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ACTIVATION OF DOPAMINE D3 RECEPTORS INHIBITS REWARD-RELATED LEARNING INDUCED BY COCAINE

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Abstract

Memories of learned associations between the rewarding properties of drugs and environmental cues contribute to craving and relapse in humans. The mesocorticolimbic dopamine (DA) system is involved in reward-related learning induced by drugs of abuse. DA D3 receptors are preferentially expressed in mesocorticolimbic DA projection areas. Genetic and pharmacological studies have shown that DA D3 receptors suppress locomotor-stimulant effects of cocaine and reinstatement of cocaine-seeking behaviors. Activation of the extracellular signal-regulated kinase (ERK) induced by acute cocaine administration is also inhibited by D3 receptors. How D3 receptors modulate cocaine-induced reward-related learning and associated changes in cell signaling in reward circuits in the brain, however, have not been fully investigated. In the present study, we show that D3 receptor mutant mice exhibit potentiated acquisition of conditioned place preference (CPP) at low doses of cocaine compared to wild-type mice. Activation of ERK and Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα), but not the c-Jun N-terminal kinase and p38, in the nucleus accumbens, amygdala and prefrontal cortex is also potentiated in D3 receptor mutant mice compared to that in wild-type mice following CPP expression. These results support a model in which D3 receptors modulate reward-related learning induced by low doses of cocaine by inhibiting activation of ERK and CaMKIIα in reward circuits in the brain.

Keywords

dopamine; D3 receptors; cocaine; reward learning; ERK; CaMKIIα

Drug addiction is a chronic brain disease and relapse to drug-seeking is the main obstacle to long-term treatment and cure for drug addiction in humans (Dackis and O'Brien, 2005). Memories of drug effects or learned associations between the rewarding properties of drugs and environmental cues contribute significantly to craving and relapse in humans (Hyman et al., 2006; Kauer and Malenka, 2007; Kalivas and O'Brien, 2008). The neurotransmitter dopamine (DA) is involved in reward-related learning (Schultz, 2002; Wise, 2008; Volkow et al., 2009). Drugs of abuse can pathologically change neuronal circuits in the

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mesocorticolimbic DA system, which projects from the ventral tegmental area to the nucleus accumbens (NAc), amygdala (AMG), prefrontal cortex (PFC) and other structures (Everitt and Robbins, 2005; Koob and Volkow, 2010). The basolateral AMG (BLA) mediates learning of conditioned associations between the rewarding effects of drugs of abuse and cues. The PFC contributes to decision-making and execution of goal-directed actions. The NAc modulates motivation for drug seeking by integrating information from the BLA and PFC and relaying it to motor output structures, and it mediates reinforcement. These different brain structures coordinate to modulate reward-related learning induced by drugs of abuse.

There are two families of DA receptors (Neve et al., 2004). The D1-like family includes D1 and D5 receptors and activation of these receptors leads to increased intracellular levels of cAMP. The D2-like family includes D2, D3 and D4 receptors and activation of these receptors is negatively linked to the cAMP production (Neve et al., 2004). D3 receptors are preferentially expressed in mesocorticolimbic DA projection areas (Le Foll et al., 2005; Heidbreder et al., 2005). We previously found that D3 receptor mutant mice exhibit potentiated acute locomotor activation compared to wild-type mice following injections of low (5 mg/kg) but not higher doses (10 and 20 mg/kg) of cocaine (Xu et al., 1997). We and others also demonstrated that a D3 receptor mutation or D3 receptor-selective agonists and antagonists can alter locomotor sensitization, discriminative stimulus and conditioned place preference (CPP) induced by stimulants (Xu et al., 1997; Carta et al., 2000; Karasinska et al., 2005; Richtand, 2006; Martelle et al., 2007; Chen et al., 2007; Beninger and Banasikowski, 2008; Chen and Xu, 2010). Self-administration of cocaine can be attenuated by the co-administration of D3 receptor-selective agonists (Neisewander et al., 2004; Martelle et al., 2007). Administration of a partial D3 receptor agonist or D3 receptor antagonists generally results in inhibition of cocaine seeking behavior (Pilla et al., 1999; Xi et al., 2006; Di Ciano, 2008; Peng et al., 2009). Taken together, these results suggest that D3 receptors contribute to the development and reinstatement of stimulant-induced behaviors.

The signaling pathways associated with DA receptors have been suggested to play a critical role in drug-induced neuroadaptations in the brain Hyman et al., 2006). D3 receptors regulate cocaine-induced cell signaling events. We previously found that activation of extracellular signal-regulated kinase (ERK) which is a member of the mitogen-activated protein kinase (MAPK) family by acute cocaine injections is inhibited by the activation of D3 receptors (Zhang et al., 2004; Chen and Xu, 2010). D3 receptors also inhibit *N*-methyl-*D*-aspartate (NMDA)-induced activation of Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα, Jiao et al., 2007). On the other hand, D3 receptors can be phosphorylated by CaMKIIα in an activity-dependent manner, resulting in the inhibition of D3 receptor function (Liu et al., 2009).

Although D3 receptors contribute to the development and reinstatement of cocaine-induced behaviors and related signaling events, how these receptors modulate cocaine-induced reward-related learning and associated changes in cell signaling in reward circuits in the brain have not been fully investigated. For example, we previous showed that a D3 receptor mutation in mice did not obviously affect the acquisition of CPP induced by relatively high doses (10 and 20 mg/kg) of cocaine (Chen and Xu, 2010). Whether these receptors inhibit reward-related learning induced by low does of cocaine remains unknown. Moreover, how D3 receptors regulate signaling involving ERK and other members of the MAPK family, c-Jun N-terminal kinase (JNK) and p38, as well as CaMKIIα in brain reward circuit is unclear. In the present study, we investigated these issues using D3 receptor mutant mice and the CPP paradigm. Our data support a model in which D3 receptors modulate reward-related learning induced by low doses (1 and 2.5 mg/kg) of cocaine by inhibiting activation of ERK and CaMKIIα in the NAc, AMG and PFC in the brain.

EXPERIMENTAL PROCEDURES

Mice

We previously generated DA D3 receptor mutant mice (Xu et al., 1997). Homozygous D3 receptor mutant mice and wild-type littermates were obtained by crossing D3 receptor heterozygous parents. The genotype of D3 receptor mutant and wild-type mice was determined by Southern blotting using a 3' gene-specific probe (Xu et al., 1997). The genetic background of all mice was initially 50% each of 129SvJ and C57BL/6J, and was subsequently bred with C57BL/6J mice for three additional generations. D3 receptor mutant and wild-type mice were group housed and were on a twelve hour light/dark cycle with food and water available *ad libitum*. Roughly equal numbers of male and female mice, 10 to 16 weeks old (mean age 12.2 weeks), were used in the current study. The temperature and humidity of the room were controlled. Animal use was in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animal and was approved by the University of Chicago Institutional Animal Care and Use Committee.

Drugs and antibodies

Cocaine hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sterile saline. All injections were administered intraperitoneally (i.p.) in a volume of 10 ml/kg body weight (Chen and Xu, 2010). Cocaine doses used in the current study were 1.0 and 2.5 mg/kg. Primary antibodies against phospho-ERK, phospho-JNK, phospho-p38, phospho-CaMKIIα, ERK, JNK, p38 and β-actin were purchased from Cell Signaling Technology (Beverly, MA). Primary antibodies for CaMKIIα and HRP-conjugated anti-rabbit or anti-goat second antibodies were purchased from Santa Cruz Technology (Santa Cruz, CA).

CPP

Eight three-chamber place preference apparati (MedAssociates, E. Fairfield, VT) were used in the present study (Zhang et al., 2006; Chen and Xu, 2010). The apparatus consisted of two large compartments (16.8×12.7×12.7 cm), and one small compartment (7.2×12.7×12.7 cm) which separated the large compartments. The two large compartments had different visual and tactile cues. One compartment was black with a stainless steel grid rod floor. The other compartment was white with a stainless steel mesh floor. The small compartment was gray with a smooth polyvinyl chloride floor. The apparatus had a clear Plexiglas top with a light on it.

We used a biased CPP procedure similar to that described before (Zhang et al., 2006; Chen and Xu, 2010). During the preconditioning phase (day 1–2, pre-test), mice were placed in the small compartment and were allowed to freely explore the 3 compartments for 20 minutes daily. The time spent in each compartment was recorded. Mice spending over 500 seconds in the small compartment or over 800 seconds in either large compartment were excluded. The next 12 days (day 3–14) were the conditioning and testing phase with one session per day. The drug-paired group received an i.p. cocaine injection and was confined in the white compartment for 30 minutes on day 3. On day 4, this group of mice received an i.p. saline injection and was confined in the black compartment for 30 minutes. As a control, the saline group received i.p. saline injections on days 3 and 4 and was confined in both compartments. On day 5 (test 1), mice were allowed to freely explore the three compartments for 20 minutes without injections, and the time spent in each compartment was recorded. Each mouse received three additional injections of cocaine plus saline and testing (tests 2–4) resulting in four conditioning sessions and four tests. All behavioral testing was performed during the light phase of the light/dark cycle (8 am–8 pm).

For the 1 mg/kg cocaine treatment, 18 wild-type mice and 16 receptor mutant mice were used; for the 2.5 mg/kg cocaine treatment, 20 each wild-type and D3 receptor mutant mice were used; for the saline treatment, 14 each mice were used. In general, 1–2 mice were excluded per group per treatment and a total of 9 out of 102 mice were excluded.

Protein extracts

Mice treated with cocaine at the 2.5 mg/kg dose and with saline were sacrificed by cervical dislocation immediately after the fourth test for CPP expression on day 14. Brains were rapidly removed, frozen on dry ice and stored at −80°C. Brains were sliced into 1 mm sections using the brain matrix as described (Chen and Xu, 2010). NAc, AMG and PFC were dissected according to a mouse brain atlas (Paxinos and Franklin, 1997). Tissues were homogenized in 300 μl ice-cold extraction buffer containing 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM EDTA, 10 mM EGTA, 2 mM sodium pyrophosphate, 4 mM paranitrophenylphosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotintin, 2 ug/ml leupeptin and 2 ug/ml pepstatin (Zhang et al., 2004). Homogenates were incubated on ice for 20 minutes and were centrifuged at 13,000 g for 20 minutes at 4°C (Chen and Xu, 2010). Supernatants were collected and protein concentrations were determined using a Bradford assay (Bio-Rad Laboratories, Hercules, CA).

Western blotting

Changes in protein levels were analyzed by using western blotting as described (Chen and Xu, 2010). Equal amounts of protein (10–20 μg) were separated by 10% SDS-PAGE. Proteins were then transferred onto polyvinylidene difluoride membranes following electrophoresis for two hours. The membranes were blocked with 5% BSA for one hour at room temperature. Membranes were then incubated overnight at 4°C with different primary antibodies. After three washes with a 0.1% Tween 20 Tris-buffered saline (pH8.4), membranes were incubated with appropriate HRP-conjugated anti-rabbit or anti-goat secondary antibodies. Signals were detected by enhanced chemiluminescence. Primary antibodies against phospho-ERK, phospho-JNK, phospho-p38 and phospho-CaMKIIα were used at 1:2000 dilutions. The secondary antibodies were used at 1:5000 dilutions. The same membranes were stripped and incubated with antibodies against total ERK, JNK, p38 and CaMKIIα (1:2000; Chen and Xu, 2010). The membranes were stripped and re-probed with an anti-actin antibody (1:2000) to assure equal loading of the samples. All western blot analyses were performed a minimum of three times.

Quantification and data analysis

The behavioral data were analyzed as time spent on the saline-paired side subtracted from time spent on the drug-paired side and were presented as mean ± SEM (Chen and Xu, 2010). Two-way repeated measure ANOVA with test as within-subjects factor, and genotype and treatment as between-subjects factors were used, followed by one-way ANOVA test with post-hoc LSD. Paired-sample *t*-test was used for within-subjects comparisons.

The results for Western blotting were analyzed using densitometry. Ratios of phospho- to total ERK, JNK, p38 and CaMKIIα densities were calculated for each sample. Saline controls were set at 1. A one-way ANOVA with post-hoc LSD was used to analyze changes in phosphorylation. The statistically significant level was set at $p<0.05$.

RESULTS

DA D3 receptor mutant mice show potentiated CPP acquisition compared to wild-type mice at low doses of cocaine

To determine the role of D3 receptors in reward-related learning, we used three groups of D3 receptor mutant and wild-type mice including two different doses of cocaine and saline (0, 1 and 2.5 mg/kg, n=12–19 mice per group) and performed a CPP study. There was a significant main effect of treatment [F (2, 92)=13.571, p<0.05] and genotype [F (1,92)=4.318, p<0.05] after conditioning (Fig. 1). Oneway ANOVA test showed that both D3 receptor mutant and wild-type mice developed CPP after conditioning [Fig. 1, F (5, 92)=14.597, p<0.05]. D3 receptor mutant and wild-type mice had developed CPP at the first [F (5, 92)=2.625, p<0.05] and third [F (5,92)=8.404, p<0.05] test sessions respectively at the 1 mg/kg dose of cocaine. At the 2.5 mg/kg dose of cocaine, both wild-type and D3 receptor mutant mice exhibited CPP at the first test session [F (5, 92)=2.625, p<0.05]. Significantly, D3 receptor mutant mice exhibited higher CPP acquisition than wild-type mice on test 4 at 1 mg/kg cocaine and both tests 3 and 4 at 2.5 mg/kg cocaine. Paired-sample t-tests indicated that D3 receptor mutant and wild-type mice do not show CPP acquisition after saline injections (Fig. 1, p>0.05). Together, these results suggest that, at very low doses of cocaine, D3 receptors inhibit acquisition of CPP induced by cocaine.

Potentiated activation of ERK, but not JNK and p38, in the NAc, AMG and PFC in D3 receptor mutant mice following CPP expression

We systematically investigated the status of all members of the MAPK family, ERK, JNK and p38 in different brain regions in D3 receptor mutant and wild-type mice immediately following CPP expression tests. Western blot analyses indicated that ERK was activated in the NAc, AMG and PFC in D3 receptor mutant mice compared to saline-treated control group [Fig. 2, NAc: F (3, 27)=32.478, p<0.05; AMG: F (3, 27)=17.728, p<0.05; PFC: F (3, 27)=22.127, p<0.05]. In wild-type mice, ERK was activated in the NAc and PFC as compared to the saline-treated group [Fig. 2, NAc: p<0.05; PFC: p<0.05], but not in the AMG (p>0.05). Notably, phospho-ERK levels are higher in all three brain regions in D3 receptor mutant mice than those in wild-type mice following CPP expression (Fig. 2, p<0.05). Levels of phospho-ERK and total ERK were similar in each of the three brain regions in D3 receptor mutant and wild-type mice following saline treatment (Fig 2).

There were no significant changes in phospho-JNK (Fig. 3) and phospho-p38 (Fig. 4) levels in any of the three brain regions in D3 receptor mutant and wild-type mice following cocaine treatment compared to that following saline treatment (p>0.05). Moreover, levels of phospho-JNK and total JNK as well as phospho-p38 and total p38 were similar in each of the brain regions in wild-type and D3 receptor mutant mice (Figs 3 and 4).

Heightened CaMKIIα activation in D3 receptor mutant mice following CPP expression

We next investigated whether there is a corresponding difference in CaMKIIα activation in the three brain regions between D3 receptor mutant and wild-type mice following CPP expression. We sacrificed D3 receptor mutant and wild-type mice (n=7 each) immediately after the fourth CPP expression test. Western blot experiments indicated that, compared to saline groups, CaMKIIα was activated in the NAc, AMG and PFC in D3 receptor mutant mice, and in the NAc in wild-type mice [Fig. 5, NAc: F (3,27)=13.346, p<0.05; AMG: F (3,27)= 9.669, p<0.05; PFC: F (3,27)=5.066, p<0.05], but not in the AMG and PFC in wild-type mice (p>0.05). Moreover, phospho-CaMKIIα levels are higher in the NAc, AMG and PFC in D3 receptor mutant mice than those in wild-type mice (Fig. 5, p<0.05). Levels of phospho-CaMKIIα and total CaMKIIα were similar in all brain regions in D3 receptor mutant and wild-type mice (Fig. 5).

DISCUSSION

We previously found that a D3 receptor mutation in mice did not obviously affect the acquisition of CPP induced by relatively high doses of cocaine (Chen and Xu, 2010). In the current study, we investigated how D3 receptors modulate CPP induction at low doses of cocaine and the associated changes in signaling events mediated by MAPK and CaMKIIα in several brain areas. We found that D3 receptor mutant mice exhibit potentiated CPP acquisition at low doses of cocaine compared to wild-type mice. Moreover, activation of ERK, but not JNK and p38, as well as CaMKIIα in brain reward circuits is also potentiated in D3 receptor mutant mice compared to that in wild-type mice after CPP expression. These data suggest a model in which D3 receptors contribute to reward-related learning induced by low doses of cocaine by inhibiting activation of ERK and CaMKIIα in the NAc, AMG and PFC.

Activation of D3 receptors inhibits cocaine-induced reward-related learning

DA is involved in reward-related learning (Schultz, 2002; Wise, 2008; Volkow et al., 2009). We previously found that D1 receptor mutant mice do not acquire CPP over a wide dose range of cocaine (2.5–20 mg/kg, Chen and Xu, 2010). This result is similar to those from pharmacological studies demonstrating that D1 receptor antagonists block cocaine-induced CPP (Tzschentke, 1998). This finding is also in agreement with those showing that D1 receptors are required for mice to learn to self administer cocaine (Caine et al., 2007). These results suggest an important role for D1 receptors in the acquisition of reward-related learning induced by cocaine. Interestingly, evidence suggests that D3 receptors play a role in cocaine-induced behavior that is opposite of D1 receptors. For example, D3 receptor mutant mice exhibit potentiated acute locomotor activation compared to wild-type mice at the 5 mg/kg dose of cocaine (Xu et al., 1997) whereas D1 receptor mutant mice fail to exhibit locomotor activation following cocaine injections over a wide dose range (Xu et al., 1994a; 1994b; 2000). D3 receptor mutant mice also exhibit opposite signaling patterns compared to those in D1 receptor mutant mice following cocaine administration (Zhang et al., 2004; Jiao et al., 2007). Whereas D1 receptor mutant mice fail to acquire CPP, D3 receptor mutant mice exhibit potentiated CPP induced by low but not high does of amphetamine or methamphetamine (Xu et al., 1997; Chen et al., 2007). At higher doses of cocaine (10 and 20 mg/kg), a D3 receptor mutation in mice does not obviously affect the acquisition of CPP (Chen and Xu, 2010). In the current study, we found that D3 receptor mutant mice exhibit potentiated CPP acquisition compared to wild-type mice at low doses of cocaine (1 and 2.5 mg/kg, Fig. 1). These findings suggest that, in contrast to D1 receptors, D3 receptors inhibit reward-related learning induced by low doses of cocaine.

D3 receptors inhibit ERK and CaMKIIα activation in brain reward circuits during acquisition of reward learning

The NAc, BLA and PFC are components of the circuitry that process learned associations between the rewarding properties of drugs and cues (Kalivas and McFarland, 2003; Di Ciano and Everitt, 2004; Weiss, 2005; Everitt and Robbins, 2005; Feltenstein and See 2008; Koob and Volkow, 2010). Conditioned cues can activate these brain regions (Neisewander et al., 2000; Ito et al, 2000; Volkow et al., 2004; 2005). In the context of CPP, the excitatory drive from the BLA to the NAc is enhanced during cocaine seeking (Miller and Marshall, 2005b). The NAc is involved in the acquisition, retrieval and reconsolidation of cocaine-paired contextual memory (Miller and Marshall, 2005a). Drug-related cues can increase DA levels (Volkow et al., 2008; Koob and Volkow, 2010). These results imply the importance of DA receptors and associated signaling mechanisms in these brain regions in establishing memories for drug-associated cues. We thus investigated signaling events in these brain regions following CPP expression.

ERK activation is oppositely regulated by D1 and D3 receptors following exposure to cocaine (Zhang et al., 2004; Chen and Xu, 2010). Inhibiting the ERK signaling pathway or an ERK mutation can attenuate cocaine-induced behavioral responses (Pierce et al., 1999; Valjent et al., 2000; 2006; Ferguson et al., 2006). The NAc, BLA and PFC express D3 receptors (Neve et al., 2004). Thus we studied D3 receptor-mediated ERK activation in these brain regions following CPP acquisition. ERK is activated in the NAc, AMG and PFC in D3 receptor mutant mice and in the NAc and PFC but not in AMG in wild-type mice following CPP acquisition (Fig. 2). These results imply that ERK activation in the NAc and PFC accompanies CPP expression induced by low doses of cocaine. Exposure to drug-associated cues increased ERK activation in the central AMG after 30 days, but not 1 day of withdrawal (Lu et al., 2005), implying that the rise in phospho-ERK levels in the AMG depends on length of withdrawal (Chen and Xu, 2010). It is possible that, in the absence of D3 receptor, the withdrawal time necessary for ERK activation in the AMG is shortened following CPP acquisition. No obvious changes in phospho-JNK and p38 levels were found in the NAc, AMG and PFC following CPP acquisition (Figs. 3 and 4). This finding is similar to those using higher doses of cocaine (Chen and Xu, 2010) or methamphetamine (Mizoguchi et al., 2004). These results suggest that activation of the ERK signaling pathway in the NAc, AMG and PFC, but not the JNK and p38 signaling pathways, via D3 receptors contributes to cocaine-induced reward learning as measured by the CPP paradigm.

Protein kinase A (PKA)-regulated CaMKIIα activation contributes to neuronal plasticity (Wayman et al., 2008). We previously found that D1 and D3 receptors can mediate and inhibit NMDA-induced CaMKIIα activation presumably by oppositely regulating PKA activity (Jiao et al., 2007). Consistent with this finding, we found potentiated CaMKIIα activation in the NAc, AMG and PFC in D3 receptor mutant mice compared to that in wild-type mice following CPP acquisition in the current study (Fig. 5). These results suggest that, in the absence of D3 receptors, there is potentiated CaMKIIα activation compared to that in wild-type mice, likely due to activation of NMDA receptors (Jiao et al., 2007; Wayman et al., 2008). Moreover, CaMKIIα activation in the NAc accompanies CPP expression induced by low doses of cocaine. Furthermore, requirement for CaMKIIα and ERK activation in different brain regions is not identical for CPP expression at low doses of cocaine.

A model for D3 receptor function in reward learning

D3 receptors have higher affinities for DA than other DA receptors, including D1 receptors (Sokoloff et al., 1992). It has been suggested that D2 family receptors like D3 receptors can respond to tonic DA release while D1 receptors are activated when there is phasic DA release (Goto et al., 2007; Grace et al., 2007). A large percentage of D3 receptor-bearing neurons co-express D1 receptors, especially in the NAc (Surmeier et al., 1996; Schwartz et al., 1998). D3 receptors are activated by basal levels of DA to inhibit adenylyl cyclase and related signaling including ERK and CaMKIIα. When DA release is enhanced and DA levels increase, CaMKIIα phosphorylates D3 receptors at the serine 229 site in the third intracellular loop (Liu et al., 2009). Consequently, the inhibitory tone of D3 receptors on cocaine-induced behavior and related signaling is apparently removed. This allows other DA receptors including D1 receptors to fully mediate behavioral activation and related signaling induced by cocaine. Our current results and those from previous studies support the above model in which D3 receptors contribute to reward-related learning induced by low doses of cocaine by regulating activation of ERK and CaMKIIα in specific areas of the brain.

Research Highlightsa

1. D3 receptors inhibit reward-related learning induced by cocaine

2. D3 receptors also inhibit cocaine-induced activation of ERK and CaMKIIα in brain reward circuits
3. D3 receptors modulate reward learning by regulating specific signaling events

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ABBREVIATIONS

(AMG)	amygdala
(CaMKII)	Ca^{2+}/calmodulin-dependent protein kinase II
(CPP)	conditioned place preference
(JNK)	c-Jun N-terminal kinase
(DA)	dopamine
(ERK)	extracellular signal-regulated kinase
(MAPK)	mitogen-activated protein kinase
(NMDA)	*N*-methyl-*D*-aspartate
(NAc)	nucleus accumbens
(PFC)	prefrontal cortex
(PKA)	protein kinase A

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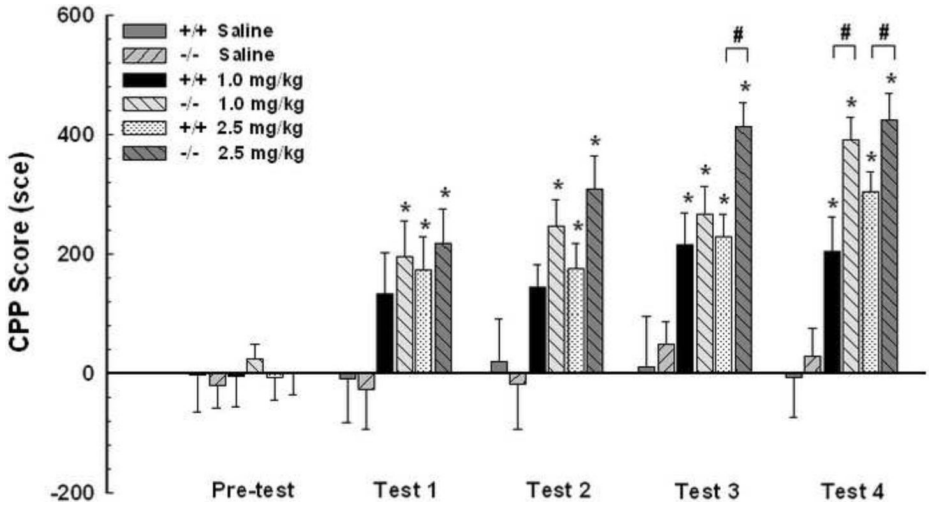

Fig. 1.

D3 receptor mutant mice show heightened CPP acquisition compared to wild-type mice. Three groups of D3 receptor mutant (−/−) and wild-type (+/+) mice received either cocaine (1 mg/kg, n=17 and 14 each; or 2.5 mg/kg, n=18 and 19 each) or saline (n=12 and 13 each) injections on alternative days, and were confined to specific compartments in a biased design. These mice were tested for place preference without injections. Both D3 receptor mutant and wild-type mice showed CPP acquisition induced by 1 and 2.5 mg/kg doses of cocaine but not by saline. Moreover, D3 receptor mutant mice exhibited higher CPP acquisition than wild-type mice at both doses of cocaine. Results represent mean ± SEM time spent on the drug-paired side minus that on the saline-paired side. *$p<0.05$ compared with the saline mouse group of the same genotype within the same test. #$p<0.05$ compared between two genotypes at the same cocaine dose and test.

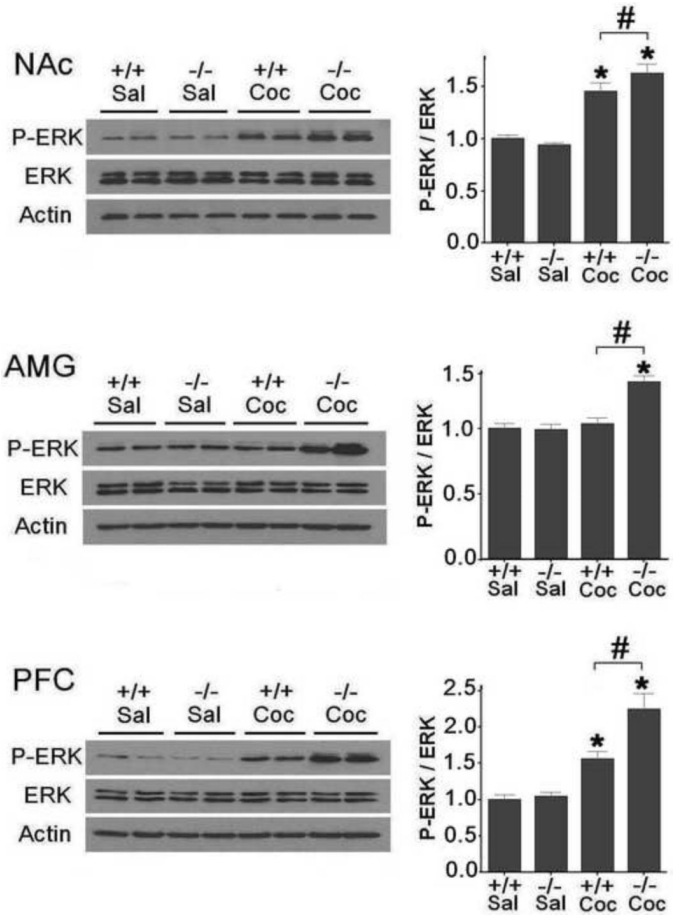

Fig. 2.

Potentiated ERK activation in the NAc, AMG and PFC in D3 receptor mutant mice compared to that in wild-type mice following CPP expression. Mice were given cocaine (Coc, 2.5 mg/kg) or saline (Sal) injections and were sacrificed at the end of the fourth CPP test. Western blotting was performed using brain samples from D3 receptor mutant (−/−) and wild-type (+/+) mice (n=7 mice each). Ratios of phospho-ERK (P-ERK) relative to total ERK protein levels in the NAc, AMG and PFC were analyzed. Data were expressed as mean ± SEM relative to saline controls that were set as 1. Actin levels were used as a loading control. **p*<0.05 compared with the saline control mouse group of the same genotype. #*p*<0.05 compared between the two genotypes.

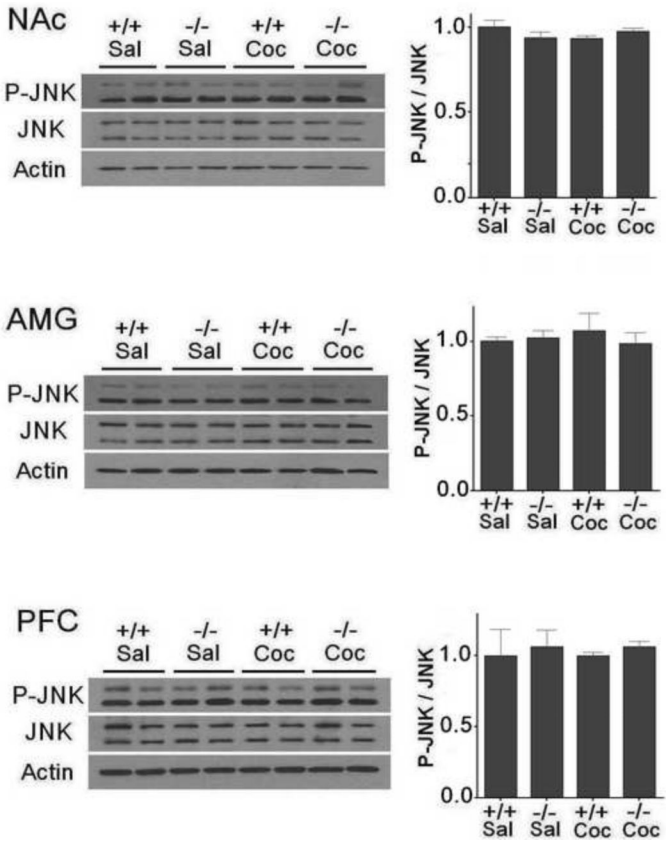

Fig. 3.

JNK is not significantly activated in the NAc, AMG and PFC in D3 receptor mutant and wild-type mice following CPP expression. Western blotting for JNK was performed using brain samples from D3 receptor mutant (−/−) and wild-type (+/+) mice (n=7 mice each) after the last CPP expression test as before. The cocaine dose used for CPP induction was 2.5 mg/kg. Ratios of phospho-JNK (P-JNK) over total JNK protein levels in the NAc, AMG and PFC were analyzed. Data represent mean± SEM relative to saline (Sal) controls and were set as 1. Actin levels were used as a loading control.

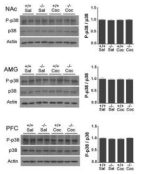

Fig. 4.

p38 is not obviously activated in the NAc, AMG and PFC in D3 receptor mutant and wild-type mice following CPP expression. Western blotting for p38 was performed using brain samples from D3 receptor mutant (−/−) and wild-type (+/+) mice (n=7 mice each) after the last CPP expression test as before. The cocaine dose used for CPP induction was 2.5 mg/kg. Ratios of phospho-p38 (P-p38) over total p38 protein levels in the NAc, AMG and PFC were analyzed. Data represent mean± SEM relative to saline (Sal) controls and were set as 1. Actin levels were used as a loading control.

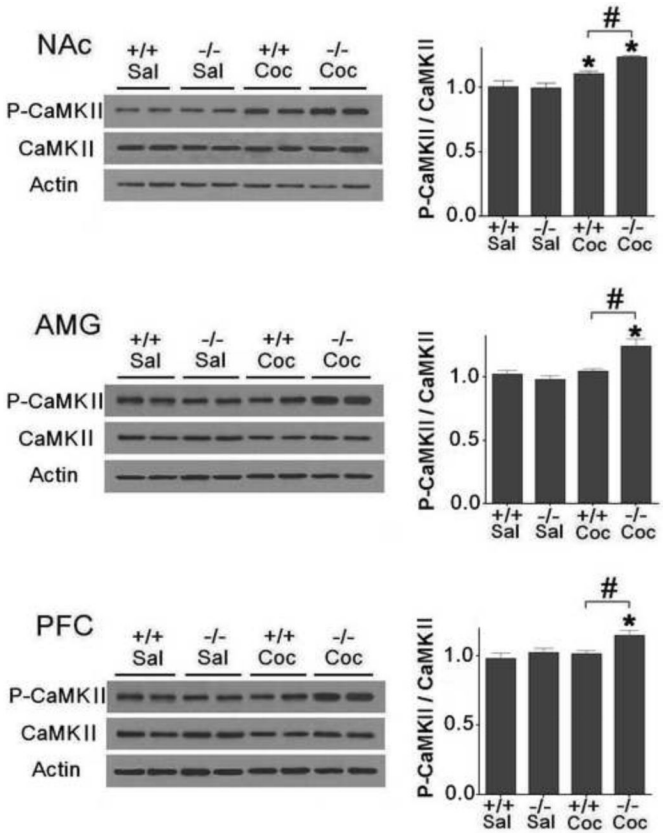

Fig. 5.

Higher CaMKIIα activation in the NAc, AMG and PFC in D3 receptor mutant than wild-type mice following CPP expression. Mice were given cocaine (Coc, 2.5 mg/kg) or saline (Sal) and were sacrificed immediately after the last CPP test. Western blotting was performed using brain samples from D3 receptor mutant (−/−) and wild-type (+/+) mice (n=7 mice each). Ratios of phospho-CaMKIIα (P-CaMKIIα) relative to total CaMKIIα protein levels in the NAc, AMG and PFC were analyzed. Data were expressed as mean± SEM relative to saline controls that were set as 1. Actin levels were used as a loading control. *p<0.05 compared with the saline control mouse group of the same genotype. #p<0.05 compared between two genotypes.