time course, in place of heroin. This regimen produces aversive motivational effects (CPA) similar to those produced by a 3-weeks of morphine administration (Bechara et al., 1995; Laviolette and van der Kooy, 2004), thus indicating a state of potent opiate dependence. An outline of exposure states is illustrated in Fig. 1A.

2.4. Place preference conditioning

An unbiased, fully counterbalanced place conditioning paradigm was used, as described previously (Lintas et al., 2011; Lyons et al., 2013). All rats (n = 99) were given one week to recover from surgery before habituation for 20 min in a motivationally neutral grey box. Rats were then randomly assigned to an experimental group, and the conditioning procedure began the following day. One conditioning environment was white with a woodchip and wire mesh floor, and the alternate environment was black with a Plexiglas floor, wiped down with 0.25 mL of 2% acetic acid. Thus, the two conditioning environments differed in terms of texture, odor and colour. As described previously, these two conditioning environments elicit no baseline preference in rats (Laviolette and van der Kooy, 2003). Rats spent an equal number of 30-min conditioning sessions in morphine-paired and vehicle-paired environments (i.e. four morphine-environment pairings and four vehicle-environment pairings over the 8-day procedure). Testing was carried out in a drug-free state 7 days following the final conditioning session. The test environment consisted of both conditioning environments separated by a neutral grey zone. Rats were placed onto the neutral zone at the start of the test and allowed to freely explore the arena for 10 min while time spent in each environment was independently recorded.

2.5. Opiate withdrawal conditioned place aversion procedure

To study the effects of the D3R and its downstream targets on the acquisition of a CPA to environments paired with opiate withdrawal, an adapted CPA paradigm was used (Laviolette et al., 2002). In the single-side opiate withdrawal procedure, rats are exposed to only one of the two conditioning environments described above. Opiate dependent/withdrawn rats are placed into the environment immediately following microinfusion 21 h following their previous heroin injection (i.e. during withdrawal), and receive a maintenance dose of heroin 2.5 to 3 h following removal from the box, as per the CPP paradigm. Withdrawal