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Cocaine regulates protein kinase B and glycogen synthase kinase-3 activity in selective regions of rat brain

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Abstract

Protein kinase B (Akt) signaling regulates dopamine-mediated locomotor behaviors. Here the ability of cocaine to regulate Akt and glycogen synthase kinase-3 (GSK3) was studied. Rats were injected with cocaine or saline in a binge-pattern, which consisted of 3 daily injections of 15 mg/kg cocaine or 1 ml/kg saline spaced one hour apart for 1, 3 or 14 days. Amygdala, nucleus accumbens, caudate putamen and hippocampus tissues were dissected 30 minutes following the last injection and analyzed for phosphorylated and total Akt and GSK3(α & β) protein levels using Western blot analysis. Phosphorylation of Akt on the threonine-308 residue was significantly reduced in the nucleus accumbens and increased in the amygdala after 1 day of cocaine treatment; however, these effects were not accompanied by a significant decrease in GSK3 phosphorylation. Phosphorylation of Akt and GSK3 were significantly reduced after 14 days of cocaine administration, an effect that was only observed in the amygdala. Cocaine did not alter Akt or GSK3 phosphorylation in the caudate putamen or hippocampus. The findings in nucleus accumbens may reflect dopaminergic motor-stimulant activity caused by acute cocaine, whereas the effects in amygdala may be associated with changes in emotional state that occur after acute and chronic cocaine exposure.

Keywords

cocaine; Akt; GSK3; rat; amygdala; nucleus accumbens

Introduction

Protein kinase B (also known as Akt) is a serine/threonine kinase that is widely expressed in brain and has classically been associated with its ability to regulate cellular growth and apoptosis (Song *et al.* 2005). Akt activity has been shown to play a key role in dopamine mediated behaviors, an effect independent of its role in cell survival and dependent on decreased phosphorylation, thus deactivation, of striatal Akt (Beaulieu *et al.* 2007a). Akt is the downstream mediator of two key signaling pathways in the cell, the inositol tri-phosphate pathway and the cAMP/protein kinase A pathway (Song *et al.* 2005). Akt activity is regulated by phosphorylation at serine-473 and threonine-308 (T308). Studies have shown that full activation of Akt in the role of cell survival pathways requires the phosphorylation of both residues (Song *et al.* 2005), while the role of Akt in dopamine-mediated behaviors is dependent on phosphorylation only at the T308 residue (Beaulieu *et al.* 2007a). Akt is

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upstream to many signaling molecules, notably glycogen synthase kinase-3 (GSK3). Akt negatively regulates the activity of GSK3 via increased phosphorylation of the inhibitory serine-9 residue in the beta form (Manning & Cantley 2007).

Originally isolated from skeletal muscle (Rylatt *et al.* 1980), GSK3 was first characterized based on its effects on glycogen synthase (Embi *et al.* 1980); however, it has been shown to be widely expressed in brain (Woodgett 1990, Leroy & Brion 1999) and its ability to regulate the transcription factors cAMP response element-binding protein (CREB) and β -catenin (Frame *et al.* 2001) have led researchers to study its role in neuronal function. GSK3 plays a key role in the Wnt signaling pathway, and it is unique in that its phosphorylation at serine-21/9 (serine-21 in GSK3 α and serine-9 in GSK3 β) leads to decreased kinase activity (Frame & Cohen 2001). GSK3 plays a critical role in diabetes, stroke, Alzheimer's disease (Frame & Cohen 2001) and schizophrenia (Alimohamad *et al.* 2005), and the mechanism responsible for the mood stabilizing effect of lithium is dependent on inhibition of GSK3 β activity (Gould *et al.* 2004).

Previous studies have documented that Akt and/or GSK3 activity is altered by dopamine receptor agonists (Brami-Cherrier *et al.* 2002), amphetamine (Beaulieu *et al.* 2004, McGinty *et al.* 2008, Svenningsson *et al.* 2003, Wei *et al.* 2007), methamphetamine (Chen *et al.* 2007), cocaine (Gil *et al.* 2003, Novikova *et al.* 2005, Wei *et al.* 2007), or by genetic deletion of the dopamine transporter (Beaulieu *et al.* 2006, Beaulieu *et al.* 2004). Typically, following dopamine receptor activation, a time-dependent decrease in phosphorylated Akt in striatum is observed which leads to increased GSK3 activity (by decreasing phosphorylated GSK3) and subsequent increase in gene transcription (Beaulieu *et al.* 2004, Chen *et al.* 2007). Although the clinical relevance of GSK3 has been noted, the role of Akt in neuropsychiatric disorders particularly psychostimulant abuse has largely gone unstudied; however, a positive association between a single nucleotide polymorphism and Akt1 haplotype in methamphetamine abusers has been identified (Ikeda *et al.* 2006). With these observations in mind, we measured the effects of acute (1 day), repeated (3 days) or chronic (14 days) binge-pattern cocaine administration on levels of phosphorylated and total Akt and GSK3 proteins in key dopaminergic-projection areas, including amygdala, nucleus accumbens, caudate putamen and hippocampus, in order to determine the temporal and spatial patterns of Akt and GSK3 regulation.

Methods

Male Sprague Dawley rats (275–375 g) were purchased from Charles River Labs (Wilmington, MA), group-housed on a 12 h light/dark cycle (7 AM – 7 PM) and given water and food *ad libitum*. Rats were acclimated to their environment for 5 days during which time they were weighed and handled daily. All procedures were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and approved by Temple University School of Medicine Institutional Animal Care and Use Committee. Cocaine HCl was provided by NIDA Drug Supply Program. All other reagents and supplies were purchased from Sigma-Aldrich (St Louis, MI) or Bio-Rad (Hercules, CA).

Rats were injected intraperitoneally (ip) with cocaine (15 mg/kg) or saline (1 ml/kg) in a binge-pattern (3 injections at 1 hour intervals beginning at 9 AM) for 1, 3 or 14 days. Binge-pattern administration is a commonly used animal model of cocaine abuse and was developed to better approximate the temporal pattern and relationship to circadian rhythm that is seen in human abuse of cocaine (Unterwald *et al.* 1992, Unterwald *et al.* 2001). Rats were killed 30 minutes after the last injection and brains were dissected rapidly on ice. The amygdala, nucleus accumbens, caudate putamen and hippocampus were dissected according to the rat brain atlas of Paxinos and Watson (Paxinos & Watson 2006). Samples were

immediately prepared by homogenizing brain regions from individual animals in boiling 1% (g/L) sodium dodecyl sulfate (SDS) using a sonicator. Homogenized samples were boiled for 5 minutes and then stored at -80°C until use.

Protein concentrations were measured using the Folin-Lowery assay (Lowry *et al.* 1951). Proteins were separated using SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to nitrocellulose for Western blot analysis of phosphorylated and total protein levels. Equal amounts (20 μg) of protein were loaded in 10% (g/L) Tris-HCl BioRad Ready-gels. Proteins were visualized by Ponceau S Red (general protein dye) to ensure transfer and equal protein loading. Nitrocellulose blots were incubated in blocking solution (tris-buffered saline with 5% (g/L) non-fat dry milk), then in primary antibody overnight at 4°C (except tubulin antibody which was incubated for 1 h at room temperature). Blots were washed with Tween-20 Tris-buffered saline (TTBS) then incubated with anti-rabbit (1:2,000 for pAkt(T308), pGSK3 α - β and total Akt) or anti-mouse (1:5,000 for total GSK3 α - β and tubulin) secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature. Blots were washed with TTBS. Nitrocellulose-bound proteins were visualized using the Pierce Supersignal enhanced chemiluminescent substrate and Fujifilm image gauge hardware and quantified using Science Lab 99 L-Process v1.96 software. To determine phosphorylated protein levels as a ratio of total protein levels, blots were first probed for phosphorylated protein (pAkt(T308) or pGSK3 α - β), then stripped using Restore Western Blot Stripping Buffer (Pierce), washed with TTBS and re-probed for total protein levels (Akt or GSK3 α - β). Primary antibodies were diluted in TBS (with 5% (g/L) non fat dry milk) and those used include phosphorylated Akt T308 [pAkt(T308); Cell Signaling 1:500], Akt [total Akt; Cell Signaling 1:1,000], phosphorylated GSK3 α - β [pGSK3 α - β Cell Signaling 1:2,000], GSK3 α - β [total GSK3 α - β Santa Cruz 1:5,000] and tubulin [Sigma 1:40,000]. The antibody used to detect GSK3 (phosphorylated and total) recognizes both a (S21, 51 kDa) and β (S9, 48 kDa) isoforms. Immunoreactivity to tubulin was used to verify equal protein loading and transfer. All data are expressed as phosphorylated protein/total protein and graphed as a percent of control. The extent of enzyme phosphorylation is used as a proxy indicator of enzymatic activity. A correlation between phosphorylation of Akt(T308) and Akt enzymatic activity has been established (Ma *et al.* 2008).

Data were analyzed by independent two-tailed t-tests comparing group means between cocaine- and saline-injected controls following 1, 3 or 14 days of treatment. Statistical significance was considered at $p < 0.05$ with a 95% confidence interval. Data were analyzed using Graphpad Prism 4. Samples sizes ranged from 6–10 and were generally 8/group.

Results

Levels of phosphorylated and total protein were determined for Akt(T308) and its downstream mediator GSK3 α - β in the amygdala, nucleus accumbens, caudate putamen, and hippocampus from rats injected with cocaine for 1, 3 or 14 days. In the amygdala, acute binge-pattern cocaine administration selectively increased pAkt(T308), while chronic cocaine administration decreased phosphorylation of both Akt(T308) and GSK3 α - β . Representative immunoblots of tissue extracts from the amygdala of saline- and cocaine-injected rats at each time point (1, 3 or 14 days) are shown in Figure 1.

Levels of pAkt (pAkt/total Akt) were significantly increased after 1 day (acute; $t_{12}=2.82$, $p=0.015$), not changed after 3 days (repeated; $p>0.05$), and decreased after 14 days (chronic; $t_{12}=2.77$, $p=0.017$) of repeated cocaine administration in the amygdala (Figure 2A). Phosphorylation of GSK3 α - β in the amygdala followed an unusual pattern compared to its upstream mediator pAkt(T308). The increase in pAkt after 1 day of binge-pattern cocaine administration (Figure 2A) was not accompanied by a significant increase in

pGSK3 α (pGSK3 α /total GSK3 α) or pGSK3 β (pGSK3 β /total GSK3 β) in the amygdala (Figure 2B; $p>0.05$). However, the decrease in pAkt after 14 days of cocaine administration (Figure 2A) was paralleled by a significant decrease in pGSK3 α (pGSK3 α /total GSK3 α) and pGSK3 β (pGSK3 β /total GSK3 β) in the amygdala (Figure 2B; $t_{13}=3.04$, $p=0.009$ and $t_{13}=2.70$, $p=0.018$, respectively).

Acute (1 day) binge-pattern cocaine administration significantly decreased pAkt (pAkt/total Akt) in the nucleus accumbens of rats ($t_{16}=2.81$, $p=0.013$), as shown in Figure 3A. No significant differences were found after 3 or 14 days of cocaine administration suggesting that tolerance develops to the regulation of Akt(T308) phosphorylation by cocaine ($p>0.05$; Figure 3A). The levels of pGSK3 α (pGSK3 α /total GSK3 α) and pGSK3 β (pGSK3 β /total GSK3 β) in the nucleus accumbens were not significantly affected by cocaine administration at any day of treatment ($p>0.05$; Figure 3B).

In the caudate putamen, the levels of pAkt(T308)/total Akt (Figure 4A) and pGSK3 α - β /total GSK3 α - β (Figure 4B) were not altered by 1, 3 or 14 days of binge-pattern cocaine ($p>0.05$). Likewise, in the hippocampus, pAkt(T308)/total Akt (Figure 5A) and pGSK3 α - β /total GSK3 α - β (Figure 5B) levels following cocaine were not statistically different from saline-injected controls ($p>0.05$). Total Akt, total GSK3 α - β , or tubulin in cocaine-injected animals were not significantly different from those in controls injected with saline for an equal number of days in any brain region tested ($p>0.05$; data not shown).

Discussion

Cocaine inhibits the function of dopamine transporters (DAT) and thus the uptake of synaptic dopamine, resulting in increased extracellular dopamine levels and increased activation of both dopamine D1 and D2 receptors. The ascending dopamine system consists of three pathways: 1) the nigrostriatal pathway extending from the substantia nigra to the caudate putamen, 2) the mesolimbic pathway projecting from ventral tegmental area to nucleus accumbens and amygdala, and 3) the mesocortical pathway originating in the ventral tegmental area and innervating cerebral cortex and hippocampus. The data presented here show that the effect of cocaine on Akt activity (as assessed by pAkt levels) is spatially restricted to the nucleus accumbens and amygdala, and does not occur in the caudate putamen or hippocampus, indicating the selective involvement of the mesolimbic dopamine system. To our knowledge, these are the first data to show a spatial pattern of Akt regulation following cocaine exposure. In addition, our data also show the temporal pattern of regulation of Akt and GSK3 activity between acute and chronic cocaine administration.

Several studies document the regulation of Akt activity by dopaminergic receptor signaling. Dopamine D1 or D2 receptor agonists rapidly increase pAkt(T308) in primary striatal neuronal cultures, an effect mediated by extracellular signal-regulated kinase (ERK) (Brami-Cherrier *et al.* 2002). While an increase in pAkt(T308) in rat striatum occurs 15 min after an amphetamine injection, a decrease is found two hours later (McGinty *et al.* 2008, Shi & McGinty 2007). The decrease in pAkt(T308) is dependent on the calcium/calmodulin-dependent protein kinase-II pathway following acute amphetamine administration (Wei *et al.* 2007) and requires an increase in ERK phosphorylation via dopamine D3 receptor following methamphetamine treatment (Chen *et al.* 2007). A decrease in Akt(T308) activity is also observed in mice lacking DAT, which have high levels of synaptic dopamine (Beaulieu *et al.* 2006, Beaulieu *et al.* 2004). Our results agree with these findings by demonstrating that pAkt(T308) levels are reduced in the nucleus accumbens (ventral striatum) following acute binge-pattern cocaine administration.

The downstream consequences of Akt activation remain to be fully characterized. However, a clear association between Akt and GSK3 has been demonstrated (Beaulieu *et al.* 2004). GSK3 has been shown to be the target of the mood stabilizer lithium (Gould *et al.* 2004) and dopamine D2 receptor-dependent antipsychotic drugs (Alimohamad *et al.* 2005). GSK3 activity is negatively regulated by Akt activity; therefore as pAkt levels decrease so do those of pGSK3 resulting in increased GSK3 enzymatic activity. It has been demonstrated that increased striatal GSK3 α - β activity (decreased pGSK3) accompanies decreased pAkt levels in DAT-knockout mice and in animals exposed to amphetamine (Beaulieu *et al.* 2004). GSK3 can regulate dopaminergic signaling and behaviors. GSK3-knockout animals have reduced dopamine-dependent locomotor behavior (Beaulieu *et al.* 2004), whereas GSK3 β -overexpressing transgenic mice display increased general locomotor activity, reduced immobility in the forced swim test (i.e. antidepressant-like effect) and most notably an upregulation of Akt expression suggesting a compensatory mechanism (Prickaerts *et al.* 2006). These studies suggest a dopamine-dependent mechanism of regulation of GSK3 activity, but it may be that GSK3 is one of several key downstream effectors of DARPP-32 in a common signaling pathway activated by dopaminergic agonists (e.g. cocaine or amphetamine), serotonergic drugs (e.g. LSD; see discussion below) and glutamatergic antagonists (e.g. PCP) (Svenningsson *et al.* 2003).

Few studies have investigated the effects of cocaine on Akt and GSK3 signaling. Mouse fetuses from cocaine-injected dams show changes in cerebral wall Akt genes (Novikova *et al.* 2005), and young rabbits (PND 20) exposed *in utero* to cocaine have increased basal levels of pGSK3 β in striatum and prefrontal cortex, but not hippocampus (Gil *et al.* 2003). One study found that cocaine does not affect pGSK3 α but blocks the amphetamine-induced decrease in pGSK3 α in striatal synaptosomes (Wei *et al.* 2007). Our data agree in that pGSK3 α (or β) levels are unaffected by acute cocaine and, in addition directly demonstrate that Akt enzymatic activity is decreased in the accumbens following acute cocaine administration.

The results of the present study also demonstrate that the effects of cocaine are dependent on the length of drug exposure. In the nucleus accumbens, Akt activity is decreased after 1 day, but not after 3 or 14 days of repeated cocaine administration. These data are in agreement with the finding that acute amphetamine decreases pAkt(T308) (Beaulieu *et al.* 2004, McGinty *et al.* 2008). However, our data show that pGSK3 α - β activity is not statistically affected by 1 day of cocaine treatment, which is consistent with the published report showing a lack of effect of cocaine on pGSK3 α (Wei *et al.* 2007), but contrary to those studies reporting an amphetamine-induced decrease in pGSK3 α - β (Beaulieu *et al.* 2004, Chen *et al.* 2007). It should be noted that the lack of effect of cocaine on striatal pGSK3 in contrast to that seen with amphetamine may be a reflection of the fact that cocaine is less effective at elevating extracellular dopamine than amphetamine (Ramsey *et al.* 2008), which not only blocks DAT but reverses its function leading to higher levels of synaptic dopamine independent of dopaminergic cell firing (Jones *et al.* 1998). The pharmacological effects of the two agents also differ in terms of their dependence on glutamatergic neurotransmission (Ramsey *et al.* 2008).

This discussion has thus far focused on the dopaminergic effects of cocaine because of the well-characterized effects of the drug on this neurotransmitter and because of the demonstrated effects of dopamine signaling on Akt or GSK3 activity (Beaulieu *et al.* 2007b, Brami-Cherrier *et al.* 2002). While it remains likely that the effects in the striatum are dopamine-dependent, the effects in other brain regions may not be dependent on dopamine receptor signaling. In fact, serotonin-deficient mice have elevated levels of pGSK3 in the frontal cortex. Further, genetic or pharmacological inhibition of GSK3 activity reduces the behavioral abnormalities induced by 5-HT deficiency in these transgenic mice (Beaulieu *et*

al. 2008). These data suggest that lack of 5-HT signaling increases GSK3-dependent behaviors, including anxiety- and depression-like behaviors. Furthermore, the effects of 5-HT deficiency were localized to the frontal cortex and not observed in striatum and hippocampus (amygdala was not studied) (Beaulieu *et al.* 2008).

The data presented herein demonstrate that cocaine alters Akt and GSK activity selectively in the nucleus accumbens and the interconnected amygdala, but not caudate putamen albeit statistically non-significant decreases in pAkt(T308) and pGSK3 levels were observed in this region. This suggests that regulation of Akt by cocaine selectively occurs in the projection fields (i.e. nucleus accumbens and amygdala) of the mesolimbic dopaminergic pathway. This is important because dopamine release onto medium spiny neurons in the accumbens and amygdala is a key regulator of initial drug perception, reinforcement, and reward, orientation and memory of emotional response to drug salience (Berke & Hyman 2000, Kalivas & Volkow 2005). On the other hand, the projection fields of the nigrostriatal and mesocortical dopamine pathways govern compulsive habit and contextual learning behaviors (Berke & Hyman 2000) and regulation of motivational salience and receives, integrates and communicates these stimuli to subcortical areas (Kalivas & Volkow 2005).

The data presented herein from the amygdala indicate that Akt activity is increased after 1 day, not changed after 3 days and decreased after 14 days of binge-pattern cocaine administration. While the levels of pGSK3 α - β showed a trend towards an elevation after 1 and 3 days, only after 14 days of cocaine administration were there significant changes in GSK3 phosphorylation and at this time point levels of pGSK3 α - β were significantly reduced. These data suggest that GSK3 may not be the sole substrate of Akt in the amygdala following acute cocaine, but rather GSK3 may be important in long-term plasticity induced by repeated cocaine exposure. The amygdala is a limbic structure classically recognized for its role in the neuronal underpinnings of emotion and mood-related behaviors in humans (Trimble 1988, Kalia 2005). Although additional studies are required, it is tempting to speculate that the temporal and opposite effects of acute versus chronic cocaine on Akt and GSK3 activity in the amygdala reflect the emotional states observed in humans. We have previously shown that acute withdrawal from 14-day binge-pattern cocaine administration induces anxiety- and depression-like behaviors in rats (Perrine *et al.* 2008). The significant decrease in pAkt and pGSK3 α - β levels and resulting increase in GSK3 activity after 14 days of cocaine exposure is consistent with the finding that GSK3 inhibitors have mood stabilizing and antidepressant-like effects (Gould *et al.* 2004). It may be that the initial euphoric and emotional perception of cocaine is dependent on dopamine-induced increase in Akt activity in the amygdala, while the anxiety and mood-related effects resulting from long-term abuse of cocaine are influenced by serotonin-mediated decreases in Akt activity and subsequent high GSK3 activity in the amygdala. Chronic cocaine administration does not decrease serotonin levels in basolateral amygdala of rat (Baumann *et al.* 1993). However, cocaine exposure upregulates serotonin transporter levels in amygdala in humans (Mash *et al.* 2000) and downregulates 5-HT_{1A} receptors in the central medial amygdala of rats (Cunningham *et al.* 1992). These latter actions of chronic cocaine on components of the 5-HT system could result in decreases in post-synaptic receptor-mediated events and potentially reduce GSK3 activity and increase aberrant behaviors.

In summary, the results presented here show that cocaine induces a spatially restricted pattern of phosphorylation of Akt and GSK3, wherein the nucleus accumbens and amygdala but not caudate putamen and hippocampus are affected. Regulation of these signaling molecules is also dependent on the length of cocaine exposure. More research is needed to understand the behavioral implications of these findings, but the results provide evidence that the Akt-GSK3 pathway is impacted by cocaine exposure. Future studies will investigate

the relationship between cocaine-mediated behavioral sensitization, anxiety- and depression-like behaviors and the involvement of Akt-GSK pathway in these effects.

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Non-standard abbreviations

Akt	protein kinase B
GSK3	glycogen synthase kinase 3

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Figure 1. Representative immunoblots of amygdala tissue from saline (sal) and cocaine (coc) treated rats at each time point (1, 3 or 14 days of cocaine or saline administration)
 Bands represent phosphorylated Akt(T308), total Akt, phosphorylated GSK3α-β, total GSK3α-β and tubulin (from top to bottom). The molecular weights of the proteins are stated on the left (kilodaltons).

Figure 2. Acute binge-pattern cocaine administration selectively increases phosphorylated Akt, while chronic treatment decreases phosphorylation of both Akt and GSK3 in the amygdala of rats

A. Levels of pAkt(T308) to total Akt (pAkt/total Akt) and **B.** pGSK3α to total GSK3α (pGSK3α/total GSK3α) and pGSK3β to total GSK3β (pGSK3β/total GSK3β) were measured by PAGE and Western blot analysis from the amygdala of rats treated with binge-pattern cocaine administration for 1, 3 or 14 days. Total-Akt/tubulin, total-GSK3α/tubulin and total- GSK3β/tubulin levels were not changed in any group (data not shown). Bars represent the mean $\pm$ SEM (N=6–10/group) and are expressed as a percent of the saline-matched control for each group. * $p < 0.05$, ** $p < 0.01$ compared to saline-matched control.

Figure 3. Acute binge-pattern cocaine administration decreases phosphorylated Akt in the nucleus accumbens of rats

A. Levels of pAkt(T308) to total Akt (pAkt/total Akt) and **B.** pGSK3 α to total GSK3 α (pGSK3 α /total GSK3 α) and pGSK3 β to total GSK3 β (pGSK3 β /total GSK3 β) were measured by PAGE and Western blot analysis from the nucleus accumbens of rats injected with cocaine in a binge-pattern for 1, 3 or 14 days. Total-Akt/tubulin, total-GSK3 α /tubulin and total- GSK3 β /tubulin levels were not changed in any group (data not shown). Bars represent the mean $\pm$ SEM (N=6–9/group) and are expressed as a percent of the saline-matched control for each group. * $p < 0.05$ compared to saline-injected controls.

Figure 4. Binge-pattern cocaine administration does not affect Akt or GSK3 α - β phosphorylation in the caudate putamen of rats

Levels of (A.) pAkt(T308) to total Akt (pAkt/total Akt) or (B.) pGSK3 α - β to total GSK3 α - β (pGSK3 α - β /total GSK3 α - β) were measured by PAGE and Western blot analysis from the caudate putamen of rats injected with binge-pattern cocaine for 1, 3 or 14 days. Total- Akt/ tubulin was not changed in any group (data not shown). Bars represent the mean $\pm$ SEM (N=8–10/group) and are expressed as a percent of the saline-matched control for each group.

Figure 5. Binge-pattern cocaine administration does not affect Akt or GSK3 α - β phosphorylation in the hippocampus of rats

Levels of (A.) pAkt(T308) to total Akt (pAkt/total Akt) or (B.) pGSK3 α - β to total GSK3 α - β (pGSK3 α - β /total GSK3 α - β) were measured by PAGE and Western blot analysis from the hippocampus of rats injected with binge-pattern cocaine for 1, 3 or 14 days. Total-Akt/tubulin was not changed in any group (data not shown). Bars represent the mean $\pm$ SEM (N=8–10/group) and are expressed as a percent of the saline-matched control for each group.