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Research Report

Surface expression of $GABA_A$ receptors in the rat nucleus accumbens is increased in early but not late withdrawal from extended-access cocaine self-administration

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ABSTRACT

It is well established that cocaine-induced changes in glutamate receptor expression in the nucleus accumbens (NAc) play a significant role in animal models of cocaine addiction. Far less is known about cocaine-induced changes in GABA transmission, despite its importance in regulating NAc output via local interneurons and medium spiny neuron (MSN) axon collaterals (GABA 'microcircuit'). Here we investigated whether $GABA_A$ receptor surface or total expression is altered following an extended-access cocaine self-administration regimen that produces a time-dependent intensification (incubation) of cue-induced cocaine craving in association with strengthening of AMPA receptor (AMPAR) transmission onto MSN. Rats self-administered cocaine or saline (control condition) 6 h/day for 10 days. NAc tissue was obtained and surface proteins biotinylated on three withdrawal days (WD) chosen to span incubation of craving and associated AMPAR plasticity: WD2, WD25 and WD48. Immunoblotting was used to measure total and surface expression of three $GABA_A$ receptor subunits (α1, α2, and α4) that are strongly expressed in the NAc. We found a transient increase in surface, but not total, expression of the α2 subunit on WD2 from cocaine self-administration, an effect that was no longer observed by WD25. The expression of α1 and α4 subunits was not altered at these withdrawal times. On WD48, when AMPAR transmission is significantly potentiated, we did not find any alteration in $GABA_A$ receptor surface or total expression. Our findings suggest that the strengthening of AMPAR-mediated glutamate transmission in the NAc is not accompanied by compensatory strengthening of GABAergic transmission through insertion of additional $GABA_A$ receptors.

1. Introduction

The nucleus accumbens (NAc) is an important structure within the limbic system that modulates goal-directed behaviors including those related to drug addiction (Groenewegen et al., 1999; Kelley, 1999). The NAc itself is composed mainly (~95%) of medium spiny neurons (MSN), which are GABAergic projection neurons that send both intra-NAc axon collaterals and efferent projections to various areas outside the NAc, including the ventral mesencephalon and ventral pallidum (Meredith, 1999; Sesack and Grace, 2010). Most of the remaining neurons are GABAergic interneurons that can be divided into multiple classes based on protein expression and electrophysiological properties (Tepper et al., 2010; Silberberg and Bolam, 2015). Extensive studies have documented alterations in glutamate receptor expression on NAc MSN after cocaine exposure (Wolf and Ferrario, 2010; Wolf, 2016). However, even though glutamatergic afferents are critical in shaping MSN activity (Meredith et al., 2008), the GABA microcircuit comprised by MSN collaterals and GABA interneurons also plays a major role (Wilson, 2007; Tepper et al., 2004; Silberberg and Bolam, 2015).

The importance of the GABA microcircuit for NAc function has been established through anatomical and electrophysiological studies. For example, one type of GABAergic interneuron, the parvalbumin-positive (PV+) fast spiking interneuron, has been shown to synapse with dorsal striatal MSN on proximal dendrites and perikarya, suggesting a strong modulatory effect on the MSN (Bennett and Bolam, 1994). Similarly, the connection between PV+ interneurons and MSN in the NAc has been established by both anatomical (Hussain et al., 1996) and electrophysiological studies (Taverna et al., 2007). This connection has physiological importance, as stimulation of these interneurons results in powerful inhibition of MSN (Pennartz and Kitai, 1991; Koos and Tepper, 1999; Gruber et al., 2009; Gittis et al., 2010). NAc function is also regulated by GABAergic MSN-to-MSN synaptic connectivity (Tunstall et al., 2002; Taverna et al., 2004; Koos et al., 2004).

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In electrophysiological studies mentioned above, $GABA_A$ receptors were implicated in mediating the action of GABA within the microcircuit based on the latency of inhibition and sensitivity to either picrotoxin or bicuculline (Pennartz and Kitai, 1991; Koos and Tepper, 1999; Gruber et al., 2009; Gittis et al., 2010). $GABA_A$ receptors are pentameric chloride channels composed mainly from various isoforms of α, β, and γ subunits (Olsen and Sieghart, 2008), and the subunit composition determines receptor function, pharmacology, and location (Olsen and Sieghart, 2009). Immunocytochemical studies have found that $GABA_A$ α2 subunits are preferentially expressed on MSN while $GABA_A$ α1 subunits are preferentially expressed on interneurons (Schwarzer et al., 2001; Boyes and Bolam, 2007). $GABA_A$ α4 subunits are located extrasynaptically on NAc MSN and are also expressed on several types of interneurons (Maguire et al., 2014). Supporting immunocytochemical studies, electrophysiological studies of MSN in the NAc have established that α2-containing $GABA_A$ receptors are present and mediate phasic inhibition of these cells (Dixon et al., 2010). Tonic inhibition of MSN through α4-containing $GABA_A$ receptors has been observed (Maguire et al., 2014) and pharmacological evidence suggests the presence of α1-containing $GABA_A$ receptor-mediated currents on striatal interneurons (Janssen et al., 2011).

A number of studies have explored the role of $GABA_A$ receptors in the effects of non-contingent cocaine administration in the NAc. It has been found that $GABA_A$ receptors containing the α2 subunit are necessary for the expression of cocaine-induced behavioral sensitization (Morris et al., 2008; Dixon et al., 2010), while repeated experimenter-administered cocaine, followed by withdrawal and a cocaine challenge, led to reduction in the expression of $GABA_A$ α2 subunits in the NAc shell (Chen et al., 2007). Studies of mice with deletion of the α4 subunit indicate that α4 $GABA_A$ receptors on D1 receptor-expressing MSN act to oppose cocaine enhancement of conditioned place preference (CPP) (Maguire et al., 2014). Fewer studies have evaluated the effects of contingent cocaine exposure on $GABA_A$ receptor expression or function. However, available data indicate that GABA transmission in the NAc shell is differently affected by contingent and non-contingent cocaine administration (Wydra et al., 2013). This is not surprising, given substantial differences in the effects of contingent and non-contingent cocaine exposure on glutamate transmission in the NAc (Wolf and Ferrario, 2010). Interestingly, one recent study found time-dependent changes in the balance between inhibitory and excitatory synaptic transmission in the NAc shell during withdrawal from limited-access cocaine self-administration (Otaka et al., 2013).

After extended-access cocaine self-administration, cue-induced cocaine craving progressively intensifies (incubates) over the first 1–2 months of withdrawal and then remains high through at least withdrawal day (WD) 90 before declining slowly (Lu et al., 2004; Pickens et al., 2011). Expression of incubated craving after 1–3 months of withdrawal depends upon strengthening of AMPA receptor (AMPAR) transmission in the NAc core through synaptic incorporation of Ca^{2+}-permeable AMPARs (CP-AMPARs; Conrad et al., 2008; Mameli et al., 2009; Loweth et al., 2014) as well as silent synapse formation and un-silencing (Lee et al., 2013; Ma et al., 2014). The goal of the present study is to determine if incubation of cocaine craving is also associated with alterations in $GABA_A$ receptor levels in the NAc. We focused on three $GABA_A$ receptor subunits that are expressed in the NAc (α1, α2, α4; see above) and examined three withdrawal times spanning the period over which incubation of craving and associated alterations in glutamate transmission are occurring.

2. Results

After extended-access self-administration of cocaine or saline (6 h/day for 10 days), rats were killed at 3 different withdrawal time-points. The time-points were chosen based on the development of AMPAR plasticity in NAc core during withdrawal from this regimen: withdrawal day (WD) 2 (before any changes in AMPAR subunit composition are detected), WD25 (when CP-AMPARs start to accumulate), and WD48 (when stable elevation of CP-AMPARs has been achieved) (Wolf and Tseng, 2012). This design was chosen so as to test the hypothesis that compensatory alterations in GABA transmission accompany changes in AMPAR transmission, as well as to encompass the period of withdrawal when cue-induced cocaine craving is progressively increasing (Lu et al., 2004). At each time-point, NAc tissue (mainly core) from cocaine and saline self-administering rats was collected, biotinylated to selectively label surface-expressed proteins, and then analyzed by immunoblotting.

We first assessed the $GABA_A$ α2 subunit, which is preferentially expressed on MSN (see Section 1). We detected a significant increase in the bound fraction on WD2 in the cocaine group compared to the saline group, indicating increased surface expression of α2-containing $GABA_A$ receptors ($t_{(22)}=2.48$; $^*p<0.05$) (Fig. 1b). This increase in surface expression was transient, as it was no longer detected by WD25 ($t_{(21)}=0.17$, $p>0.05$) (Fig. 1d) or WD48 ($t_{(19)}=0.78$, $p>0.05$) (Fig. 1f). There was no alteration in the total expression of the α2 subunit at any of the three withdrawal times (WD2: $t_{(22)}=0.81$, $p>0.05$; WD25: $t_{(17)}=0.01$, $p>0.05$; WD48: $t_{(19)}=0.48$, $p>0.05$) (Fig. 1a, c, e). Two-way ANOVA revealed no significant difference between cocaine and saline groups over different withdrawal times (surface: $F_{(1,59)}=1.99$, $p>0.05$; total: $F_{(1,53)}=0.01$; $p>0.05$).

We then examined the expression of the $GABA_A$ α4 subunit, which is a component of extrasynaptic receptors expressed on MSN but is also expressed by interneurons (see Section 1). There was no alteration in total (WD2: $t_{22}=0.76$, $p>0.05$; WD25: $t_{19}=0.84$, $p>0.05$; WD48: $t_{19}=0.64$, $p>0.05$) or surface (WD2: $t_{22}=0.11$, $p>0.05$; WD25: $t_{19}=0.77$, $p>0.05$; WD48: $t_{19}=0.75$, $p>0.05$) expression of the $GABA_A$ α4 subunit at any of the withdrawal time-points examined (Fig. 2). Two-way ANOVA revealed no significant difference between cocaine and saline groups over different withdrawal times (surface: $F_{(1.57)}=0.13$, $p>0.05$; total: $F_{(1,55)}=0.27$, $p>0.05$).

Finally, we examined the expression of the $GABA_A$ α1 subunit, which is preferentially expressed on interneurons (see Section 1). There was no alteration in total (WD2: $t_{20}=0.34$, $p>0.05$; WD25: $t_{20}=0.67$, $p>0.05$; WD48: $t_{19}=0.30$, $p>0.05$) or surface (WD2: $t_{22}=0.79$, $p>0.05$; WD25: $t_{16}=0.31$, $p>0.05$; WD48: $t_{19}=0.32$, $p>0.05$) expression of the $GABA_A$ α1 subunit at any of the withdrawal time-points examined (Fig. 3). Two-way ANOVA revealed no significant difference between cocaine and saline groups over different withdrawal times (surface: $F_{(1,54)}=0.06$, $p>0.05$; total: $F_{(1,56)}=0.21$, $p>0.05$).

3. Discussion

The state of drug addiction results from complex neuroplasticity including homeostatic cascades that can both promote and oppose drug craving (Koob and Volkow, 2010; Huang et al., 2011). We chose to investigate potential plasticity of GABA transmission using the 'incubation of cocaine craving' model. This model is relevant to a common pattern of human drug abuse in which users undergo a period of heavy drug-taking that is followed by a period of abstinence (imposed by hospitalization or incarceration); during abstinence, incubation of cue-induced cocaine craving may