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Surface Expression of GABAA Receptors in the Rat Nucleus Accumbens is Increased in Early but Not Late Withdrawal from Extended-Access Cocaine Self-Administration

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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.

Keywords

abstinence; biotinylation; cocaine self-administration; $GABA_A$ receptors; incubation of craving; nucleus accumbens

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Conflict of interest

The authors declare no conflicts of interest.

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 & 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 & Bolam, 2015). Extensive studies have documented alterations in glutamate receptor expression on NAc MSN after cocaine exposure (Wolf & 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 & 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 & 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 & Kitai, 1991; Koos & 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).

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 & Kitai, 1991; Koos & 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 & Sieghart 2008), and the subunit composition determines receptor function, pharmacology, and location (Olsen & Sieghart 2009). In the NAc, the most commonly expressed $GABA_A$ receptor subtypes are α2βγ2, α1βγ2, and α4βδ (Pirker et al., 2000). 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 & 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-ccontaining $GABA_A$ receptors (Santhakumar et al., 2010; Maguire et al., 2014) and the presence of α1-containing

$GABA_A$ receptor-mediated currents on interneurons have also been observed (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 $GABA_A$ 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 & 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 at high levels 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 & 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 Introduction). 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 Introduction). 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 Introduction). 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 & 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 occur and increase vulnerability to relapse (Reichel & Bevins, 2009; Pickens et al., 2011). In fact, incubation of craving has been observed in clinical studies of humans addicted to nicotine (Bedi et al., 2009), methamphetamine (Wang et al., 2013) and alcohol (Li et al., 2015).

Using an extended-access cocaine self-administration regimen leading to incubation of craving, our lab previously discovered that CP-AMPAR levels increase in excitatory

synapses onto NAc core MSN after ~1 month of abstinence and thereafter mediate the expression of incubated cocaine craving (Conrad et al., 2008; Loweth et al., 2014). Since CP-AMPARs exhibit higher conductance compared to Ca^{2+}-impermeable AMPARs, it is expected that the NAc response to afferent glutamatergic excitation will be enhanced (for review, see: Isaac et al., 2007; Lee et al., 2012). In fact, we found a significant increase in the responsiveness of NAc core MSN to synaptic stimulation after withdrawal periods sufficient to lead to increased synaptic levels of CP-AMPARs (Purgianto et al., 2013). Other work, mainly focused on glutamate inputs to NAc shell MSN, has demonstrated pathway-specific plasticity after withdrawal from cocaine self-administration (Suska et al., 2013; Lee et al., 2013; Ma et al., 2014; Pascoli et al., 2014; Terrier et al., 2015).

Based on the observations described above, it is reasonable to ask whether there is a homeostatic response to enhanced glutamate drive in the NAc of “incubated rats”, perhaps mediated by alteration of the GABAergic microcircuit. We focused on NAc core, where a causal relationship between elevated CP-AMPAR levels and incubation of craving is well established (Conrad et al., 2008; Loweth et al., 2014). Other evidence also implicates NAc core MSN in the incubation of cocaine craving (Hollander et al., 2005; Hollander et al., 2007; Guillem et al., 2014). We began by studying two $GABA_A$ α receptor subunits (α2 and α4) that are expressed by NAc MSN (Schwarzer et al., 2001; Boyes & Bolam 2007; Maguire et al., 2014).

We discovered that there was an elevation of $GABA_A$ α2 subunit surface expression on WD2 but not WD25 or WD48, suggesting a transient increase in inhibitory tone onto MSN early in withdrawal. This could contribute to low levels of cue-induced cocaine seeking in early withdrawal. Our results on $GABA_A$ α2 subunit expression may also be suggestive of a failure of homeostasis, both at long withdrawal times (when we failed to observe an increase in $GABA_A$ receptor surface expression that might compensate for increased AMPAR transmission) and at short withdrawal times as well. Thus, on WD1, biochemical evidence indicates that the responsiveness of NAc core MSN to excitatory inputs may be decreased due to a reduction in cell surface GluA1 levels (Conrad et al., 2008). The increase in $GABA_A$ α2 surface expression observed on WD2 would serve to further reduce NAc responsiveness. Another potential interpretation is that the up-regulation of α2 expression is a protective mechanism. Dixon et al. (2014) observed that the level of cocaine intake in wild-type animals decreased over the course of 10 self-administration sessions, while the level of intake of global $GABA_A$ α2 knock-out animals stayed constant. Extrapolating from these behavioral data, our observed increase in α2 surface expression may represent a compensatory mechanism that limits drug intake. However, behavioral data from knock-out animals may not be directly comparable to data from wild-type animals, since genetic deletion of the subunit may lead to compensatory mechanisms not seen on WT animals.

Our findings indicate that the surface and total expression of the $GABA_A$ α4 subunit was not altered during withdrawal from extended-access cocaine self-administration. No prior studies have examined the effect of cocaine on α4 subunit expression in the NAc, although Heiman et al. (2008) found a significant increase in $GABA_A$ α4 gene expression in dorsal striatum soon after (~4h) the last of fifteen of daily cocaine injections. Functionally, α4-containing $GABA_A$ receptors exert a tonic inhibitory influence onto MSN of the NAc and

appear to be important in suppressing the rewarding effect of cocaine, since pharmacological stimulation of this receptor blocks cocaine-induced enhancement of CPP while their genetic deletion enhances cocaine-induced CPP (Maguire et al., 2014). Two microdialysis studies found that NAc levels of extracellular GABA, which is the most likely source for $GABA_A$ α4 activation, are altered after discontinuing cocaine exposure (Xi et al., 2003 – core/shell placements; Wydra et al., 2013 – shell) and adaptations in the expression of $GABA_A$ α4 might therefore be expected. However, depending on the regimen, different changes in GABA levels were observed (Xi et al., 2003; Wydra et al., 2013). Our cocaine regimen differs in many respects from these prior studies, making it difficult to predict whether any alterations in extracellular GABA levels would be expected under our experimental conditions.

We also did not find any alteration in $GABA_A$ α1 subunit surface expression after our cocaine self-administration regimen. In the NAc, it has been suggested that this type of receptor is mainly expressed on interneurons (Schwarzer et al., 2001). The most likely pre-synaptic GABA sources to NAc interneurons are either MSN axon collaterals or projection neurons from globus pallidus (Tepper et al., 2010). Although the amount of GABA neurotransmitter released from these sources may be altered by cocaine exposure (Xi et al., 2003; Wydra et al., 2013), our study suggests GABA receptors expressed on interneurons in the NAc do not undergo an adaptation in response to such changes. We have also failed to find evidence for altered activity of GABAergic interneurons after >48 days of withdrawal from the same cocaine regimen used herein (Purgianto et al., 2014). No study has been done to evaluate the functional role of the $GABA_A$ α1 subunit in the context of cocaine self-administration.

While our study focused on tissue obtained primarily from the NAc core, a recent study of NAc shell found evidence for dynamic changes in the balance of GABA and glutamate transmission during withdrawal from limited-access cocaine self-administration (Otaka et al., 2013). On WD1, they observed a decrease in the relative weight of excitatory to inhibitory synaptic inputs to NAc shell MSN (defined operationally as the ratio of the peak amplitude of EPSCs divided by the peak amplitude of IPSCs). If the decrease in $GABA_A$ α2 subunit surface expression that we detected in core on WD2 also occurs in shell, this could help explain their WD1 results. On WD21, Otaka et al. (2013) found that the excitatory/inhibitory ratio in NAc shell was increased, probably reflecting a combination of decreased amplitude of $GABA_A$R mIPSCs and enhanced AMPAR transmission detected at this withdrawal time (Otaka et al., 2013). It will be important to conduct similar recording studies in NAc core MSN.

Overall, two main conclusions can be drawn from our study. First, surface expression of the $GABA_A$ receptor subunits α1, α2, and α4 is not altered during the late withdrawal period when we have previously demonstrated enhanced AMPAR transmission in the NAc and elevated cue-induced cocaine craving. These results suggest the absence of homeostatic changes involving GABA transmission. Therefore, it may be of interest to investigate whether enhancement of GABA transmission during abstinence can attenuate cocaine craving and prevent relapse by offsetting increased AMPAR transmission. Many studies have shown that the $GABA_B$ agonist baclofen attenuates cocaine-related behaviors in animal

models of addiction, including tests of drug seeking during abstinence (Filip et al., 2015), and some results of clinical trials suggest that baclofen may be of greater benefit during abstinence as compared to during active cocaine use (Shoptaw et al., 2003; Kahn et al., 2009). Second, our observation of a transient elevation in α2 subunit surface expression in early withdrawal (WD2), which returned to normal levels by WD25, could help explain low levels of cocaine seeking in early withdrawal, although its functional significance remains to be examined. More generally, it may serve as a component of maladaptive processes that pave the way for incubation of cocaine craving and accompanying adaptations observed later in withdrawal.

4. Experimental Procedures

4.1. Jugular catheterization surgery and self-administration

Our procedures for jugular catheterization surgery and cocaine self-administration have been described in detail previously (Conrad et al., 2008). Briefly, adult male Sprague-Dawley rats (250–275 g on arrival; Harlan, Indianapolis, IN) were housed singly in standard rat cages (lights on at 1900 hours, off at 0700 hours; food and water available *ad libitum*). After ~1 week to acclimate to the colony, rats were surgically implanted with a jugular catheter (PlasticsOne, Roanoke, VA) under ketamine-xylazine anesthesia (80–10mg/kg, i.p., respectively) to enable intravenous drug self-administration. Rats received the analgesic flunixin meglumine (2mg/kg, s.c; Henry Schein, Melville, NY) before surgical procedures. Immediately after surgery and each day during the recovery period (5–7 days), intravenous antibiotic was administered (Cefazolin; 100mg/ml, 0.15ml; Moore Medical, Farmington, CT) and the catheters were flushed with sterile saline solution to ensure patency. After this recovery period, rats began self-administration training in operant chambers (MED Associates, St Albans, VT). All self-administration sessions were conducted during the dark cycle. Nose-poking in the inactive hole had no consequences, whereas nose-poking in the active hole delivered an infusion of saline or cocaine (0.5 mg/kg in a 100 μl/kg volume over 3 s), paired with a 20 s light cue inside the nose-poke hole. All procedures were performed in accordance with the USPHS Guide for Care and Use of Laboratory Animals and EC Directive 86/609/EEC, and were approved by the Rosalind Franklin University of Medicine and Sciences Institutional Animal Care and Use Committee.

4.2 Biotinylation

All samples were collected and processed as described previously (Ferrario et al., 2011). Briefly, animals were decapitated and brains were rapidly removed. The NAc (mainly core, but sometimes including a portion of lateral shell; see diagram in McCutcheon et al., 2011) was punched from a 2 mm coronal section obtained using a brain matrix. Bilateral pieces of NAc tissue from each rat were minced with a scalpel. Tissue was then added to eppendorf tubes containing ice-cold aCSF with 1mM sulfo-NHS-S-S-Biotin (Thermo Scientific, Rockford, IL) and incubated at 4°C with gentle agitation for 30 min. Samples were incubated with 100mM glycine at 4°C for 10 min to quench the reaction. They were then pelleted, re-suspended in ice-cold lysis buffer [25mM HEPES pH 7.4, 500mM NaCl, 2mM EDTA, 20mM NaF, 10mM NaPPi, 1mM PMSF, 0.1% NP-40 (v/v), 1mM NaOV, 1μM okadaic acid, 1μM microsystin-LF, 1× protease inhibitor cocktail (Calbiochem 539131,

EMD Millipore, Billerica, MA)], sonicated, and stored at −80°C. An aliquot of each sample (100μg) was added to 37.5 μg of high capacity NeutrAvidin agarose beads (Thermo Scientific, Rockford, IL) and incubated overnight at 4°C on an end-over-end rotator. Biotinylated proteins bound to NeutrAvidin beads (bound fraction) were isolated from the nonbiotinylated (unbound) fraction by centrifugation (3000 RPM, 1min) and washed several times with 1× PBS. The biotinylated fraction was then dissolved in Laemmli sample buffer with DTT (100mM) and heated at 97°C for 3 min to release the biotinylated protein from the beads. The samples were then spun at 10,000 RPM for 5 min on a centrifugal filter unit (0.45μm, UFC30HV00, EMD Millipore, Billerica, MA) to remove the NeutrAvidin beads from the solution. Samples were used for immunoblotting as described below.

4.3 SDS-PAGE and immunoblotting

Samples were heated to 70°C in Laemmli sample treatment buffer and electrophoresed on 4–12% bis-tris gradient gels (Cat# 345-0125; BioRad, Des Plaines, IL) under reducing conditions. Proteins were then transferred to PVDF membranes (Amersham Biosciences, Piscataway, NJ). Using the SNAP-ID 2.0 protein detection system (EMD Millipore, Billerica, MA), PVDF membranes were incubated with blocking solution (0.5% non-fat milk and 1% goat serum in TBS-Tween20 (TBS-T); 10 min), which was followed by incubation with primary antibodies for $GABA_A$ receptor subunits α1 (1:333; 75–136; Neuromab, Davis, CA), α2 (1:333; AB72445; ABCAM, Cambridge, MA), or α4 (1:10; 73–383; Neuromab, Davis, CA) for 30 min. Membranes were washed 4 times with TBS-T, followed by incubation with secondary antibodies (HRP-conjugated anti-rabbit or anti-mouse; 1:3000; Invitrogen, Carlsbad, CA). Membranes were then washed 4 times with TBS-T and immersed in chemiluminescence (ECL) detecting substrate (GE Healthcare, Piscataway, NJ). Images were acquired with an Amersham Imager 600 (GE Healthcare, Piscataway, NJ) and quantified with TotalLab software (TotalLab; Newcastle, UK). Data were excluded if there were problems that interfered with band analysis such as bubbles. A background value was obtained and diffuse densities for bands of interest in each lane were determined. For data obtained from starting material, diffuse densities were normalized to either total protein in the lane as determined by Ponceau staining (P7170-1L; Sigma-Aldrich, St. Louis, MO) or a loading control (GAPDH; CB1001; EMD Millipore, Billerica, MA). Controls were performed to verify that intracellular proteins such as tyrosine hydroxylase were not detected in the bound fraction.

4.4 Statistical analysis

Results are expressed as mean ± SEM. Two-tail unpaired t-tests were used to assess group differences (cocaine vs. saline) in $GABA_A$ subunit expression at each withdrawal time-point. Two-way ANOVA was used to analyze differences (cocaine vs. saline) through the course of withdrawal (WD2, WD25, and WD48).

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Highlights

- Cocaine craving incubates during withdrawal from cocaine self-administration (SA).
- GABA is critical for regulating NAc output but its role in incubation is unknown.
- $GABA_AR$ α1, α2, and α4 subunits were measured during withdrawal from cocaine SA.
- In early withdrawal (day 2), α2 surface expression was increased in NAc core.
- No alterations in $GABA_AR$ subunits were found in late withdrawal (25 or 48 days).

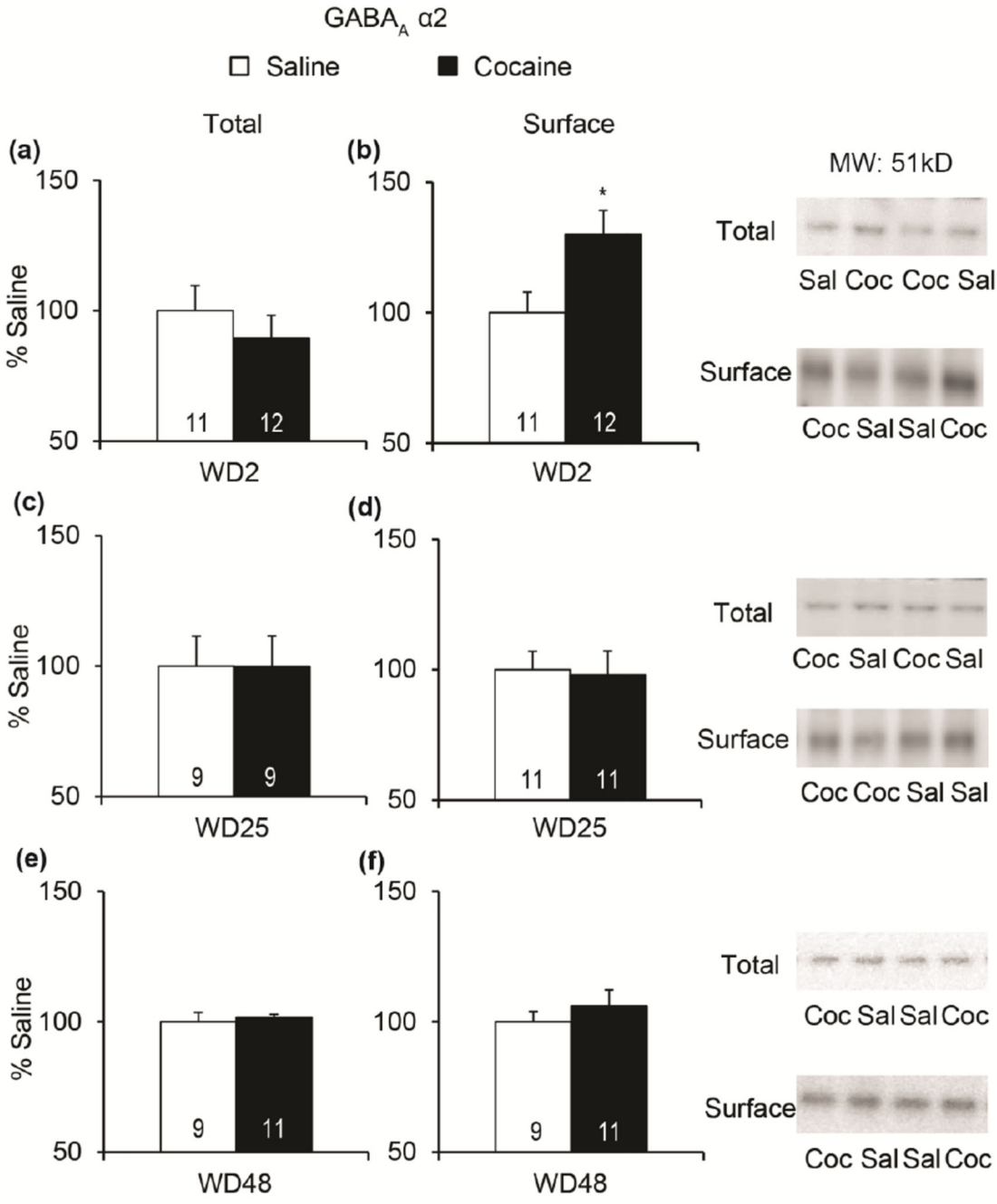

Figure 1.

Effect of extended-access cocaine administration and different periods of withdrawal on the expression of GABA$_A$ α2 receptor subunits in the NAc. (a,b) On WD2 after discontinuing cocaine self-administration, total expression of GABA$_A$ α2 subunits was unchanged (t-test, $p>0.05$), while there was a significant increase in surface expression of this subunit (t-test, $*p<0.05$). (c,d) On WD25, total expression of GABA$_A$ α2 subunits remained unchanged while surface expression of the subunit returned to a level comparable to saline controls (t-tests, $p>0.05$). (e,f) On WD48, total and cell surface levels of GABA$_A$ α2 subunits did not

differ between cocaine and saline groups (t-tests, $p>0.05$). Two-way ANOVA revealed no significant difference between cocaine and saline groups over different withdrawal times ($p>0.05$). Representative blots show data from 4 different animals: 2 cocaine animals and 2 saline animals. Numbers within the bars indicate the number of rats in each experimental group.

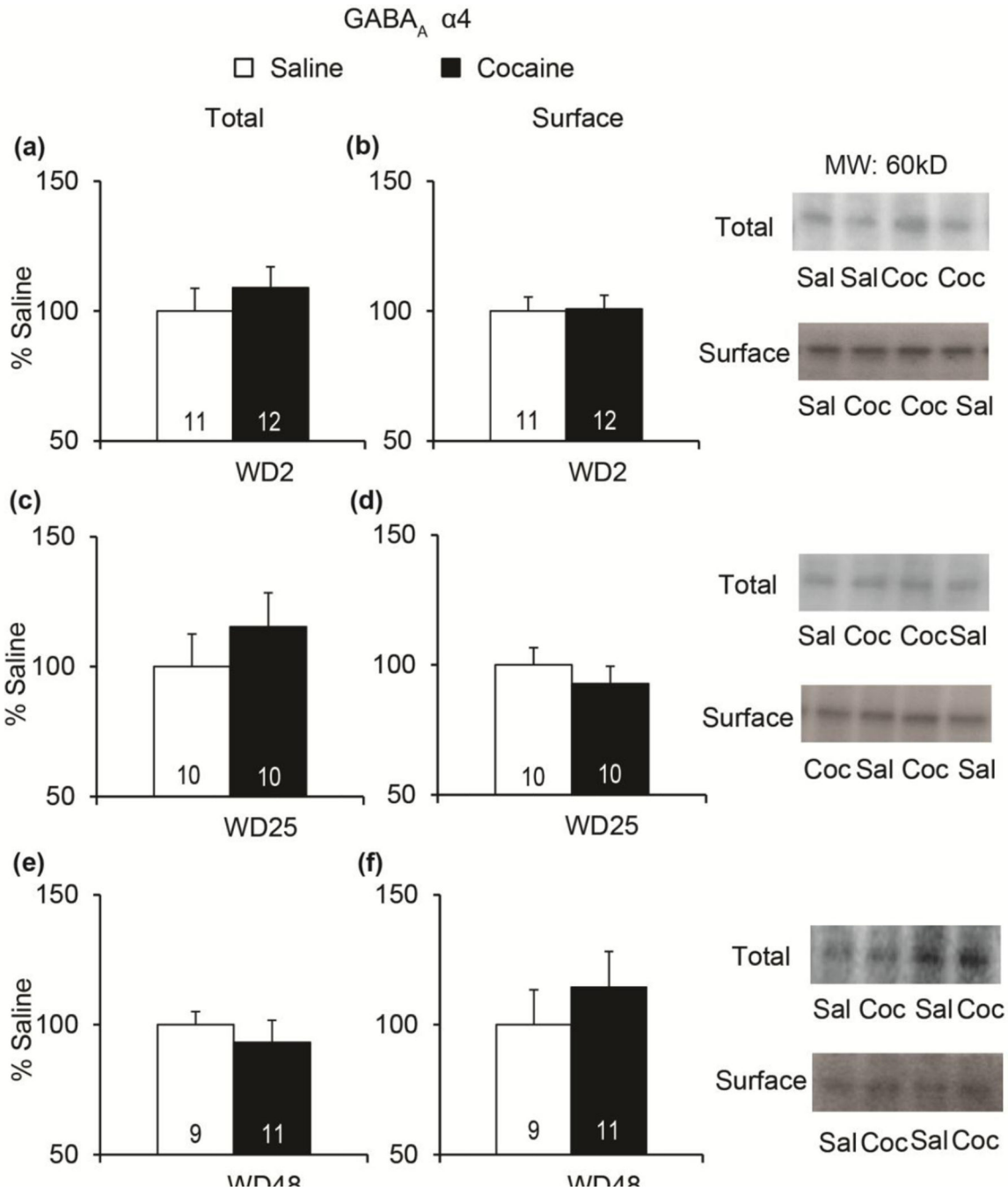

Figure 2.

Effect of extended access cocaine administration and different periods of withdrawal on the expression of GABA$_A$ α4 subunits in the NAc. Saline and cocaine rats did not differ in either surface or total expression of GABA$_A$ α4 subunits on WD2 (a,b), WD25 (c,d), or WD48 (e,f) (t-tests, $p>0.05$). Two-way ANOVA revealed no significant difference between cocaine and saline groups over different withdrawal times ($p>0.05$). Representative blots show data from 4 different animals: 2 cocaine animals and 2 saline animals. Numbers within the bars indicate the number of rats in each experimental group.

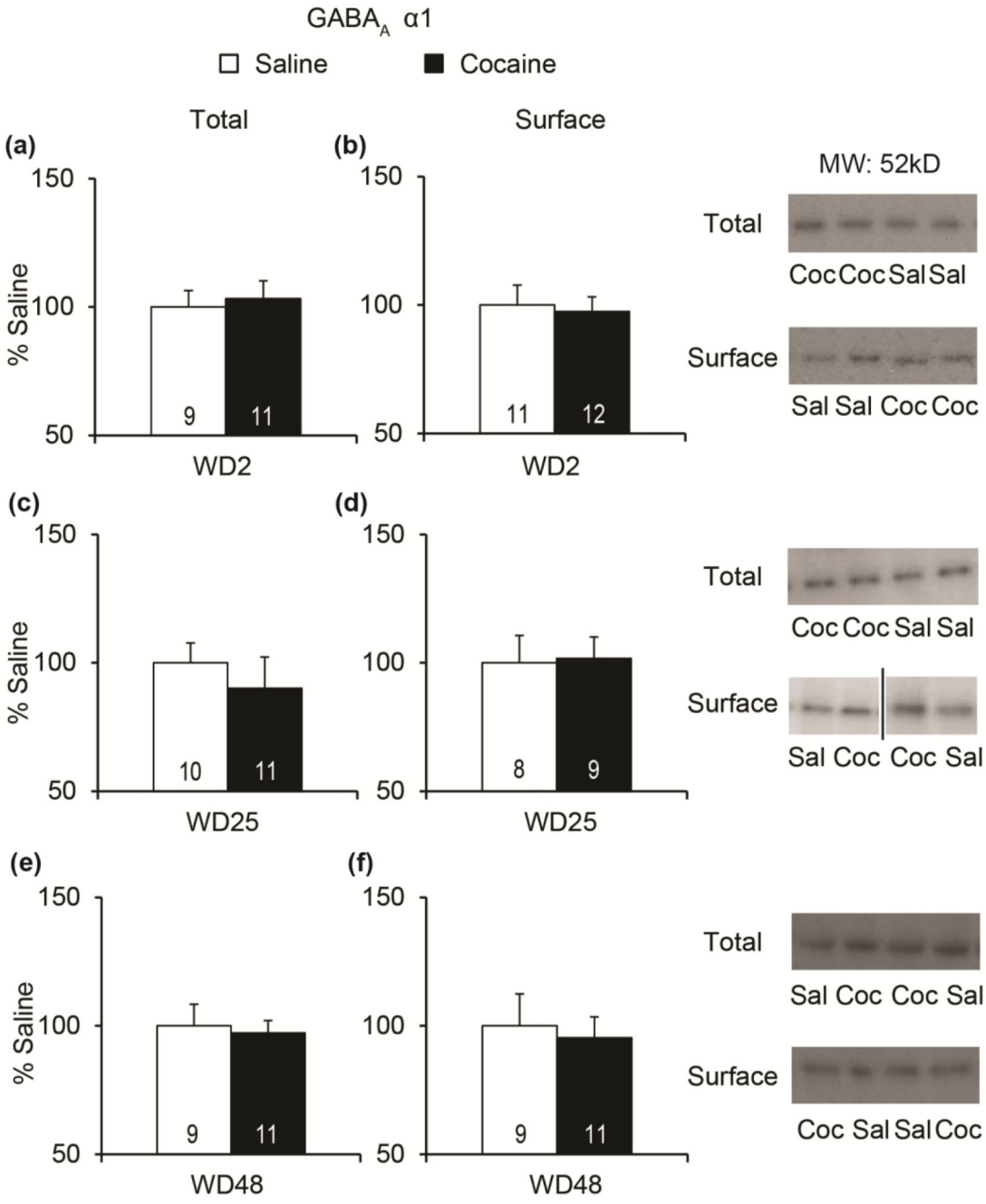

Figure 3.

Effect of extended access cocaine administration and different periods of withdrawal on the expression of GABA$_A$ α1 subunits in the NAc. Saline and cocaine rats did not differ in either surface or total expression of GABAA α4 subunits on WD2 (a,b), WD25 (c,d), or WD48 (e,f) (t-tests, $p>0.05$). Two-way ANOVA revealed no significant difference between cocaine and saline groups over different withdrawal times ($p>0.05$). Representative blots show data from 4 different animals: 2 cocaine animals and 2 saline animals. Vertical line in the surface blot in the middle panel indicates that lanes were not adjacent in the original

immunoblot. Numbers within the bars indicate the number of rats in each experimental group.