The influence of estrous cycle and gonadal hormones on cocaine-induced PKA-mediated intracellular changes

By

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Abstract

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An extensive body of literature provides evidence for sexual dimorphism in drug abuse pattern, behavioral activation, and neurochemical levels. Female rats display a greater behavioral locomotor response to acute cocaine administration, condition faster and at lower doses, and also show greater hypothalamic-pituitary adrenal axis (HPA) activation and reward effects. However, the mechanisms underlying the sexually dimorphic responses to cocaine remain unclear. One biological basis for the observed gender differences in the behavioral response to cocaine may be hormonal fluctuations during the menstrual/estrous cycle; hormonal fluctuations during the estrous cycle have been shown to affect the response to cocaine. To date, no study has addressed which cellular mechanisms may affect the responses to cocaine in females. Both acute and chronic cocaine-induced stimulation of the dopaminergic system has been shown to affect various levels of DARPP-32/PP-1 and PKA/CREB pathways. Our hypothesis is observed gender differences in the behavioral response to cocaine may, in part, be regulated by differential activation of proteins in DARPP-32/PP1 or PKA/CREB pathways. We postulate that protein activation in PKA-mediated pathways may be sexually dimorphic and/or differentially activated by the estrous cycle. Because of the crucial role of ovarian hormones on female biological responses, a correlate of this hypothesis is that estrogen and progesterone differentially alter cocaine intracellular

activation. In the NAc, cocaine-induced sex differences were observed in PKA-mediated intracellular cascades: males had higher PKA protein levels than females, while females had higher P-Thr-34-DARPP-32 protein levels than males. Cocaine-induced estrous cycle differences were observed in PKA, pCREB, PP-2B, P-Thr-34-DARPP-32 protein levels: 15 min after acute cocaine, PP-2B (CNA1) protein levels increased in estrus females, while PKA and pCREB levels decreased in estrus females. One hour after cocaine administration, P-Thr-34-DARPP-32 protein levels increased in the NAc of estrus and diestrus females, and in the CPu of diestrus females. Moreover, region-specific estrous cycle differences in P-Thr-34-DARPP-32 protein levels were observed: estrus females had higher accumbal P-Thr-34-DARPP-32 protein levels than diestrus females, whereas, in the CPu, diestrus females had higher P-Thr-34-DARPP-32 protein levels than estrus. Furthermore, cocaine-induced hormone replacement differences were observed in PKA, pCREB, and ΔFosB protein levels: accumbal PKA protein levels increased in vehicle and E+P co-administration group, while accumbal pCREB protein levels increased in vehicle and decreased in E+P co-administration group. Additionally, ΔFosB protein levels decreased in vehicle females. Moreover, when analyzed to their respective saline controls, accumbal PKA and pCREB protein levels were higher in the vehicle group when compared to hormone replacement groups and E+P co-administration group had lower pCREB protein levels compared to all other groups. Understanding how the estrous cycle and gonadal hormones affect cocaine-induced intracellular changes should allow for more efficient treatment for female addicts, which represent one-third of the current cocaine users.

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Name	Abbreviation
Adenosine 5'-triphosphate	ATP
alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	AMPA
Analysis of variance	ANOVA
Anterior pituitary	AP
Anteroventral periventricular nucleus	AVPV
Calcium	Ca^{+2}
cAMP-dependent protein kinase	PKA
cAMP, response element-binding protein	CREB
Caudate putamen	CPu
c-fos protein	c-fos
Cyclin-dependent kinase 5	Cdk5
Conditioned place preference	CPP
D1-like family of dopamine receptors	D1/D5
D2-like family of dopamine receptors	D2/D3/D4
Delta FosB	ΔFosB
Dihydroxyphenylacetic acid	DOPAC
Deoxyribonucleic acid	DNA
Dopamine transporter	DAT
Dopamine	DA
Dopamine and cAMP-regulated~32 kDa phosphoprotein	DARPP-32
Dithiothreitol	DTT
Estrogen	E
Estrogen + Progesterone co-administration	E+P
Hypothalamic-pituitary-adrenal axis	HPA axis
Follicle-stimulating hormone	FSH
Gamma-aminobutyric acid	GABA
Glutamate	GLU
Gonadotropin-releasing hormone	GnRH
Immediate early gene	IEG
Knockout mice	KO mice
Luteinizing hormone	LH
N-methyl-D-aspartic acid	NMDA
Norepinephrine	NE
Nucleus accumbens	NAc
Ovarianectomized	OVX
Progesterone	P
Phosphorylated DARPP-32 at threonine 34	p-Thr-34-DARPP-32
Phenylmethylsulphonyl fluoride	PMSF
Protein-phosphatase-1	PP-1
Protein-phosphatase-2A	PP-2A
Protein-phosphatase-2B (calcineurin)	PP-2B
Protein phosphatase 2B (catalytic subunit)	CNA1
Protein phosphatase 2B (regulatory subunit)	CNB1
Standard error of the mean	SEM
Sodium Fluoride	NaF
Tris-buffere saline tween-20	TBST
Vehicle	V
Ventral tegmentum area	VTA

I. Background and Significance

Cocaine is an alkaloid compound extracted from the leaves of Erythroxylon coca, a plant that is native to the Andes and western South America [98]. Coca has been used by the Andean Indians for over a thousand years who chew the leaves, to release cocaine and its stimulant properties, to offset symptoms associated with living in high altitudes including dizziness and nausea [83]. In the late 19th century, Karl Koller discovered that cocaine was an excellent anesthetic to the surface of the eye, and experimentation of injecting cocaine near nerves to block pain shortly followed [130]. Pure cocaine was used in the 1880's as a vasoconstricting local anesthetic agent beneficial for eye, nose, and throat surgeries [3]. Drs. William A. Hammond in America and Sigmund Freud in Europe extolled cocaine as an almost safe and non-addicting drug for many personality disorders [129]. However, the medicinal use of cocaine declined rapidly since 1880-1900, when the increase in production of refined cocaine, led to an equally marked increase in medical reports of cocaine-related toxicity and mortality [213]. The Harrison Narcotic Act of 1914 prohibited access to cocaine without a prescription [131].

Cocaine resurfaced as a drug of abuse in the 1970s and 1980s due to an increase in supply and a decrease in production costs. Its use tended to be limited to young, urban males, earning higher than average wages [213]. Recently, this statistic has dramatically changed. During 2002, there were an estimated one million new cocaine users in the United States [3]. According to the 2004 National Survey on Drug Use and Health, approximately 34.9 million Americans ages 12 and older had tried cocaine at least once in their lifetime [3]. Among students surveyed, 3.4% of eighth graders, 5.4% of tenth graders, and 8.1% of twelfth graders report using cocaine at least once during their

lifetimes [3]. In 1998, the National Household Survey on Drug Abuse reported that 33% of the 1.8 million Americans that currently use cocaine were women [1]. The number of emergency room visits increased; specifically, a nine per cent increase in women was observed [2].

DA System

Dopamine (DA) is commonly associated with the 'pleasure system' of the brain, and is thought to underlie reward processes, motivation, and addictive behaviors [102]. The DA mesolimbic circuitry includes dopaminergic cell bodies in the ventral tegmentum area (VTA) that project to the prefrontal cortex, hippocampus, amygdala, and nucleus accumbens (NAc) [Figure 1]. The mesolimbic pathway is thought to be involved in producing pleasurable feeling and is often associated with feelings of reward and desire. Therefore, it is heavily implicated in neurobiological theories of addiction. The NAc is a heterogeneous structure made up of two functionally and anatomically distinct subregions, the central “core” and the surrounding “shell,” which are thought to be a site for integration of limbic information related to memory, drive, and goal-directed motor behaviors [221]. Thus, it has been widely postulated that the NAc plays a pivotal role on the control of motor activation.

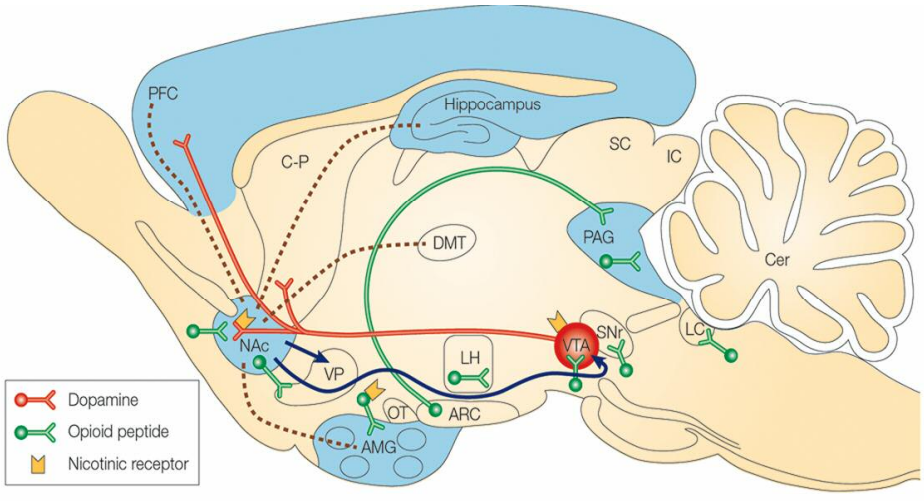


Figure 1: Dopamine pathways in the brain. The mesolimbic pathway links the area in the midbrain to the NAc in the limbic system. The mesolimbic pathway is thought to be involved with reward and pleasure. [134]

Dopamine transmission is mediated by membrane receptors belonging to the seven transmembrane domaine, G-protein-coupled family of receptors. The dopamine receptor subtypes, designated D1 through D5 [Figure 2], can be broken down further into two receptor classes based on homology and pharmacology, D1-like (D1 and D5), or D2-like (D2, D3, D4) [179]

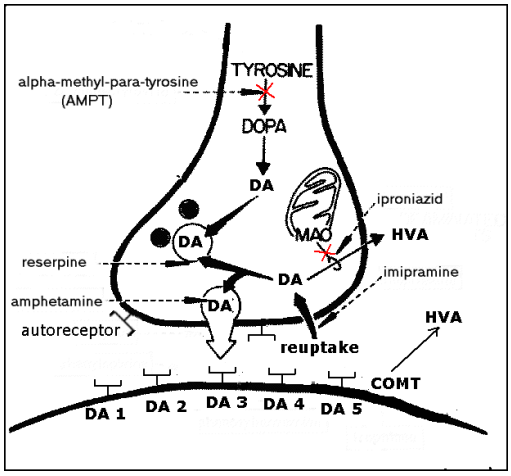


Fig 2: DA receptors in the human nervous system can be classified into 5 different subtypes: D1, D2, D3, D4 and D5. D1 and D5 receptors are excitatory, which means that they stimulate the formation of cAMP by activation of the G-protein. On the other hand, D3, D4 and D5 receptors are inhibitory and inhibit the formation of cAMP [56].

DA Intracellular activities

D1-like and D2-like receptors have opposing intracellular effects due to the activation of their respective G-proteins and their subsequent cascade of effects. Dopamine signaling through the activation of D1-like receptors activates G protein dependent ($G_s$alpha/$G_o$alpha), which in turn stimulates adenylyl cyclase and protein kinase A [64]. Adenylate cyclase promotes 3',5' cyclic adenosine monophosphate (cAMP) formation through ATP hydrolysis, and cAMP activates the catalytic subunits of cAMP-dependent protein kinase (PKA) to amplify DA signaling cascades [54]. Activity of the transcription factors, cAMP, and cAMP response element-binding protein (CREB) increases as a result of the increase in PKA [119]. In contrast, D2-like receptors lead to inhibition of PKA signaling by activating $G_i$alpha/$G_o$alpha, which in turn, inhibits adenylyl cyclase [179]. Furthermore, dopamine receptor subtypes interact additionally with other signal transduction pathways besides adenylyl cyclase that affects calcium stores [7].

PKA/CREB/Fos Pathway

Stimulation of D1 receptors and adenylyl cylase increases activation of PKA. PKA phosphorylates CREB (cAMP response element-binding) on a single serine residue, Ser 133 [149]. Once phosphorylated, CREB proteins are transcription factors which bind to certain sequences called cAMP response elements (CRE) in DNA and thereby regulate gene expression [119;176]. For example, once phosphorlyated, CREB dimers bound to specific CREB sites on target genes, and can produce proteins, such as dynorphin, which cuts off dopamine release and temporarily inhibits the reward circuit. Additionally,

phosphorylated CREB has been shown to increase c-Fos and ΔFosB expression in the NAc and striatum [119;176]. c-Fos and ΔFosB are members of the Fos family, belonging to the immediate early gene (IEG) family of transcription factors, which are thought to play a role in mediating both short- and long-term in neuronal function [132].

DARPP-32/PP-1/PP-2B pathway

D1 receptor activation regulates dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) via an increase of cAMP production and stimulation of PKA, resulting in phosphorlyation of DARPP-32 at Thr34 [204]. DARPP-32 is found in medium-spiny inhibitory neurons, which make up 95% of the cell type in the striatum and has been shown to be a key mediator of the biochemical, electrophysical, transcriptional, and behavioral effects of dopamine [139;143] [Refer to Table III]. Phosphorylation of DARPP-32 at Threonine 34 converts DARPP-32 into a potent inhibitor of the serine/threonine protein phosphatase, PP-1 [139]. PP-1 is thought to modulate the phosphorylation state and activity of a variety of downstream effector molecules critical in several regulatory cellular processes; including cell division, gene expression, synaptic plasticity, apoptosis, as well as activity including various neurotransmitter receptors and voltage-gated ion channels [61].

D1 receptor activation also decreases phosphorylation of DARPP-32 at Thr75, mediated by PKA-dependent phosphorylation of protein-phosphatase-2A (PP-2A). Thus, PP-2A further promotes D1 receptor-mediated stimulation of cAMP pathway by removing P-Thr75-DARPP-32 inhibition on PKA, resulting in a positive feedback loop [18;138;198]. D2 receptor activation leads to inhibition of PKA signaling by activating

$Ca2^{+}$-dependent activation of calcineurin, a protein phosphatase also known as (PP2B), which plays an important role in dephosphorlyation of Thr34 of DARPP-32, thus, counteracting the effects of PKA by blocking the DARPP-32 pathway [138;139;189].

Furthermore, cross-talk occurs throughout the intracellular cascade of PKA-mediated pathways. For example, dephosphorylation of CREB is under the control of PP-1 [77]. Moreover, after D1 receptor-mediated activation in DARPP-32 KO mice, CREB, c-Fos, and ΔFosB levels are strongly attenuated [62;188;219]. Additionally, ΔFosB can activate cyclin-dependent kinase 5 (Cdk5), which phosphorylates DARPP-32 at Threonine 75, converting DARPP-32 into an inhibitor of PKA, reducing its ability to phosphorylate DARPP-32 and other substrates [17]. Taken together, PKA-mediated intracellular cascades have a complex and dynamic compensatory relationship.

II. Cocaine's effects on the CNS at the behavioral and cellular level

A. Cocaine effects on the dopaminergic system

Cocaine acts pharmacologically to inhibit the reuptake of DA, serotonin (5-HT), and norepinephrine (NE) at monoaminergic transporters in the synaptic cleft, thus, elevating extracellular levels of these neurotransmitters [159] [Figure 4]. Although serotonin and norepinephrine play a significant role in cocaine addiction, in this study we will concentrate on the effects of dopamine.

It is widely known that cocaine effects dopaminergic function, primarily through blocking the reuptake of dopamine in the synaptic cleft through the presynaptic dopamine transporter (DAT), and subsequent DA accumulation in the synapse [159]. An extensive body of literature suggests that the reinforcing and psychomotor effects of cocaine are

primarily mediated by increased dopamine within the mesocorticolimbic pathway [90]. Evidence suggests that the greater effects of cocaine in the NAc are caused by higher rates of DA release compared with those in the CPu [216].

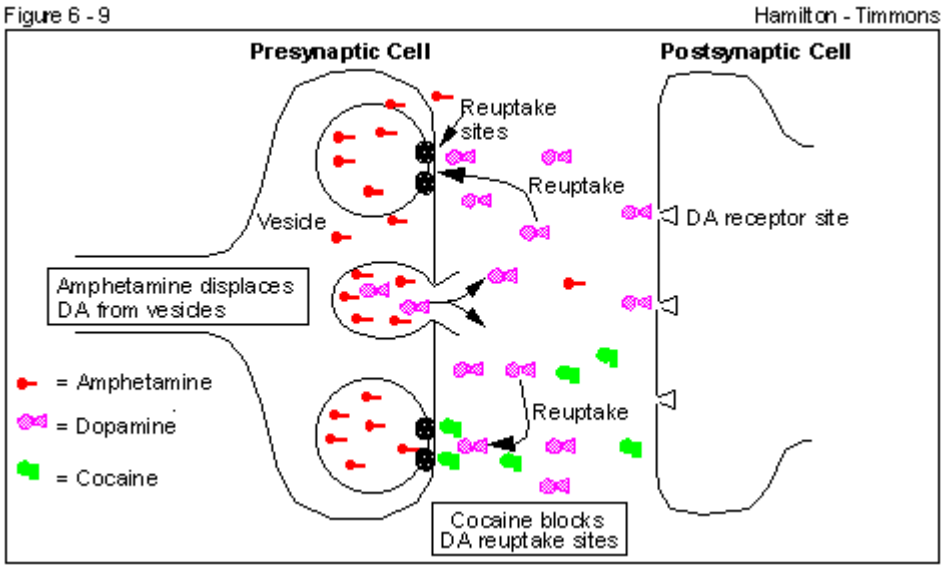


Figure 3: Cocaine's effects at the synapse. Under normal conditions, the DAT removes dopamine from the synapse. Cocaine binds to the transporter and increases DA in the synapse by preventing re-uptake [193].

B. Effects in the DA system after acute and chronic cocaine administration.

The involvement of the dopaminergic system on cocaine-induced behavioral effects has been extensively studied. A wide body of evidence shows that acute cocaine administration increases the concentration of dopamine [214]. DA receptor modulation affects simple behaviors such as locomotion and stereotypies [69;197]. For example, D1 and D2 receptor antagonists attenuate acute cocaine-induced locomotor activity and stereotypies in rats [8;197]. In addition, when dopaminergic neurons in the NAc are lesioned, cocaine-induced locomotor activity is abolished [100].

Moreover, acute cocaine administration produces a dose-dependent increase in motor activity by elevating extracellular DA content, primarily through blocking the

reuptake of DA in the synaptic cleft through the presynaptic dopamine transporter (DAT), resulting in a subsequent DA accumulation in the synapse [159] [Figure 3]. For example, i*n vivo* dialysis in the conscious rat showed that acute cocaine enhanced DA release in the NAc and striatum [47]. Activation of D1 receptors, subsequently, results in an increase in adenylyl cyclase activity [99;229] and an increase in PKA in the nucleus accumbens, which in turn increases activity of the transcription factors, cAMP, CREB, and rapid and transient induction of several Fos proteins in the NAc and dorsal striatum [72;119;135;190;196;220].

Chronic administration of cocaine augments locomotor responses to cocaine. This is known as sensitization [51;96;151]. On the other hand, tolerance, attenuation of a behavior to other components to cocaine is also developed after chronic cocaine administration. It has been hypothesized that alterations at the dopamine system are an important neural substrate critical for the development of sensitization [164].

Effect of acute and chronic cocaine administration on PKA/CREB/FosB pathway

Cocaine-induced changes in intracellular signaling are directly associated with behavioral effects seen after acute administration. Intraccumbal infusions of a PKA activator, were shown to augment the acute locomotor activating effects of cocaine, while intraccumbal infusions of a PKA inhibitor attenuated the acute locomotor activating effects of cocaine [124]. Acute administration of cocaine causes a rapid and transient induction of c-Fos proteins in the NAc and dorsal striatum [72;94;135] [Figure 4].

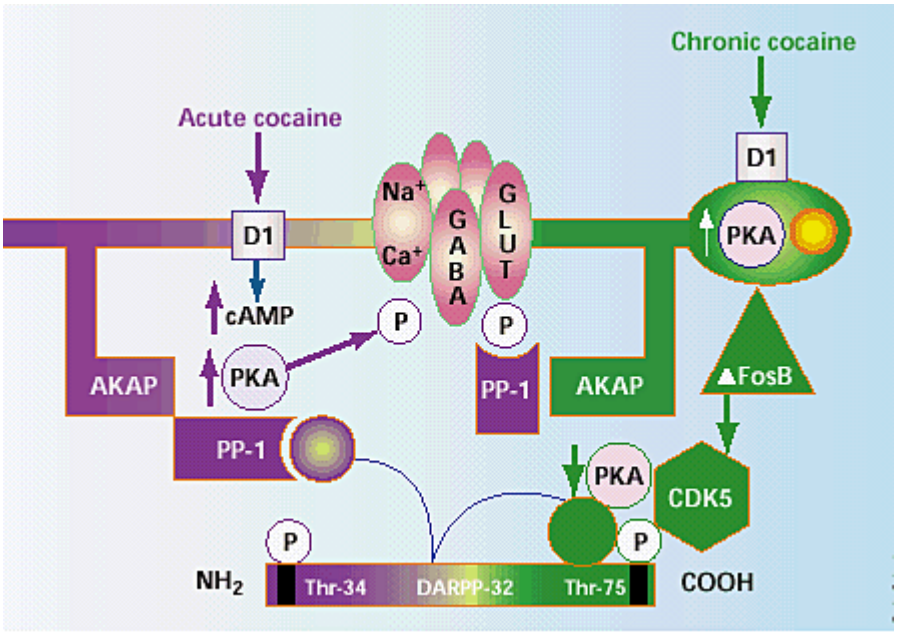


Figure 4: Acute cocaine administration increases D1 receptor signaling by blocking the presynaptic re-uptake of dopamine. D1 receptor activation increases cAMP levels, leading to activation of PKA. PKA substrates include voltage-gated Na+ and Ca^{2+} channels, ligand-gated GABA-A channels (GABA), and glutamate channels (GLU) such as AMPA/kainate and NMDA channels. PKA phosphorylates DARPP-32 at Thr-34 causes it to inhibit phosphatase-1 (PP-1), allowing for PKA activation. Chronic cocaine administration upregulates D1/PKA signaling, and also leads to the activation of the transcription factor ΔFosB. ΔFosB induces the cyclin-dependent protein kinase Cdk5. Alternatively, Cdk5 phosphorylates DARPP-32 at Thr-75, which then inhibits PKA [212].

Repeated exposure to cocaine has been shown to upregulate the cAMP-signaling pathway [190]. Upregulation of cAMP system in the NAc plays a role in chronic mediation and modulation of cocaine-induced effects on locomotor activity [124] [Table II]. Animals infused with PKA activators show enhanced sensitized locomotor activity in response to systemic cocaine administration, while PKA inhibitors decreased the sensitized cocaine-induced activity compared to saline-infused animals [124]. As discussed previously, IEG's, like CREB, c-Fos, and ΔFosB can regulate gene transcription, possibly mediating both short- and long-term effects of drugs of abuse within the pathways that underlie addiction [80;81;144].

In the NAc, chronic cocaine administration results in an increase in CREB

activity that produces a negative feedback adaptation which may decrease an individual's sensitivity to the rewarding effects of cocaine; a possible mechanism underlying tolerance [25]. PKA activators, which increase CREB phosphorylation within the NAc, decrease rewarding effects of cocaine, whereas PKA inhibition has the opposite effect [172]. Overexpression of CREB in mice show reduced sensitivity to drugs of abuse and stress, whereas mice expressing mCREB show increased sensitivity [120;137;168]. CREB-KO mice exhibit enhanced cocaine locomotor sensitization and CPP compared to wild-type mice [209]. Compared with c-Fos, which is induced rapidly and transiently after acute administration of cocaine, ΔFosB gradually accumulates in the NAc after repeated exposure to cocaine and persists in these regions for weeks or months after discontinuation of drug exposure. As discussed previously, ΔFosB activates Cdk5, which increases P-Thr75-DARPP-32, converting DARPP-32 into an inhibitor of PKA, dampening the DARPP-32/PP-1 cascade [17]. The accumulation of ΔFosB may be a mechanism by which chronic drug exposure that can lead to gene expression that persists long after the cessation of drug taking behavior [135;136].

Table I: Effects of acute and chronic cocaine on intracellular signal transduction pathways

acute cocaine	Result	cocaine dose	model	reference
CREB	↑ pCREB levels in the NAc	10 mg/kg i.p.	Mice	Walters et al., 2003
c-Fos	↑ c-fos expression in striatum	25 mg/kg i.p. ,15mg/kg i.p.	Rats	Graybiel et al., 1990;
		20mg/kg i.p.		Moratalla et al., 1993
				Torres and Rivier, 1994
				Grabus et al., 2004
	↓induction of c-fos mRNA levels in NAc in DARPP-32 THr-34 mutant	10 mg/kg	Mice	Zachariou et al., 2006
DARPP-32	↑ phosphorylation of Thr34 in CPu, hippocampus, prefrontal cortex	10mg/kg, i.p.	Rats	D'Addario et al., 2007
	↑ phosphorylation of Thr34	20 mg/kg i.p.	Mice	Nishi et al., 2000
	↓ phosphorylation at Thr75			
	↓ cocaine-induced locomotor activity in DARPP-32 KO compared to wild-type	10 mg/kg i.p.;	Mice	Fienberg et al., 1998
	↓ cocaine-induced locomotor activity in DARPP-32 Thr-34 mutant	20 mg/kg, i.p.	Mice	Zachariou et al., 2006
	-- No affect on cocaine-induced locomotor activity in DARPP-32 KO	5 mg/kg and 20 mg/kg	Mice	Zacahariou et al., 2002
Chronic	**Result**	**cocaine dose**	**model**	**Reference**
cAMP	↑cAMP in NAc and CPU	15 mg/kg i.p., 3x daily for 14 days	Rats	Unterwald et al., 1996
		15 mg/kg i.p., 2x daily for 14 days	Rats	Terwilliger et al., 1991
PKA	PKA activator ↑ cocaine-induced activity	10 mg/kg i.p., 3 days pretreatment	Rats	Miserendino & Nestler, 1995
	PKA inhibitor ↓ cocaine-induced activity	+ challenge		
	↑ PKA activity	20 mg/kg i.p., 8 days	Mice	Bibb et al., 2001
CREB	↓ sensitivity to cocaine w/overexpression of CREB in NAc	5 mg/kg i.p. 3-7 days (CPP)	Rats	Carlezon et al., 1998
	↓ in pCREB levels in CPu	30µg/day i.c.v., 8 days	Rats	Di Benedetto et al., 2007
ΔFosB	↑ ΔFosB in NAc compared saline-treated controls	15 mg/kg i.p., 2x daily, 14 days	Rats	Brenhouse and Stellar, 2006
		pretreament+ challenge		
	↑ locomotor and CPP in FosB mutants compared to wild-type	20 mg/kg, i.p. 6 -7 days	Mice	Hiroi et al., 1997
	↓ induction of ΔFosB in NAc of Thr-34 mutant	20 mg/kg, i.p. for 4-8 days		Zachariou et al., 2006
DARPP-32	↑ locomotor sensitization in KO compared to wild-type	20 mg/kg, i.p. for 6 days	Mice	Hiroi et al., 1999
	↑ locomotor sensitization in , ↓ CPP in DARPP-32 Thr-34 mutant	20 mg/kg, i.p. for 4-8 days	Mice	Zachariou et al., 2006
	No locomotor sensitization, no effect on CPP, in DARPP-32 Thr-75 mutant			
	↓ reward and ↓ ability to develop acquisition of self-administration in DARPP-32 Thr-34 mutant	1.o mg/kg catheter	Mice	Zhang et al.,2006
	↓ in phospho-Thr-34-DARPP-32 levels in CPu	30µg/day i.c.v., 8 days	Rats	Di Benedetto et al., 2007
	↓phosphorylation of Thr34 in striatum	40mg/kg, i.p. for 14 days	Rats	Scheggi et al., 2004
	↑phosphorylation at Thr75	pretreatment + 10 mg/kg challenge		
		20 mg/kg, I.p., for 8 days	Mice	Bibb et al., 2001

Effect of acute and chronic cocaine administration on DARPP-32/PP-1/PP-2B

DARPP-32 is regulated by cocaine [132]. Experiments with male DARPP-32 knockout mice showed attenuated locomotor responsiveness to a single injection of cocaine compared to wild-type mice [62]. Acute cocaine treatment in mice increases the phosphorlyation of DARPP-32 at Thr34 and decreases the phosphorylation at Thr75 [38;138] [Figure 5].

Subsequent to chronic cocaine administration, DARPP-32 knockout mice have increased locomotor sensitization as compared to wild-type mice [82]. These observations further suggest that the PKA-DARPP-32-PP-1 axis is likely to dampen the effects of chronic cocaine exposure [76]. As discussed previously, ΔFosB protein levels increase after chronic cocaine. ΔFosB induces cyclin-dependent kinase 5 (Cdk5) which phosphorylates DARPP-32 on Thr75, inhibiting PKA/DARPP-32 signaling axis. Thus, in summary, as shown in Figure 5, after both acute and chronic cocaine administrations (acute- thin line, chronic- bold line), DA receptors and second messenger systems are activated, but the direction of and the proteins activated differ.

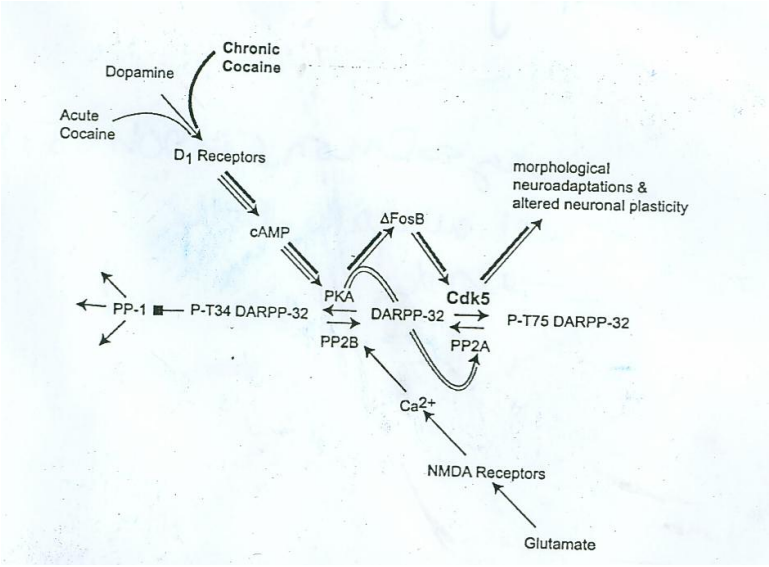


Figure 5: Schematic representation of cellular process targeted by cocaine. Thin line depicts signaling machinery associated with acute cocaine exposure. Heavy line details cellular pathways targeted by chronic cocaine exposure. Intracellular modulation of DARPP-32 phosphorlyation/ dephosphorlyation is central to the cellular response to D1 receptor activation during cocaine use [16].

Sex differences in response to cocaine administration

Both humans and experimental animals demonstrate gender differences to cocaine [60;173] [Table III]. Women achieve a faster "high" and higher peak of plasma cocaine levels, and report more episodes of euphoria than males [111]. Cocaine also produces a greater level of "nervousness" [103], and, hence, greater levels of drug craving upon introduction of cocaine cues in women compared to men, suggesting that females may be more sensitive to cocaine than males [160].

Similar to women, female rats display greater behavioral locomotor responses to acute and chronic cocaine administration than male rats and greater hypothalamic-pituitary adrenal axis (HPA) activation [31;34;60;107;166;173;202;205;206]. Females sensitize to cocaine with faster and at lower doses [34;68;150;202]. Moreover, female rats have also been shown to condition faster and at lower doses to develop CPP [166]. In chronic administration paradigms, repeated intermittent administration of cocaine in

females has been suggested to result in 'enhanced' sensitization in comparison to males [68;202]. Thus, cumulatively, these findings suggest that females are more sensitive to the behavioral effects of cocaine than males [31;34;60;67;166;173;202;205;206]. However, the mechanisms underlying the sexually dimorphic responses to cocaine remain unclear.

Sex differences in DA

In the NAc, sex differences in D1 levels, have been reported; female rats have lower D1 receptors levels, while in the caudate putamen (CPu), females have greater striatal DA release and re-uptake then male rats [6;14;208]. Robust differences in monoaminergic levels were found in the NAc after acute cocaine administration [60]. Specifically, female rats showed reduced levels of DA, DOPAC and DOPAC/DA turnover [60]. Differences in dopamine receptor function may contribute to differences in the behavioral effects of cocaine in male and female rats [178]. Gender differences in cocaine metabolism could underlie the gender differences found in cocaine responsiveness, but no gender differences in brain or plasma levels of cocaine were found, suggesting that cocaine metabolism to not seem to account for the enhanced sensitivity of female rats to cocaine [21]. However, Festa et al (2003) found in female rats, that norcocaine levels, a bioactive metabolite of cocaine, in the brain and serum were higher in females than in male rats. This indicates that observed sex differences in behavioral responses to acute cocaine administration may, in part, be explained by pharmacokinetic differences.

Table II: Sex differences in cocaine-induced behavior following acute or chronic cocaine administration

State of Animal			Male vs. Female Rats	Cocaine Dose	References
Acute Cocaine					
		↑	Females have greater cocaine-induced locomotor and/or stereotypic activity than male rats	10, 20, 40 mg/kg, i.p. 5, 15 mg/kg. i.p. 15 mg/kg, i.p. 5,15,20,30 mg/kg, i.p. 3.0 mg/kg, i.v. for 13 days	Walker et al., 2001 Sell et al., 2000 Chin et al., 2002 Festa et al., 2004 Harrod et al., 2005a
		↑	Females have greater cocaine-induced ambulatory and rearing than male rats	20mg/kg, i.p.	Festa et al., 2006
		↓	D1 antagonist decreased ambulatory and stereotypic activity in female rats at lower dose than males	20mg/kg, i.p.	Festa et al., 2006
Chronic Cocaine		↑	Females have greater sensitized response to cocaine than male rats	15 mg/kg 1, 10mg/kg, s.c. 3.0 mg/kg, i.v. for 13 days	Chin et al., 2002 van Haaren and Meyer, 1991 Harrod et al., 2005b
		↑	Females acquire CPP after fewer pairings and at lower doses of cocaine than male rats	5-20 mg/kg, i.p., 4-day or 8-day paradigms	Russo et al., 2003
		Δ	Rotational behavior in female rats sensitizes to a single cocaine injection	20 mg/kg, I.p.	Glick and Hinds, 1984
			GDX vs. Intact		
Acute Cocaine					
	Males	--	Did not affect cocaine-induced activity	15 mg/kg, i.p. 5, 10, 20, mg/kg, i.p.	Chin et al., 2002 Hu and Becker, 2003
		↑	Cocaine-induced ambulatory activity	10, 20, 40 mg/kg, i.p.	Walker et al.,2001
	Females	↓	OVX attenuated cocaine-induced locomotor activity compared to intact females	3.0 mg/kg, i.v., for 13 days	Harrod et al., 2005a
		↓	OVX attenuated cocaine-induced ambulatory activity compared to intact females	10-40 mg/kg, i.p. 10, 20, 40 mg/kg, i.p.	Chin et al., 2002 Walker et al., 2001
Chronic Cocaine	Males	↓	Cocaine-induced activity as compared to intact males	15 mg/kg, i.p. 14 days pre-treatment + challenge	Chin et al., 2002
		--	No difference in cocaine-induced activity between castrated and intact males	3.0 mg/kg, i.v. for 13 days	Harrod et al., 2005b
		--	Rotational behavior in male rats does not sensitize	1, 10, 20 mg/kg, i.p.	Hu and Becker, 2003
	Females	↓	Cocaine-induced activity as compared to intact rats	15 mg/kg, i.p. 1, 10 mg/kg, s.c.	Chin et al., 2002 van Haaren and Meyer, 1991
		--	OVX did not affect cocaine-induced activity as compared to intact rats	3.0 mg/kg, i.v. for 13 days	Harrod et al., 2005

↑ Represents an increase in behavioral activity. ↓ Represents a decrease in behavioral activity.
Δ Represents an effect observed only in female rats

Adapted from Festa et al., 2004 [59]

Hormonal fluctuations of the menstrual/estrous cycle in humans and rats

During the estrous cycle, both estrogen and progesterone vary in their concentrations [Figure 6]. Although the estrous cycle of a rat is only 4 days, the endocrinological changes closely resemble those seen in humans. It has been hypothesized that the fluctuating hormones within the estrous cycle may act as neuromodulators to facilitate or inhibit the effects of cocaine in females, by affecting the regulation of DA [24].

In humans [Figure 6A], the cycle begins with the follicular phase, which is characterized by the development of the egg, and the pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In the periovulatory period, the egg matures, the rate of estrogen secretion increases and induces the release of a pulse of gonadotropin-releasing hormone (GnRH) from the hypothalamus which in turn induces a pulse of LH release by the pituitary, inducing a preovulatory surge of progesterone from the ovaries, resulting in ovulation. Once the follicle has ruptured and expelled its egg, it is transformed into a new endocrine organ known as the corpus luteum, which releases progesterone. This period is known as the luteal phase. Lastly, the menstrual phase occurs with the fall in progesterone and estrogen secretion, associated with regression of the corpus luteum, and the sloughing of the endometrieum, resulting in uterine bleeding [Fig 6A].

In rodents [Figure 6B], estrus describes the day of the estrous cycle on which ovulation actually occurs, lasts from 9 to 15 hours, is characterized by a high rate of running activity, and a period of heightened estrogen secretion [27]. Metestrus, occurs on the day following ovulation, lasts for 10 to 14 hrs, and is characterized by reduced

hormonal and behavioral activity. Diestrus occurs on the third day of the cycle, lasts for 60-70 hours, and is associated with the onset of follicular activity and estrogen secretion. Proestrus is the day before estrus and includes the major hormonal events, GnRH and LH surges, which induce behavioral estrus, and ultimately, trigger eventual ovulation [27].

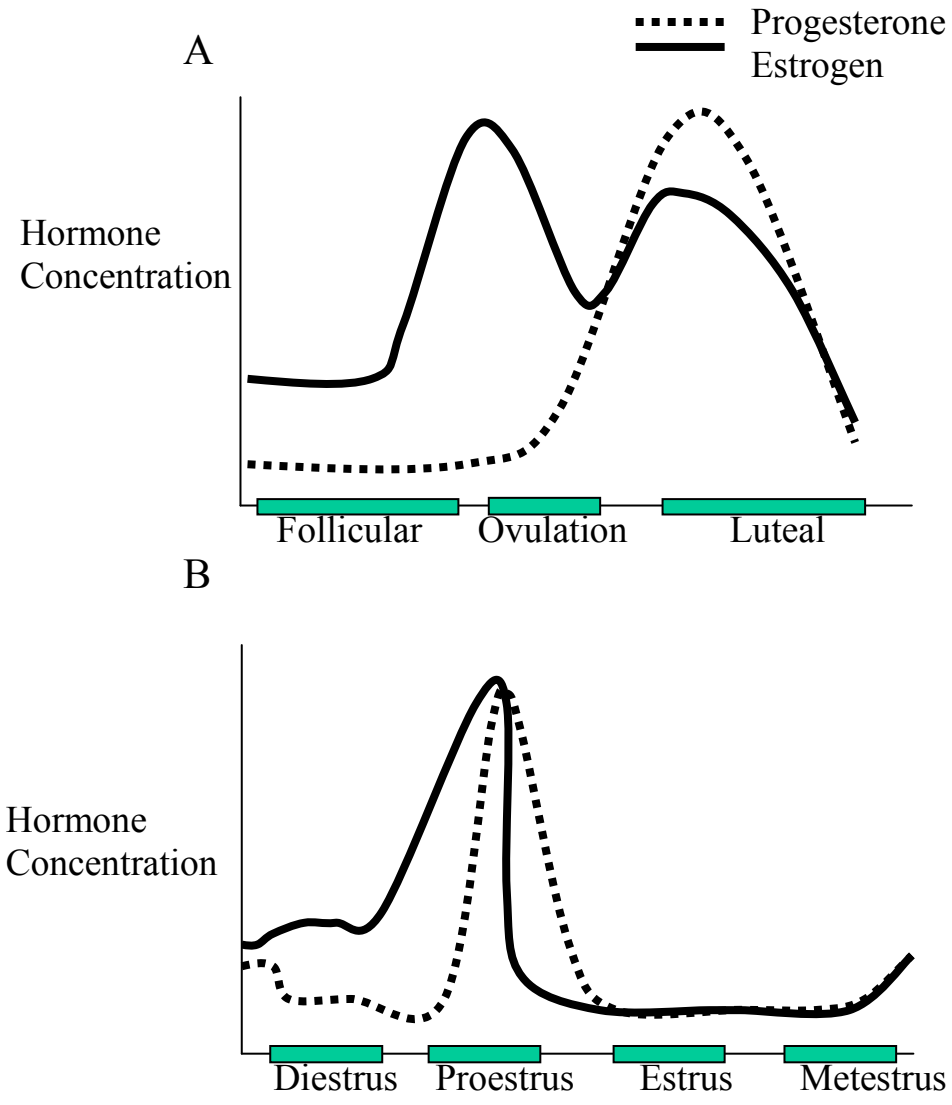


Figure 6: Phases of the menstrual and estrous cycles. During the human menstrual (A) and rat estrous (B) cycle, levels of the ovarian hormones estrogen and progesterone fluctuate across 3 phases (follicular, ovulation, and luteal) for the menstrual cycle and 4 phases (metestrus, diestrus, proestrus, and estrus) for the female rat estrous cycle. Data were redrawn from those presented in [27] [59].

Estrous cycle differences in dopaminergic system

Several reports suggest that estrous cycle stage, and fluctuating estradiol concentrations, may modulate the dopaminergic system, in the same regions where behavioral activities are controlled [11]. Levesque and Di Paolo (1990) demonstrated that the density of D1 receptors were modulated over the estrous cycle; during both proestrus and estrus an increase in striatal D1DA receptor density was observed as compared to rats during diestrus [109].

Estrous cycle differences and cocaine administration

In humans, self-administration of cocaine, by smoking or intranasally, in the follicular phase, exhibit higher peak plasma cocaine levels than do women during the luteal phase of the cycle [111;184]. Concurrent with cocaine plasma levels, women during the luteal phase had an attenuated subjective response to smoked cocaine and less desire to smoke cocaine than those in the follicular phase [184].

In rats, overall cocaine-induced activity is the lowest during diestrus when compared to proestrus or diestrus [152;173;208]. Quinones-Jenab et al (1999) found acute 'binge' cocaine administration produced a greater locomotor response in female rats during the estrus stage of their cycle than the other stages of their cycle, whereas Sell et al. (2000) and Walker et al (2002) observed similar levels of behavioral activity during both estrus and proestrus subsequent to acute cocaine injection. Rats in estrus demonstrate an increased motivation to self-administer cocaine compared to rats in other stages of the cycle [162]. However, Walker et al (2000) did not find that cocaine-stimulated locomotion differed across the estrous cycle. Vaginal lavage completely

blocked the high cocaine-induced responding observed in proestrus and estrus. The mechanisms underlying these changes remain unclear [207].

OVX versus gonadal hormone replacement: the role of estrogen and progesterone on cocaine-stimulated behavioral activation:

Previous studies have shown that the neuromodulatory effects of estrogen and progesterone alter cocaine responding in various behavioral paradigms. However, their underlying mechanisms are not well understood. Estrogen and progesterone have been postulated to underlie the sex differences in cocaine behavioral responses [59]. Estrogen and progesterone replacement in OVX females augments cocaine-induced locomotor responses [148;155;173]. Moreover, estradiol modifies agonist-mediated stereotypy, stimulant-induced behavioral sensitization, and paced mating behavior [29;46;145;217]. Enhanced response to cocaine was demonstrated in the presence of pharmacologically administered estradiol [173]. In addition, estradiol administration to female rats decreased cocaine's toxic effects, resulting in significantly lower levels of cocaine in plasma compared to females who did not receive estradiol [158;174]. Moreover, estradiol has been shown to alter basal and stimulated dopamine release increase, decrease DA turnover, modulate both presynaptic and postsynaptic DA receptors, and the number of DA uptake sites in the striatum [9;12;13;20;28;44;48;53;57;118;121;127]. Furthermore, in the NAc, estradiol administration resulted in changes in DA levels and D2-type receptor density, suggesting estrogen may modulate DA release and reuptake [157;177].

In humans, an acute dose of progesterone administered before smoking cocaine attenuates cocaine's subjective effects as compared to placebo-treated females [183]. Moreover, progesterone affects rearing activity dose-dependently when given 24h before cocaine administration: at 50μg and 500μg progesterone inhibited rearing activity, 100μg and 250μg progesterone increased cocaine-induced rearing activity [142]. Progesterone replacement in Silastic capsules attenuates CPP as compared to OVX females [166]. Also, an acute-repetitive, or single doses of cocaine administration significantly increases plasma levels of progesterone in intact female rats, suggesting that cocaine has a transient effect on progesterone plasma levels [153]. Evidence suggests that at certain concentrations, progesterone can attenuate some cocaine-induced behavioral effect in female rats.

In acute cocaine administration paradigms, co-administration of estrogen and progesterone augments cocaine-induced activity as compared to OVX females [152;173;181]. Additionally, co-administration of estrogen and progesterone also potentiates cocaine CPP. In chronic cocaine administration paradigms, co-administration of estrogen and progesterone augments behavioral sensitization to cocaine as compared to rats receiving either estrogen or progesterone alone [148;181].

The role of estrogen and progesterone on cocaine-stimulated intracellular signal transduction:

Both estrogen and progesterone modulate signal transduction pathways in the brain. Estrogens alter DA substrates involved in the behavioral effects of cocaine [122]. OVX decreased D1 receptor adenylyl cyclase activity in the striatum of female rats [106].

In contrast, treatment with estrogen to primary striatal cultures enhanced DA activated D1 receptor adenylyl cyclase and suppresses D2 receptor adenylyl cyclase activity [118]. Moreover, estrogens have been shown to modulate expression of mRNA and protein for DA [127;191;226;227]. Chronic estrogen in OVX rats significantly increased D1 receptor mRNA levels in the hypothalamus and increased D2 receptor mRNA levels in the midbrain, and after three weeks of implant treatment, there was increased hyperactivity induced by cocaine [226]. Estrogen was also shown to increase phosphorylated CREB immunoreactivity in the anteroventral periventricular nucleus (AVPV) compared to OVX control females [75]. Studies assessing the role of progesterone on cocaine-induced intracellular signaling are limited. Antisense oligonucleotides to DARPP-32 inhibited D1 agonist and progesterone-facilitated sexual receptivity in estradiol-primed female rats [116]. Progesterone enhances DA-mediated cAMP and PKA activation and DARPP-32 phosphorylation at Theronine-34 in the hypothalamus [116]. Taken together, estrogen and progesterone affect DA-mediated intracellular transduction pathways. However, the effects of gonadal steroids on the signal transduction mechanisms involved in cocaine's actions in brain regions such as the NAc and CPu have yet to be elucidated.

Significance of work

Understanding the underlying mechanisms of the known sex differences in cocaine's actions in males and females is crucial to evolving more effective therapeutic treatments for cocaine addiction. It is known that 30% of cocaine abusers are females and that females experience different subjective effects to cocaine than males. Sex and estrous cycle differences in the behavioral response to cocaine are apparent in several animal behavioral paradigms. However, no studies have addressed how the fluctuating hormones of the estrous cycle and hormone replacement may modulate the cellular mechanisms and signal transduction pathways of the DA system. Understanding the intracellular mechanisms underlying these sex/estrous cycle differences may help the scientific community develop more effective pharmacological treatments for addiction.

The specific aim of this project is to determine how the estrous cycle and gonadal hormones influence cocaine-induced PKA-mediated signal transduction mechanisms. *Our general hypothesis is that gender differences in cocaine-induced behavioral effects may be due to estrogen and progesterone modulations of PKA-mediated pathways.* To test this hypothesis the following aims were proposed:

Specific Aim 1: To test the hypothesis that PKA-mediated intracellular cascades are involved in the sexually dimorphic response after cocaine. To this end, males and females received acute injection of saline or cocaine and sacrificed after 15 min or 1h. Protein levels were measured using Western Blot technique. We predict that acute cocaine administration will differentially alter PKA-mediated protein levels in males and females.

Specific Aim 2: To test the hypothesis that PKA-mediated intracellular cascades are differentially activated during the estrous cycle. To this end, intact females are lavaged in order to determine estrous cycle stage. Females received acute injection of saline or cocaine and sacrificed after 15 min or 1h. Protein levels were measured using Western Blot technique. We predict that estrus females will have different PKA-mediated protein levels after acute cocaine administration when compared to females during other stages of the estrous cycle.

Specific Aim 3: To test the hypothesis that PKA-mediated intracellular cascades are differentially altered by OVX/hormone replacement after cocaine: To this end, OVX females were administered injections of vehicle, estrogen only, progesterone only, or co-administration of estrogen + progesterone. Females received acute injection of saline or cocaine and sacrificed after 15 min or 1h. Protein levels were measured using Western Blot technique. We predict that estrogen and progesterone will differentially alter PKA-mediated protein levels after acute cocaine administration when compared to vehicle groups.

II. Methods

2.1 Sex differences

2.1a Subjects: Eight-week old male and female rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chamber (20 x 20 x 41 cm) layered with beta chips. Rats were given free access to standard lab chow and water and were maintained on a 12 hour light/dark cycle (lights on at 8am). All rats were weighed and handled for five days. Animals were randomly assigned to experimental groups and experiments were conducted in two different cohorts with a total of (n= 4/group). Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.1b Drug, Antibodies, and Reagents: The primary antibody for DARPP-32 was purchased from Cell Signaling (Beverly, MA). P-Thr34-DARPP-32 antibody was purchased from PhosphoSolutions (Aurora, CO). PKA_c antibody was purchased from BD Bioscience Pharmigen (San Diego, CA). PP-1α (catalytic subunit of PP1) antibody was purchased Sigma Aldrich (St. Louis, MO). CN-A1 (catalytic subunit of PP2β) antibody was purchased from Sigma Aldrich (St. Louis, MO). CN-B1 (regulatory subunit of PP2β) antibody was purchased from Anti-phospho-CREB (Ser133) antibody was purchased from Upstate Cell Signaling Solutions (Charlottesville, VA). Fos proteins; (ΔFosB and c-Fos) and α-tubulin antibodies were purchased from Santa Cruz Technologies (Santa Cruz, CA). All appropriate secondary antibodies purchased from Amersham Pharmacia (Piscataway, NJ).

2.1c Cocaine administration paradigm and protein measurements: Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intro-peritoneally (i.p.). Rats were given an injection of saline or cocaine (20 mg/kg). This dose produces a midrange of behavioral activation [58;60;142]. Animals were sacrificed 15 or 60 minutes after drug treatment by decapitation (following a brief 20 s exposure to CO_2). Rat brains were removed, immediately frozen in 2-methylbutane (-40°C), and stored at -80°C until used. The CPu and NAc were dissected from coronal sections (1mm thick) using a matrix (ASI Instruments, Houston Texas) and homogenized in a lysis buffer [Tris-HCl (50mM), NaCl (150 mM), EDTA (2mM), Glycerol (10%), Triton X-100 (1%), Igepal CA-630 (1%), sodium deoxycholic acid (1%), Pepstatin, Leupeptin, DTT (1mM), Apoprotenin, PMSF (100mM), NaF (50mM), and Na_3VO_4 (1mM)]. Total protein content was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

2.1d Protein Measurements: Protein samples (25 μg) were boiled in Lammeli buffer containing 1% β-mercaptonethanol, centrifuged, and loaded onto Ready Gel Precast Gel (4-15%). Gels were transferred overnight onto nitrocellulose membranes, and then blocked for 30 min with 5% non-fat dry milk in tris-buffer-saline-tween (TBST) at room temperature. Membranes were probed overnight a 4°C with DARPP-32 (1:000), p-T34-DARPP-32 (1:1000), PP-1 (1:4000), CN-A1 (1:15,000), CN-B1 (1:5000), pCREB (1:1000), or FosB (1:1000) antibodies. After three washes with TBST, membranes were then incubated with their appropriate secondary antibody (1:1000) for 60 min at room temperature followed by three more washes with TBST. Antibody binding was detected

using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia, Piscataway, NJ). Resulting films were scanned and intensity of protein bands was quantified with a computer densitometer and Image Quant Program (Molecular Dynamics, GE Health Care Biosciences Corp, Piscataway, NJ).

2.1e Statistical analysis of data: Western blot data are represented as a ratio of the antibody band over α-tubulin. Data are presented as the group mean ± SEM. % saline is calculated as a ratio of individual cocaine animal/average of respective control group, presented as group mean ± SEM. Unpaired-sample t-tests were conducted to determine differences in drug treatment to their respective control. One-way ANOVA was conducted to determine basal and % saline differences between groups. One-way ANOVA was also conducted to determine ratio of activity for DARPP-32 within each time point and brain region. Determination of statistically significant differences was considered at the 0.05 probability level.

2.2 Estrous Cycle

2.2a Subjects: Eight-week old intact female rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chamber (20 x 20 x 41 cm) layered with beta chips. Rats were given free access to standard lab chow and water and were maintained on a 12 hour light/dark cycle (lights on at 8am). All rats were weighed, handled, and lavaged for twelve days. Animals were randomly assigned to experimental groups and experiments were conducted in three different cohorts with a total of (n= 4/group). Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH

publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2b Estrous Cycle Determinants: Estrous cycle stage was monitored by analysis of cell types in vaginal lavages. Vaginal lavages were collected 30 min after the lights went on and were allowed to dry on microscope slides. Slides were then fixed with ethanol and stained with cresyl violet acetate (Sigma, St. Louis, MO). Cells were classified into four identifiable stages: proestrus (predominance of mature epithelial cells), estrus (fully cornified epithelial cells), metestrus (cornified cells with few leukocytes), and diestrus (predominance of leukocyes with a small number of epitheilial cells) [66]**.** Animals that could not be reliably staged, or that did not produce two consecutive cycles, were not included in any statistical analysis.

2.2c Drug, Antibodies, and Reagents: The primary antibody for DARPP-32 was purchased from Cell Signaling (Beverly, MA). P-Thr34-DARPP-32 antibody was purchased from PhosphoSolutions (Aurora, CO). PKA_c antibody was purchased from BD Bioscience Pharmigen (San Diego, CA). PP-1α (catalytic subunit of PP1) antibody was purchased Sigma Aldrich (St. Louis, MO). CN-A1 (catalytic subunit of PP2β) antibody was purchased from Sigma Aldrich (St. Louis, MO). CN-B1 (regulatory subunit of PP2β) antibody was purchased from Anti-phospho-CREB (Ser133) antibody was purchased from Upstate Cell Signaling Solutions (Charlottesville, VA). Fos proteins; (ΔFosB and c-Fos) and α-tubulin antibodies were purchased from Santa Cruz Technologies (Santa

Cruz, CA). All appropriate secondary antibodies purchased from Amersham Pharmacia (Piscataway, NJ).

2.2d Cocaine administration paradigm and protein measurements: Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intro-peritoneally (i.p.). Rats were given an injection of saline or cocaine (20 mg/kg). This dose produces a midrange of behavioral activation [58;60;142]. Animals were sacrificed 15 min after drug treatment by decapitation (following a brief 20 s exposure to CO_2). Rat brains were removed, immediately frozen in 2-methylbutane (-40°C), and stored at -80°C until used. The CPu and NAc were dissected from coronal sections (1mm thick) using a matrix (ASI Instruments, Houston Texas) and homogenized in a lysis buffer [Tris-HCl (50mM), NaCl (150 mM), EDTA (2mM), Glycerol (10%), Triton X-100 (1%), Igepal CA-630 (1%), sodium deoxycholic acid (1%), Pepstatin, Leupeptin, DTT (1mM), Apoprotenin, PMSF (100mM), NaF (50mM), and Na_3VO_4 (1mM)]. Total protein content was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

2.2e Protein Measurements: Protein samples (25 μg) were boiled in Lammeli buffer containing 1% β-mercaptonethanol, centrifuged, and loaded onto Ready Gel Precast Gel (4-15%). Gels were transferred overnight onto nitrocellulose membranes, and then blocked for 30 min with 5% non-fat dry milk in tris-buffer-saline-tween (TBST) at room temperature. Membranes were probed overnight a 4°C with DARPP-32 (1:000), p-T34-DARPP-32 (1:1000), PP-1 (1:4000), CN-A1 (1:15,000), CN-B1 (1:5000), pCREB

(1:1000), or FosB (1:1000) antibodies. After three washes with TBST, membranes were then incubated with their appropriate secondary antibody (1:1000) for 60 min at room temperature followed by three more washes with TBST. Antibody binding was detected using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia, Piscataway, NJ). Resulting films were scanned and intensity of protein bands was quantified with a computer densitometer and Image Quant Program (Molecular Dynamics, GE Health Care Biosciences Corp, Piscataway, NJ).

2.2f Statistical analysis of data: Western blot data are represented as a ratio of the antibody band over α-tubulin. Data are presented as the group mean $\pm$ SEM. % saline is calculated as a ratio of individual cocaine animal/average of respective control group, presented as group mean $\pm$ SEM. Unpaired-sample t-tests were conducted to determine differences in drug treatment to their respective control. One-way ANOVA was conducted to determine basal and % saline differences between groups. One-way ANOVA was also conducted to determine ratio of activity for DARPP-32 within each time point and brain region. Determination of statistically significant differences was considered at the 0.05 probability level.

2.3 Hormone Replacement

2.3a Subjects: Eight-week old OVX female rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chamber (20 x 20 x 41 cm) layered with beta chips. Rats were given free access to standard lab chow and water and were maintained on a 12 hour light/dark cycle (lights on at 8am). All rats were weighed, handled, and lavaged for

twelve days. Animals were randomly assigned to experimental groups and experiments were conducted in one cohort with a total of (n= 4/group). Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.1.b Hormone Replacement Determinants: Rats received subcutaneous injections of estrogen (50 μg) 48h prior to experimentation, progesterone (500 μg) 4h prior to experimentation, or co-administration of both estrogen (50 μg) 48h and progesterone (500 μg) 4h before administration of cocaine or saline [Table IV]. Vehicle (sesame oil) was administered to control for the time at which the hormone was injected. Control groups received vehicle treatment at 48h, 4h, or both, before drug treatment to control for the time at which the hormone was administered. The timing of both estrogen and progesterone administration were chosen on the basis of previous studies by our group [141;147]. Furthermore, 50 μg of estrogen and 500 μg of progesterone produced the most pronounced effects on behavioral activity after cocaine administration [142]. Moreover, these doses fall within the range of doses used in previously published studies that aimed to determine the role of gonadal hormones in responses to cocaine [88;146;155;181].

Table III: Hormone Replacement Paradigm

	Vehicle		Estrogen		Progesterone		E+P	
48h	V	V	E	E	V	V	E	E
4h	V	V	V	V	P	P	P	P
Drug	S	C	S	C	S	C	S	C

2.3c Drug, Antibodies, and Reagents: Estradiol, progesterone, sesame oil, and cocaine hydrochloride was purchased from Sigma Chemical Company (St. Louis, MO). The primary antibody for DARPP-32 was purchased from Cell Signaling (Beverly, MA). P-Thr34-DARPP-32 antibody was purchased from PhosphoSolutions (Aurora, CO). PKA_c antibody was purchased from BD Bioscience Pharmigen (San Diego, CA). PP-1α (catalytic subunit of PP1) antibody was purchased Sigma Aldrich (St. Louis, MO). CN-A1 (catalytic subunit of PP2β) antibody was purchased from Sigma Aldrich (St. Louis, MO). CN-B1 (regulatory subunit of PP2β) antibody was purchased from Anti-phospho-CREB (Ser133) antibody was purchased from Upstate Cell Signaling Solutions (Charlottesville, VA). Fos proteins; (ΔFosB and c-Fos) and α-tubulin antibodies were purchased from Santa Cruz Technologies (Santa Cruz, CA). All appropriate secondary antibodies purchased from Amersham Pharmacia (Piscataway, NJ).

2.3d Cocaine administration paradigm and protein measurements: Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intro-peritoneally (i.p.). Rats were given an injection of saline or cocaine (20 mg/kg). This dose produces a midrange of behavioral activation [58;60;142]. Animals were

sacrificed 15 min after drug treatment by decapitation (following a brief 20 s exposure to CO_2). Rat brains were removed, immediately frozen in 2-methylbutane (-40°C), and stored at -80°C until used. The CPu and NAc were dissected from coronal sections (1mm thick) using a matrix (ASI Instruments, Houston Texas) and homogenized in a lysis buffer [Tris-HCl (50mM), NaCl (150 mM), EDTA (2mM), Glycerol (10%), Triton X-100 (1%), Igepal CA-630 (1%), sodium deoxycholic acid (1%), Pepstatin, Leupeptin, DTT (1mM), Apoprotenin, PMSF (100mM), NaF (50mM), and Na_3VO_4 (1mM)]. Total protein content was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

2.3e Protein Measurements: Protein samples (25 μg) were boiled in Lammeli buffer containing 1% β-mercaptonethanol, centrifuged, and loaded onto Ready Gel Precast Gel (4-15%). Gels were transferred overnight onto nitrocellulose membranes, and then blocked for 30 min with 5% non-fat dry milk in tris-buffer-saline-tween (TBST) at room temperature. Membranes were probed overnight a 4°C with DARPP-32 (1:000), p-T34-DARPP-32 (1:1000), PP-1 (1:4000), CN-A1 (1:15,000), CN-B1 (1:5000), pCREB (1:1000), or FosB (1:1000) antibodies. After three washes with TBST, membranes were then incubated with their appropriate secondary antibody (1:1000) for 60 min at room temperature followed by three more washes with TBST. Antibody binding was detected using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia, Piscataway, NJ). Resulting films were scanned and intensity of protein bands was quantified with a computer densitometer and Image Quant Program (Molecular Dynamics, GE Health Care Biosciences Corp, Piscataway, NJ).

2.3f Statistical analysis of data: Western blot data are represented as a ratio of the antibody band over α-tubulin. Data are presented as the group mean ± SEM. % saline is calculated as a ratio of individual cocaine animal/average of respective control group, presented as group mean ± SEM. Unpaired-sample t-tests were conducted to determine differences in drug treatment to their respective control. One-way ANOVA was conducted to determine basal and % saline differences between groups. One-way ANOVA was also conducted to determine ratio of activity for DARPP-32 within each time point and brain region. Determination of statistically significant differences was considered at the 0.05 probability level.

III. Results

3.1 Sex difference in PKA-mediated intracellular proteins after saline or cocaine administration: In the NAc, 15 minutes after saline or cocaine administration, no sex differences in PKA, pCREB, c-Fos, ΔFosB, DARPP-32, P-Thr-34-DARPP-32, PP-1, or PP2B protein levels were observed [Fig: 7,9,11,13,15,17,19,21,23]. In the NAc, 15 min after drug treatment, PP-1 protein levels decreased in males when compared to their respective saline controls [$t=2.13$, $p<0.05$; Fig: 19A]. However, 1h after cocaine administration, when normalized to their respective saline controls, sex differences in accumbal PKA and P-Thr-34-DARPP-32 protein levels were observed; males had higher PKA protein levels than females [$t=-2.35$, $p<0.05$; Fig: 8B], and females had higher P-Thr-34-DARPP-32 protein levels than males [$t=2.40$, $p<0.05$; Fig: 18B].

In the CPu, no sex differences in protein levels were observed either 15 min or 1h after drug treatments [Fig: 25-42].

3.2 Estrous cycle differences in PKA-mediated intracellular proteins after saline or cocaine administration: Overall, in the NAc, 15 minutes after saline or cocaine administration, the stage of estrous cycle did not alter PKA, c-Fos or ΔFosB, DARPP-32, P-Thr-34-DARPP-32, PP-1, PP2B protein levels [Fig: 43,47,49,51,53,55,57,59]. However, pCREB protein levels decreased in estrus-females after cocaine administration when compared to saline controls [$t=-2.51$, $p<0.05$; Fig: 45A], while CNA1 protein levels increased in estrus-females after cocaine administration when compared to saline controls [$t=-2.51$, $p<0.05$; Fig: 57A]. In the NAc, 1h after drug treatment, PKA protein levels decreased in estrus-females when compared to saline controls [$t=1.95$, $p<0.05$; Fig: 44A].

Moreover, P-Thr-34-DARPP-32 protein levels increased in both cocaine-treated estrus- and diestrus-females when compared to their respective saline controls [t=-1.96, p<0.05; t=-2.04, p<0.05; respectively; Fig: 54A]. When data was normalized to % of saline, estrous cycle differences in P-Thr-34-DARPP-32 protein levels were observed [F(2,9)=6.16, (p<0.05); estrus-females had higher P-Thr-34-DARPP-32 protein levels than proestrus-females [p<0.01; Fig 54B].

In the CPu, 15 min after drug treatment, no estrous cycle differences in protein levels were observed [Fig: 61,63,65,67,69,71,73,75,77]. However, in the CPu, 1h after drug treatment, P-Thr-34-DARPP-32 protein levels increased in cocaine-treated diestrus-females when compared to their respective saline controls [t=-2.25, p<0.05; Fig: 72A]. When data was normalized to % of saline, a significant estrous cycle interaction was observed [F(2,9)=5.1, p<0.05]; diestrus-females had higher P-Thr-34-DARPP32 protein levels than estrus-females [Fig: 72B].

3.3 Estrogen and progesterone effects on PKA-mediated intracellular proteins after saline or cocaine administration: In the NAc, 15 min after cocaine administration, no differences in c-fos, ΔFosB, DARPP-32, P-Thr-34-DARPP-32, PP-1, or PP2B protein levels were observed [Fig: 81-87]. However, PKA protein levels increased in vehicle and estrogen + progesterone-treated groups when compared to their respective controls [t=-2.38, p<0.05; t=-2.93, p<0.05; respectively; Fig: 79A]. A two-way ANOVA shows a significant drug effect [F(1,24)=5.182, p<0.05]; where vehicle-treated cocaine groups had higher PKA protein levels than estrogen-treated cocaine groups. Furthermore, E+P-treated cocaine groups had higher PKA protein levels than estrogen-treated cocaine

groups ($p<0.05$, for all comparisons). Although it failed to reach significance; an interaction between drug x stage was observed [$F(3,24)=2.813$, $p=0.06$]. When data was normalized to % of saline, a main effect of drug in PKA protein levels was observed [$F(3,12)=5.84$, $p<0.01$]; vehicle-treated groups had higher PKA protein levels than estrogen, progesterone, or estrogen + progesterone-treated groups [$p<0.005$; $p<0.01$; $p<0.05$; respectively; Fig: 79B]. pCREB protein levels increased in vehicle-treated rats [$t=-2.61$, $p<0.05$; Fig: 80A], while pCREB protein levels decreased in the estrogen + progesterone-treated groups when compared to their saline controls [$t=2.07$, $p<0.05$; Fig: 80A]. When data was normalized to % of saline, a main effect of drug in pCREB protein levels was observed [$F(3,12)=13.11$, $p<0.0005$]; vehicle-treated groups had higher pCREB protein levels than estrogen, progesterone, or estrogen + progesterone-treated groups [$p<0.01$; $p<0.00005$; $p<0.00005$; respectively; Fig: 80B]. Furthermore, estrogen + progesterone treatment lowered pCREB protein levels further than vehicle, estrogen, or progesterone-treated rats [$p<0.00005$; $p<0.01$; $p<0.01$; respectively; Fig: 80B].

In the CPu, after estrogen and progesterone replacement, no differences in PKA-mediated protein levels were observed [Fig: 88-96]. Overall, in all three experimental paradigms, protein levels in the NAc were higher than protein levels in the CPu.

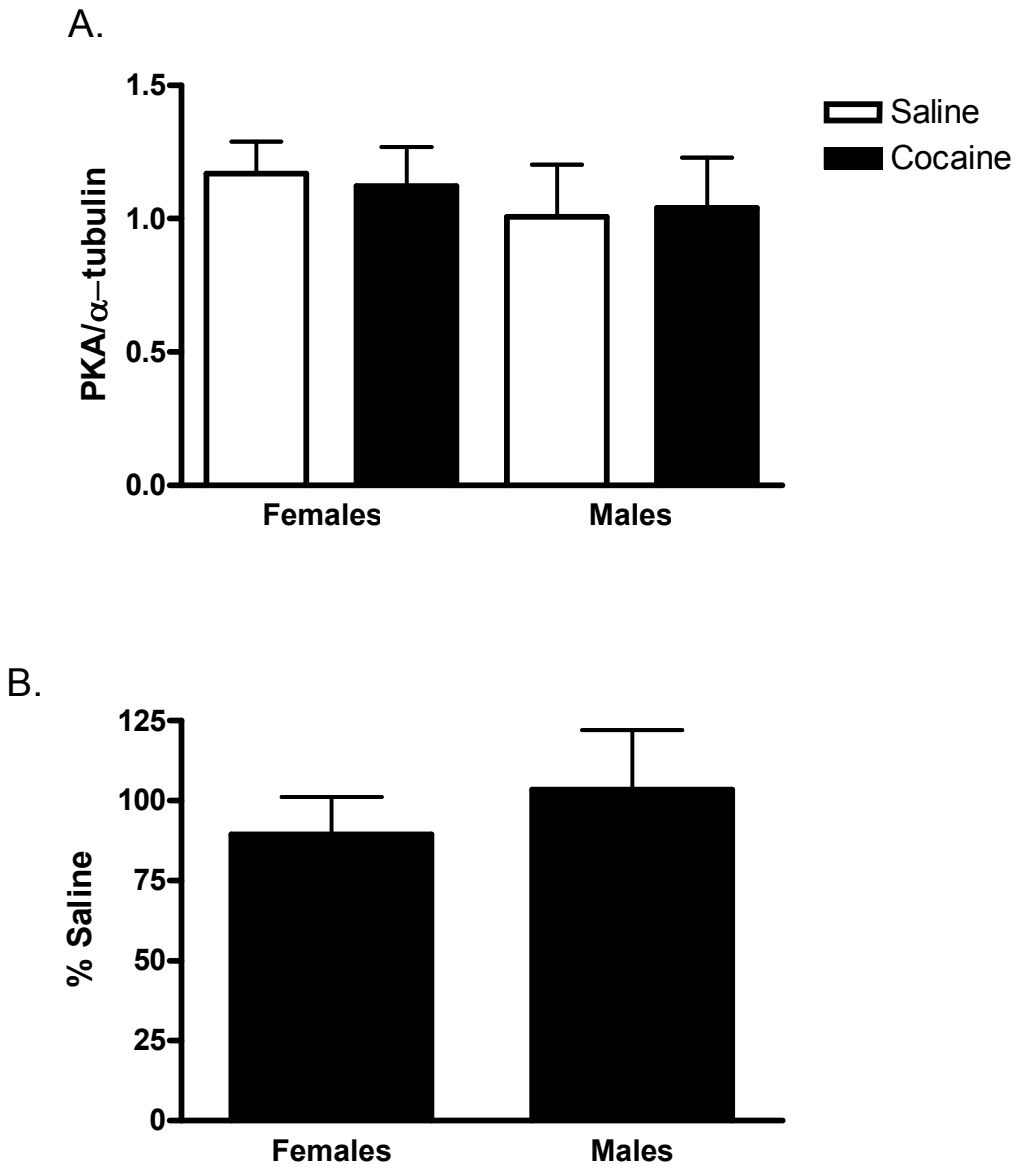


Figure 7: Males versus females in comparison before and after acute cocaine in the NAc, PKA protein levels15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

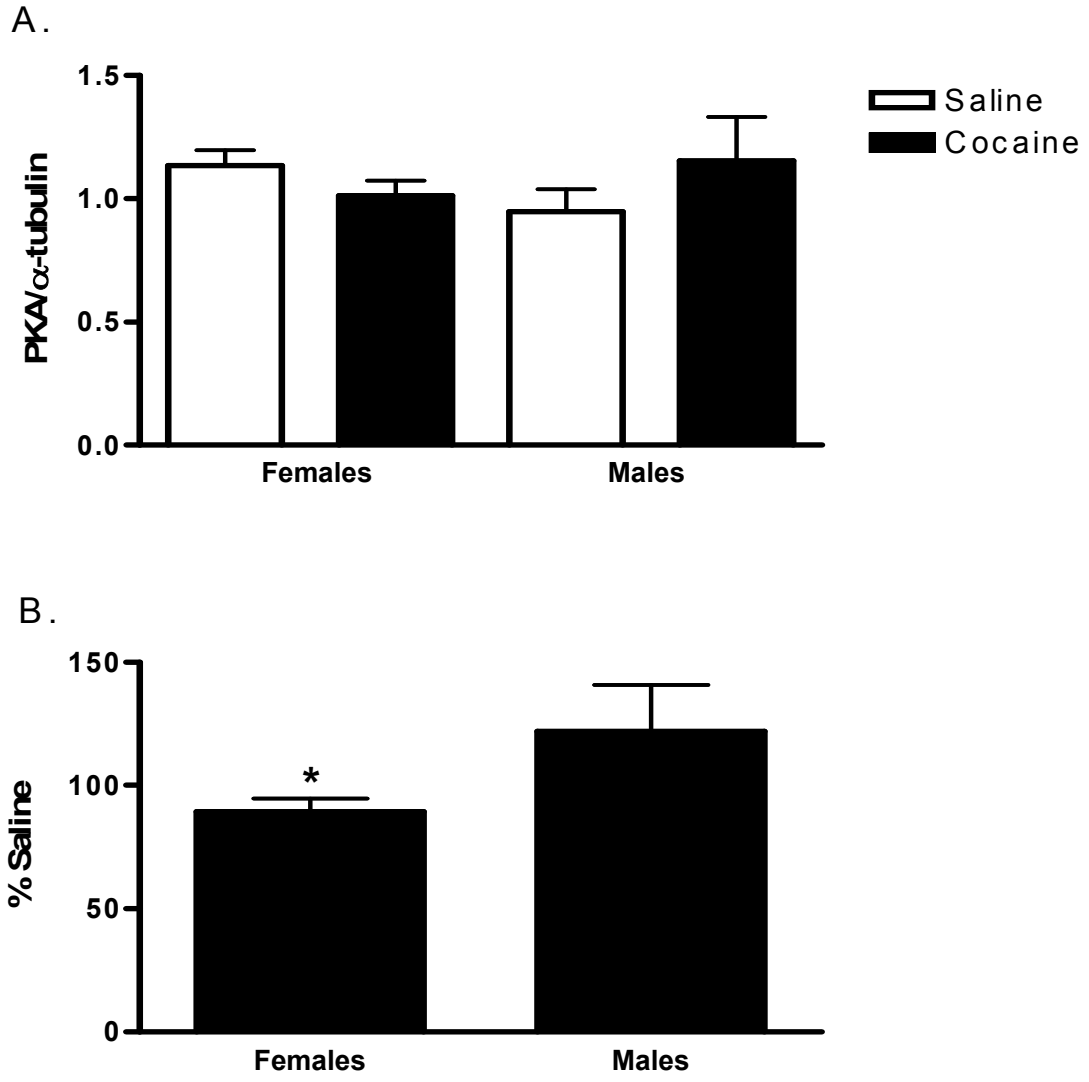


Figure 8: Males versus females in comparison before and after acute cocaine in the NAc, PKA protein levels 1h after saline (white bars) or cocaine administration (black bars) in the NAc. **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin**. (B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). * Represents statistically significant difference between males and females.

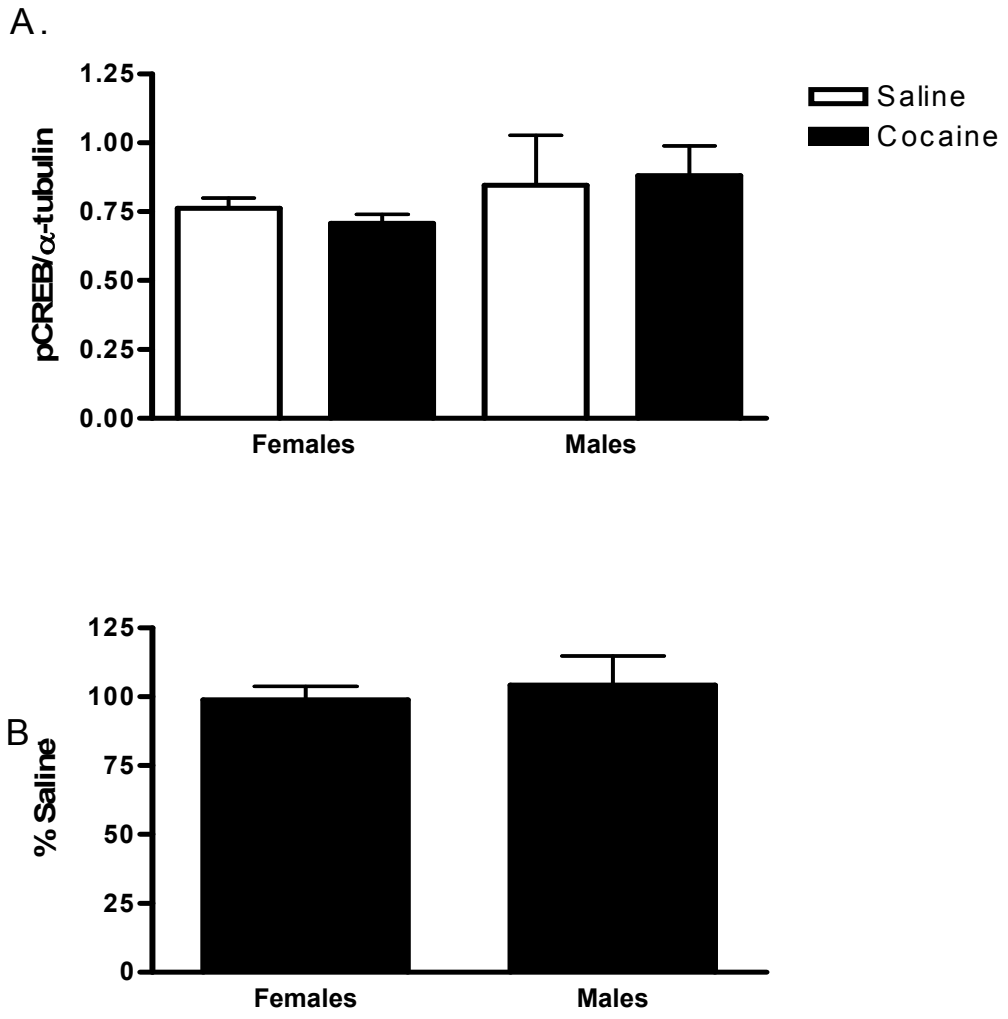


Figure 9: Males versus females in comparison before and after acute cocaine in the NAc, pCREB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

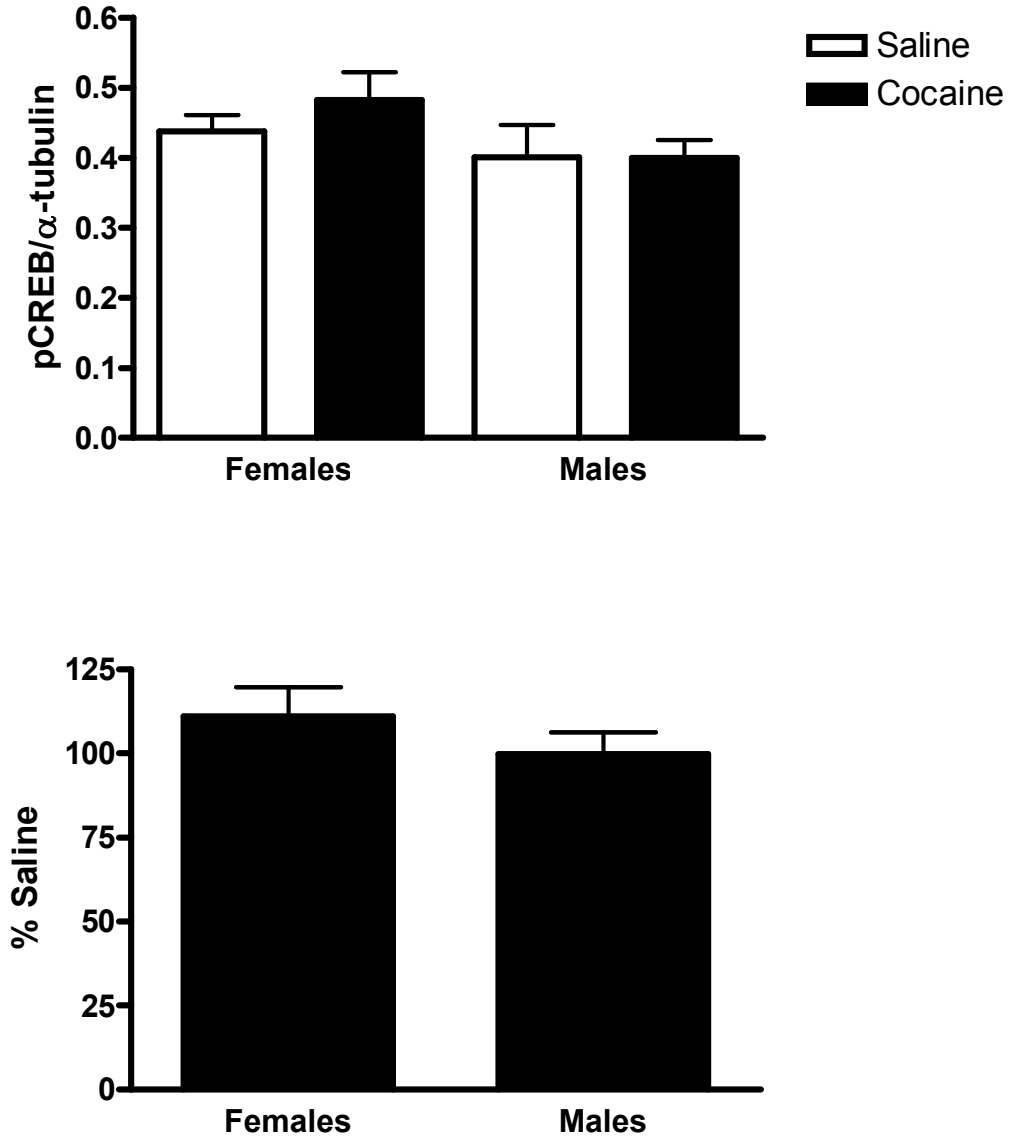


Figure 10: Males versus females in comparison before and after acute cocaine in the NAc, pCREB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

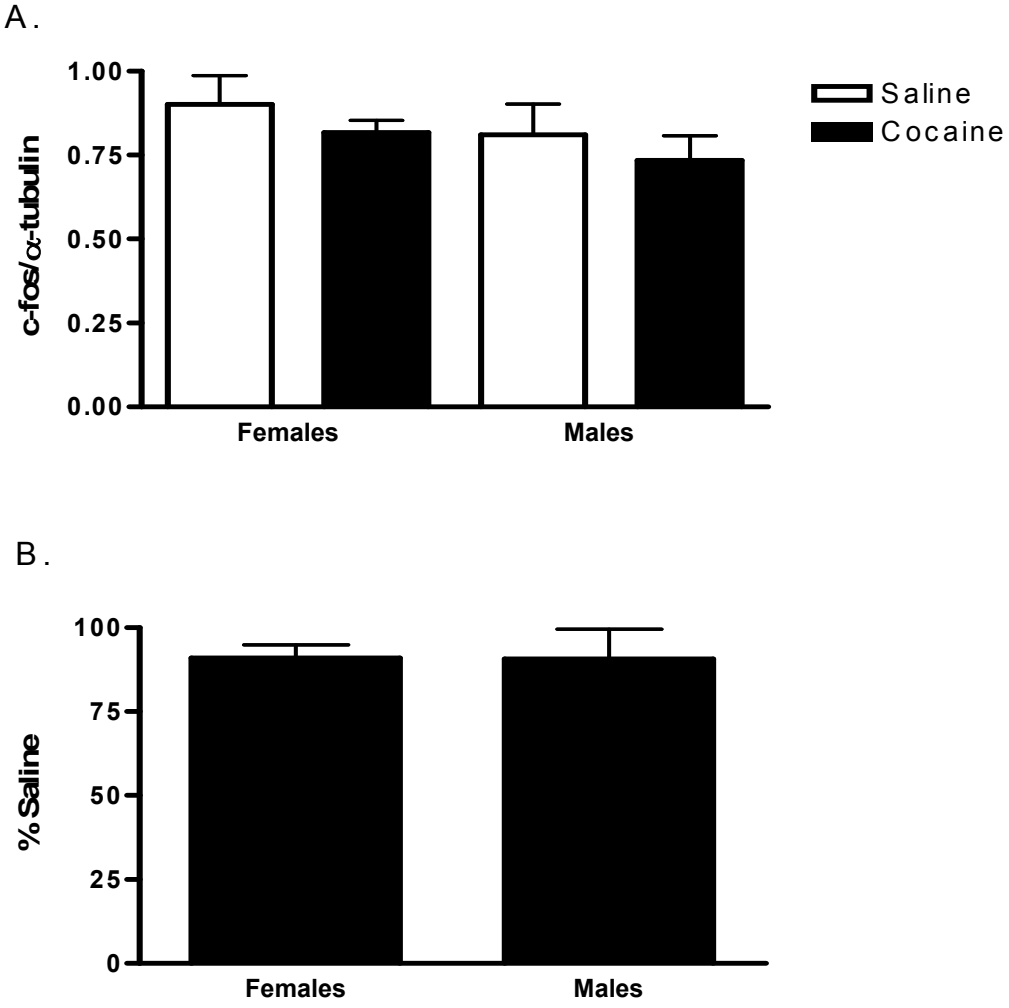


Figure 11: Males versus females in comparison before and after acute cocaine in the NAc, *c-fos* protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

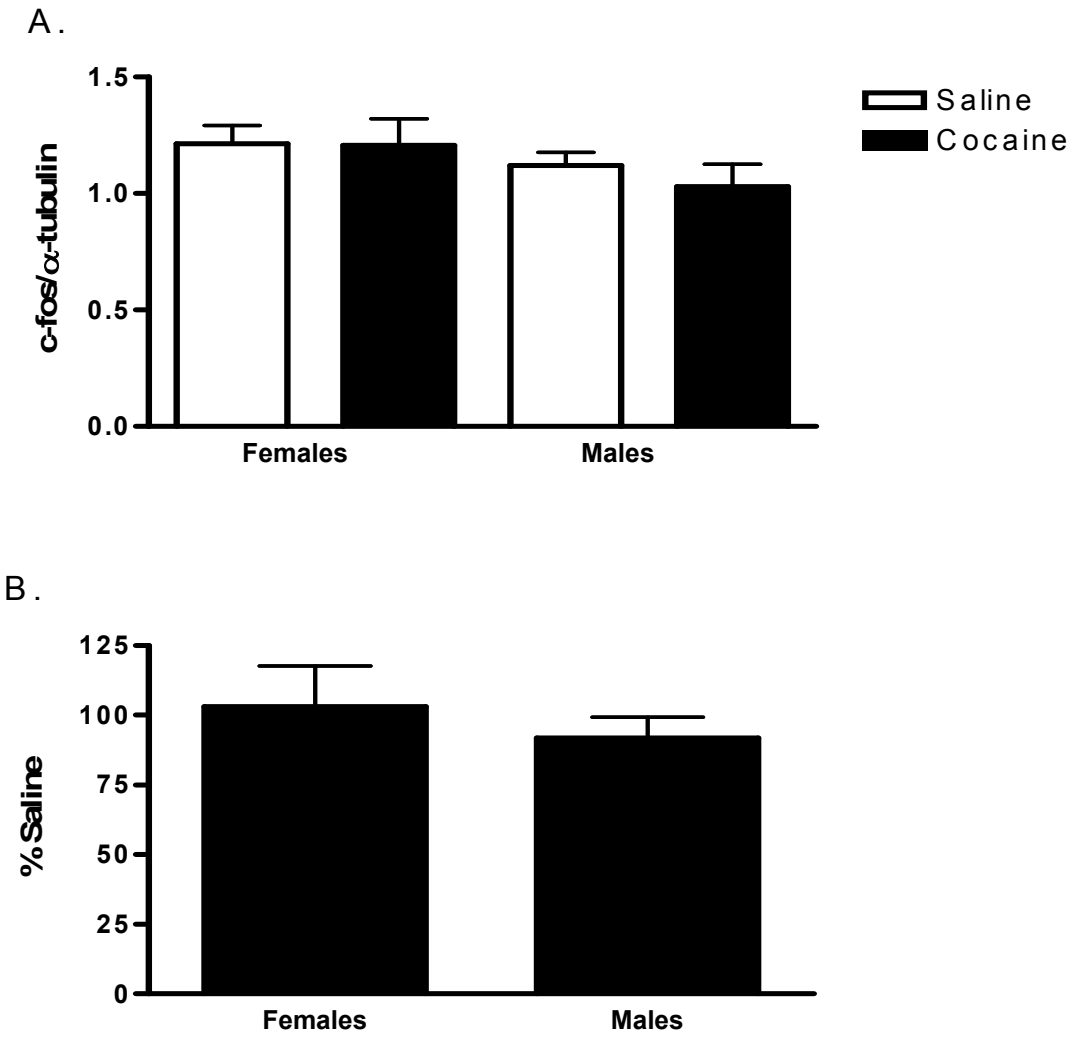


Figure 12: Males versus females in comparison before and after acute cocaine in the NAc, *c-fos* protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

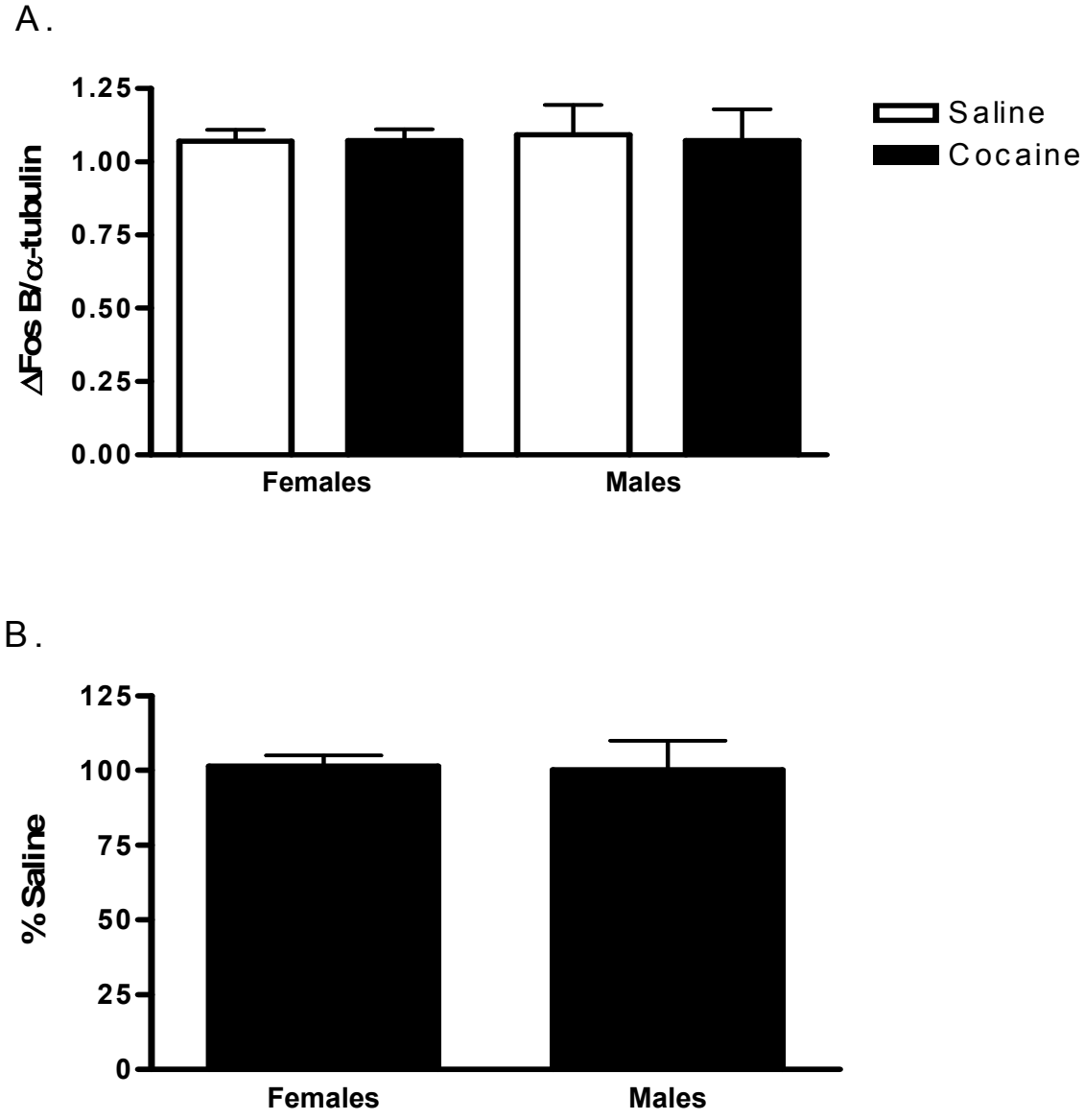


Figure 13: Males versus females in comparison before and after acute cocaine in the NAc, ΔFosB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

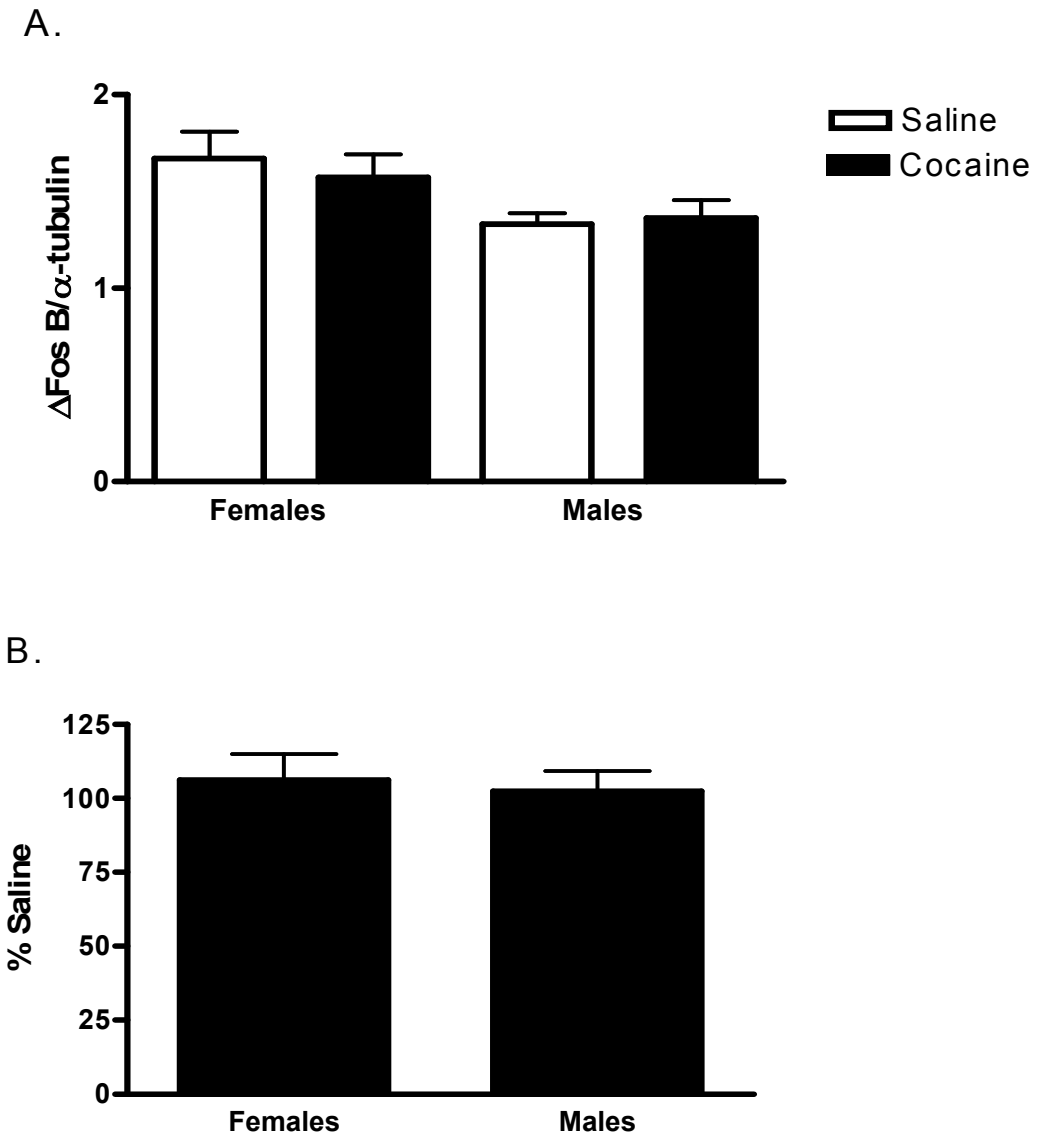


Figure 14: Males versus females in comparison before and after acute cocaine in the NAc, ΔFosB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

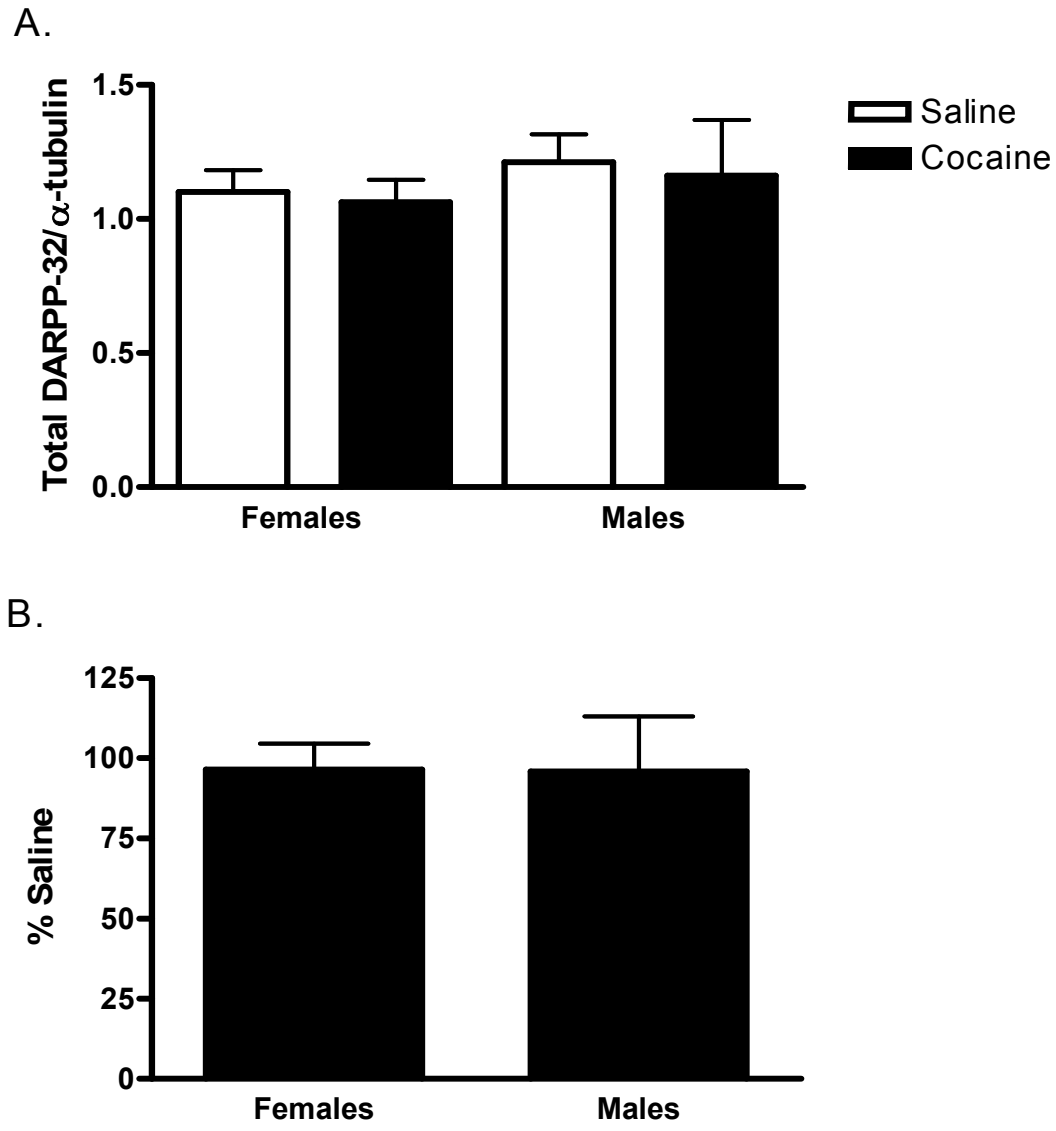
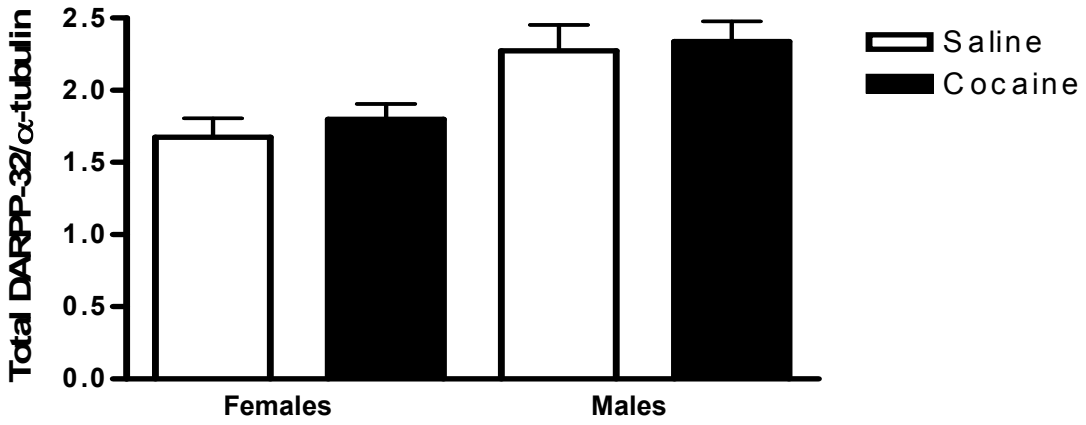


Figure 15: Males versus females in comparison before and after acute cocaine in the NAc, Total DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

A.



B.

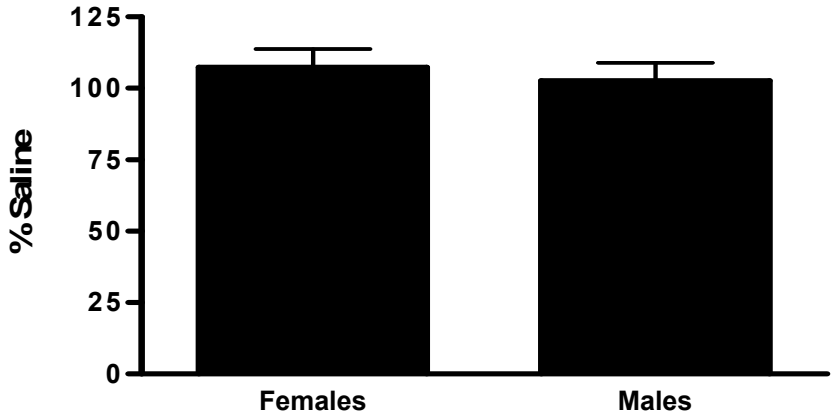


Figure 16: Males versus females in comparison before and after acute cocaine in the NAc, Total DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

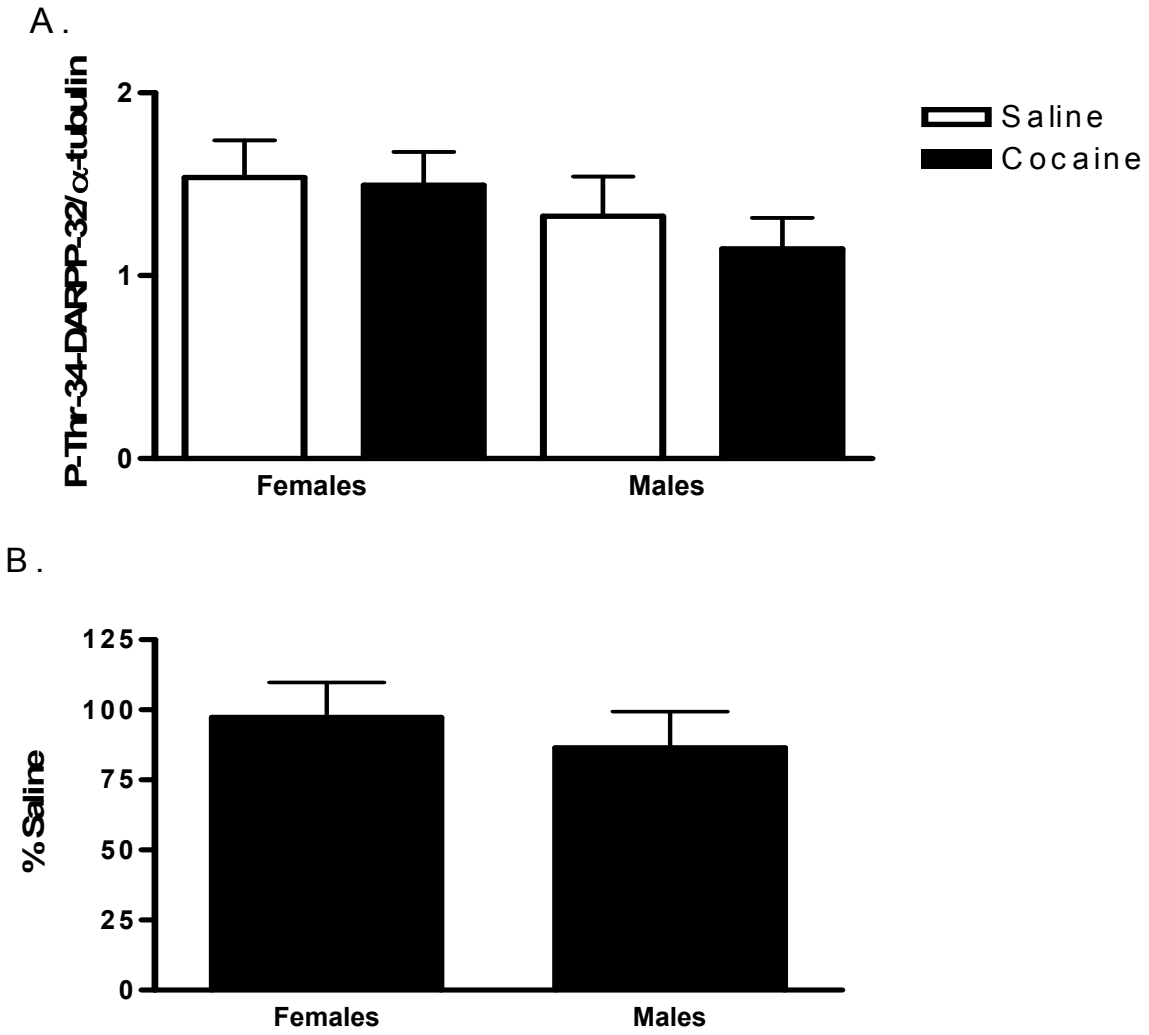


Figure 17: Males versus females in comparison before and after acute cocaine in the NAc, P-Thr-34-DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

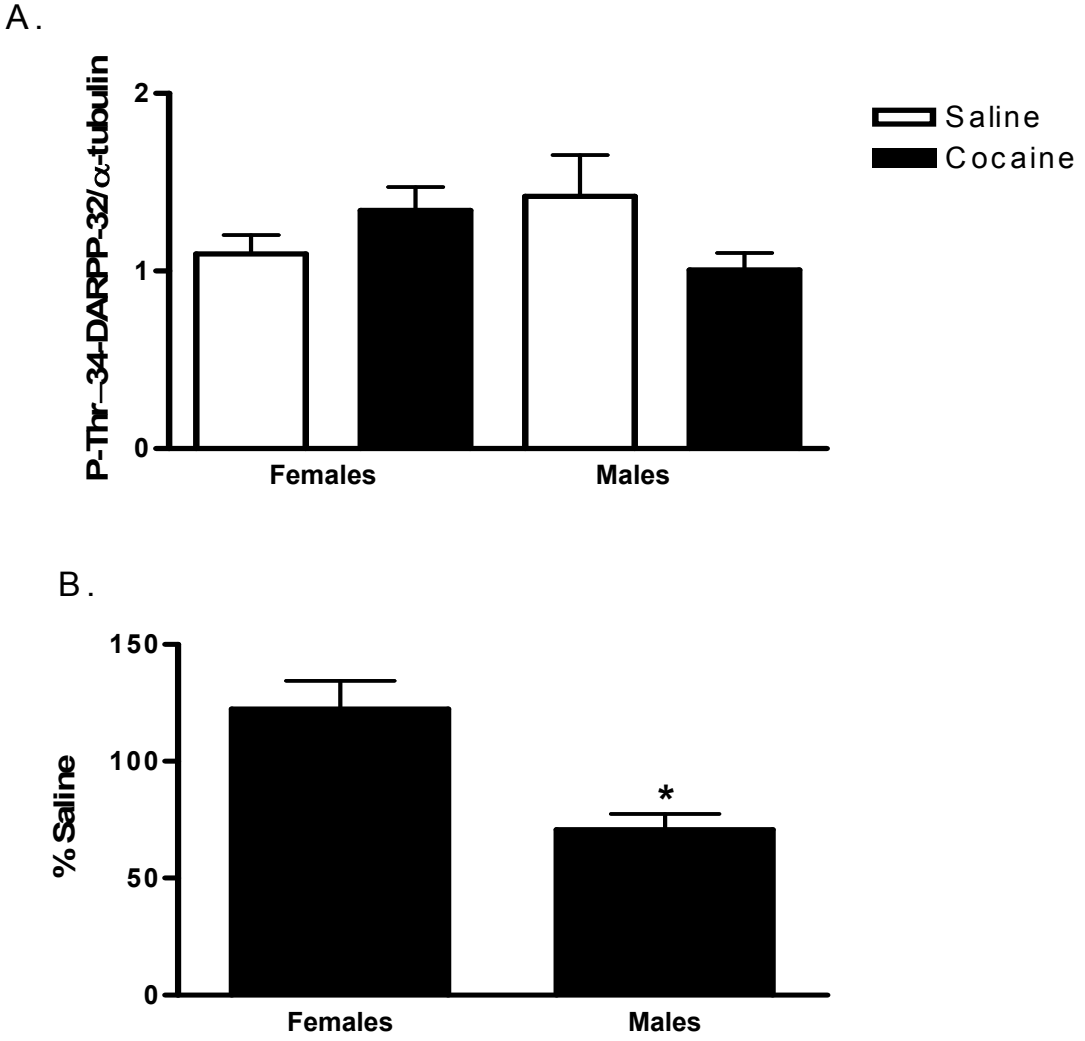


Figure 18: Sex differences in the NAc, P-Thr-34-DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).
* Represents statistically significant difference between males and females.

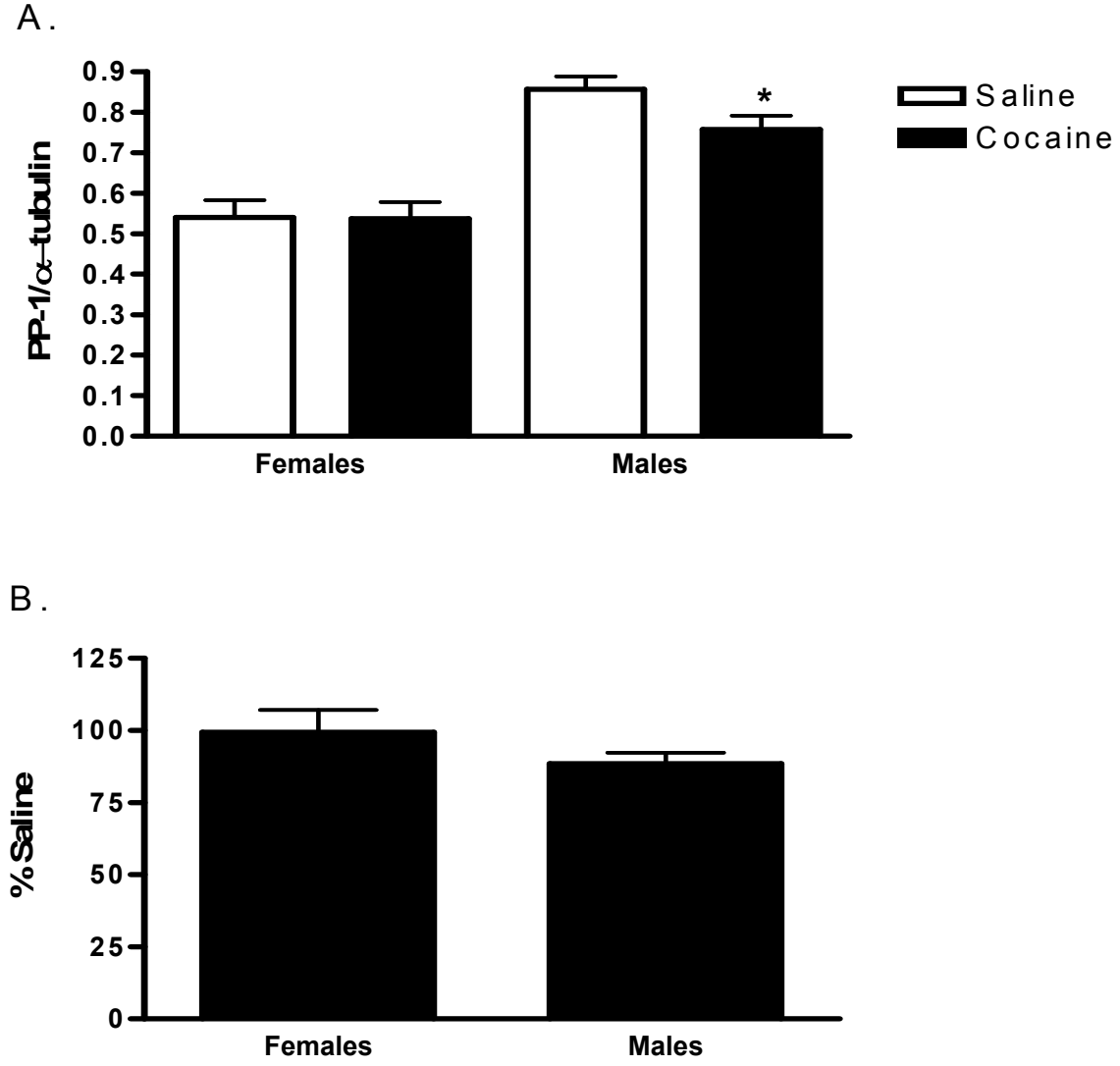


Figure 19: Males versus females in comparison before and after acute cocaine in the NAc, PP-1 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. * Represents statistically significant difference between saline and cocaine males **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

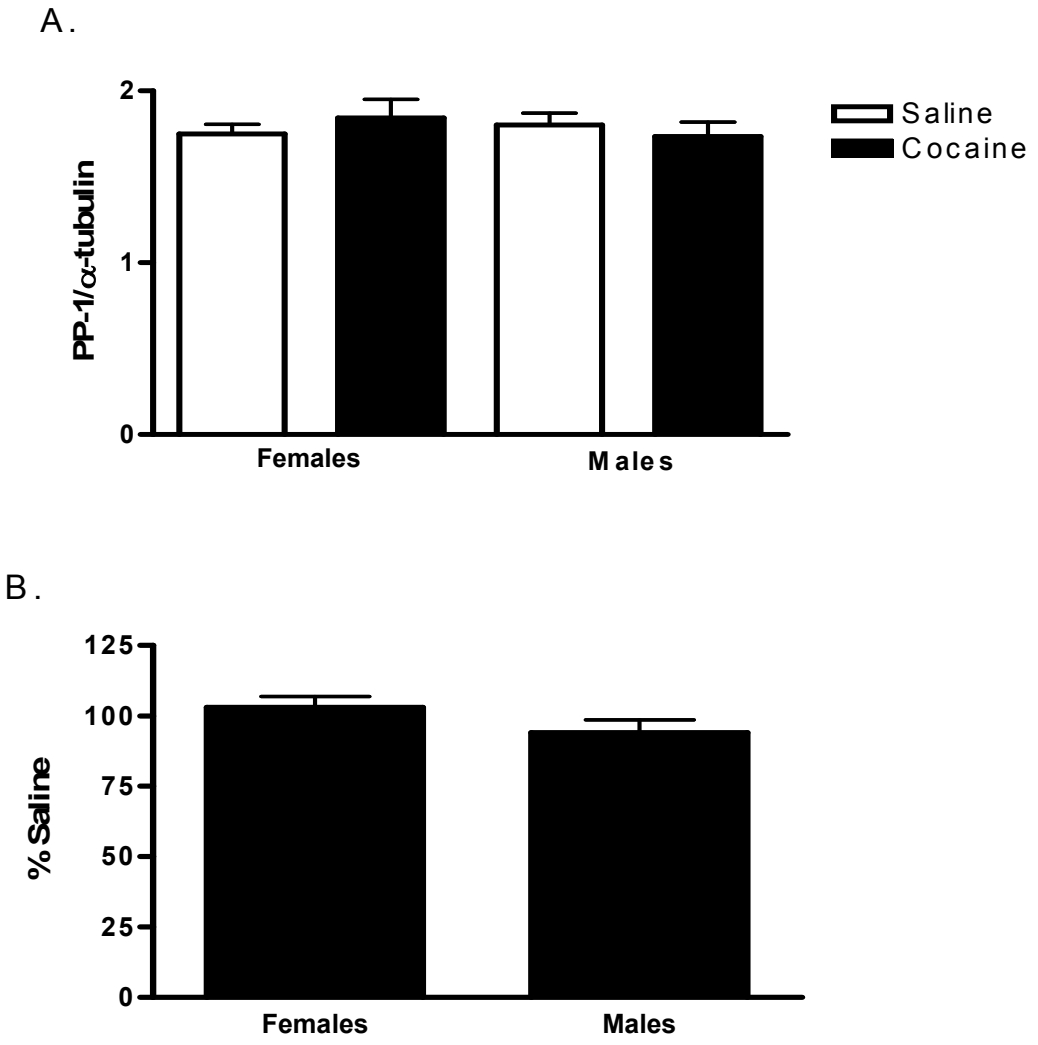


Figure 20: Males versus females in comparison before and after acute cocaine in the NAc, PP-1 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

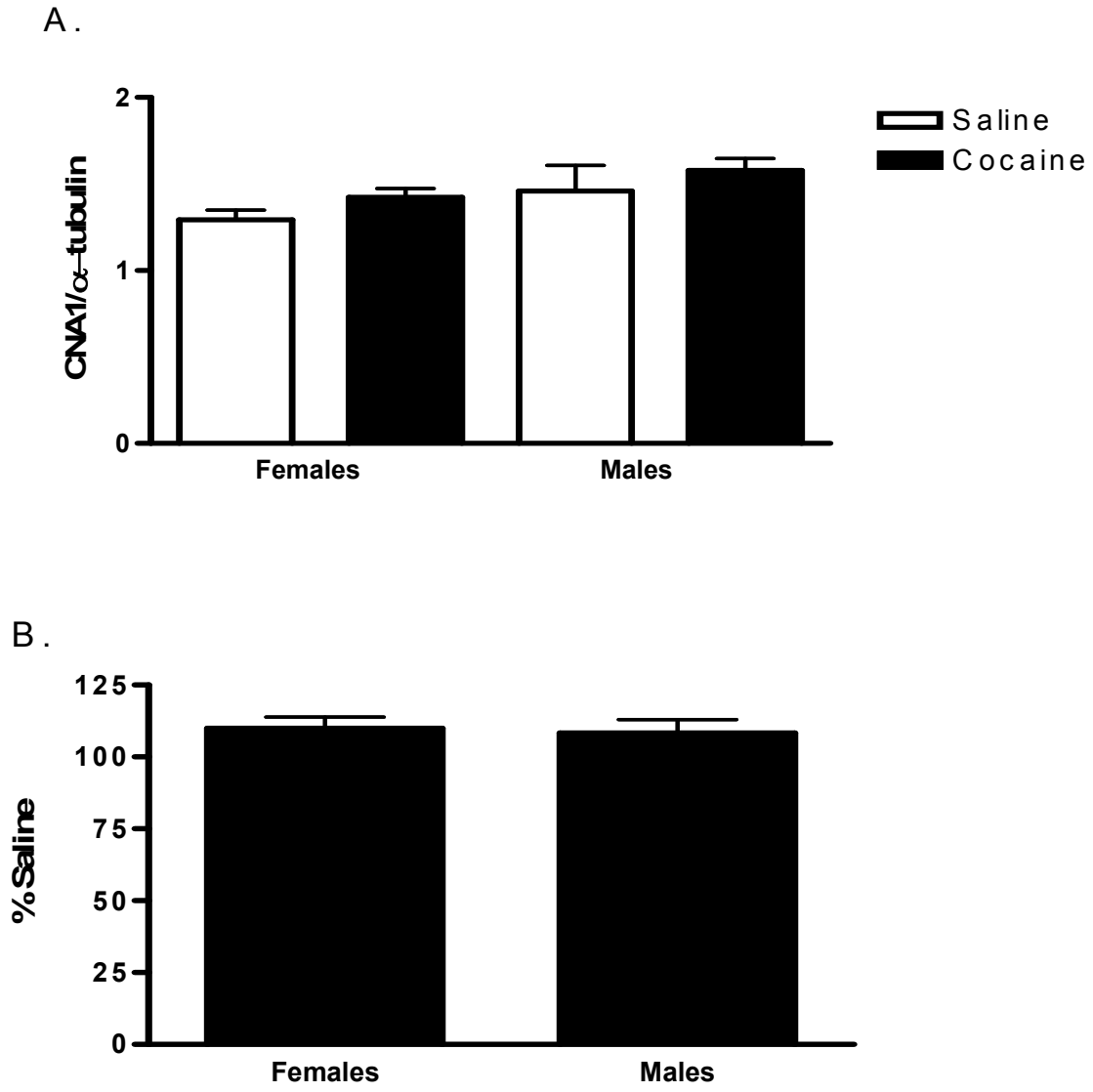


Figure 21: Males versus females in comparison before and after acute cocaine in the NAc, PP-2B (CNA1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

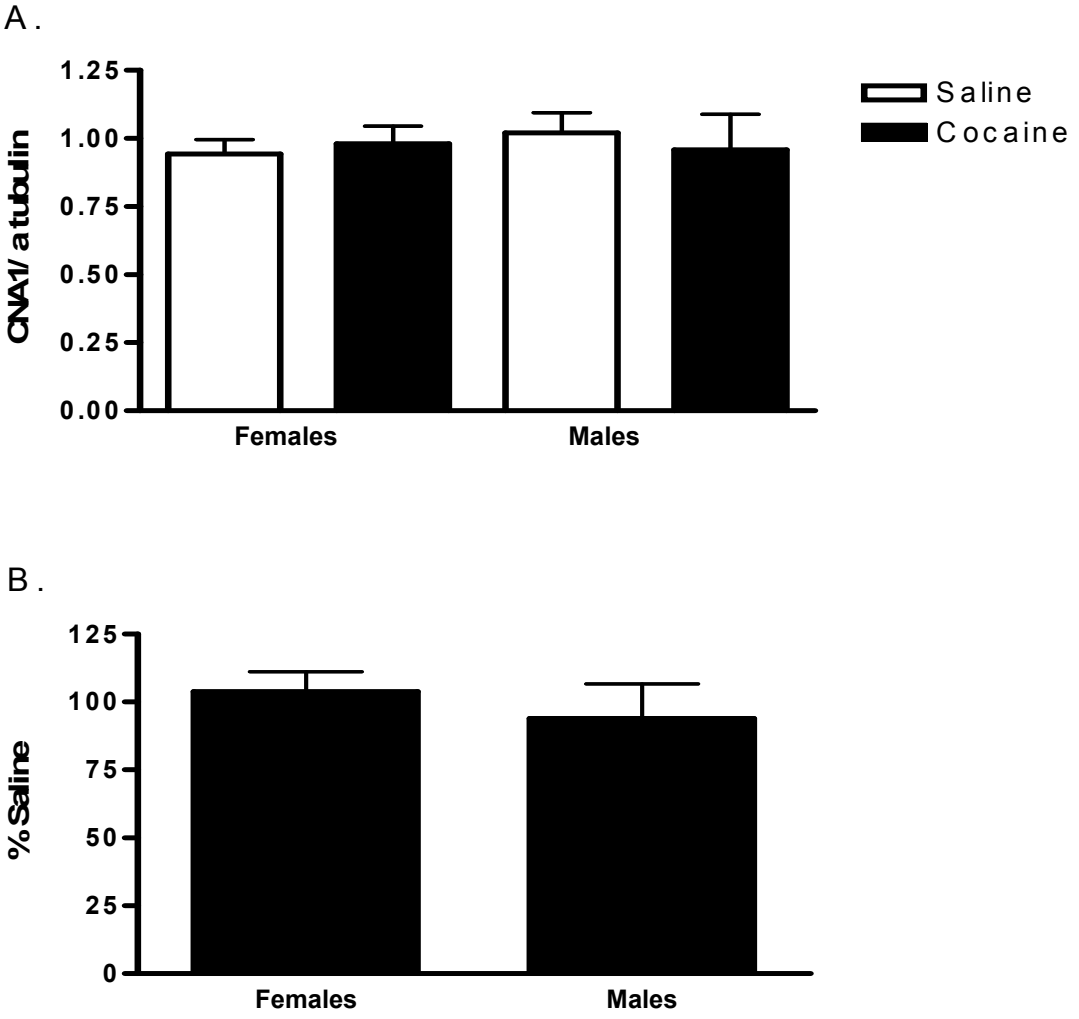


Figure 22: Males versus females in comparison before and after acute cocaine in the NAc, PP-2B (CNA1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

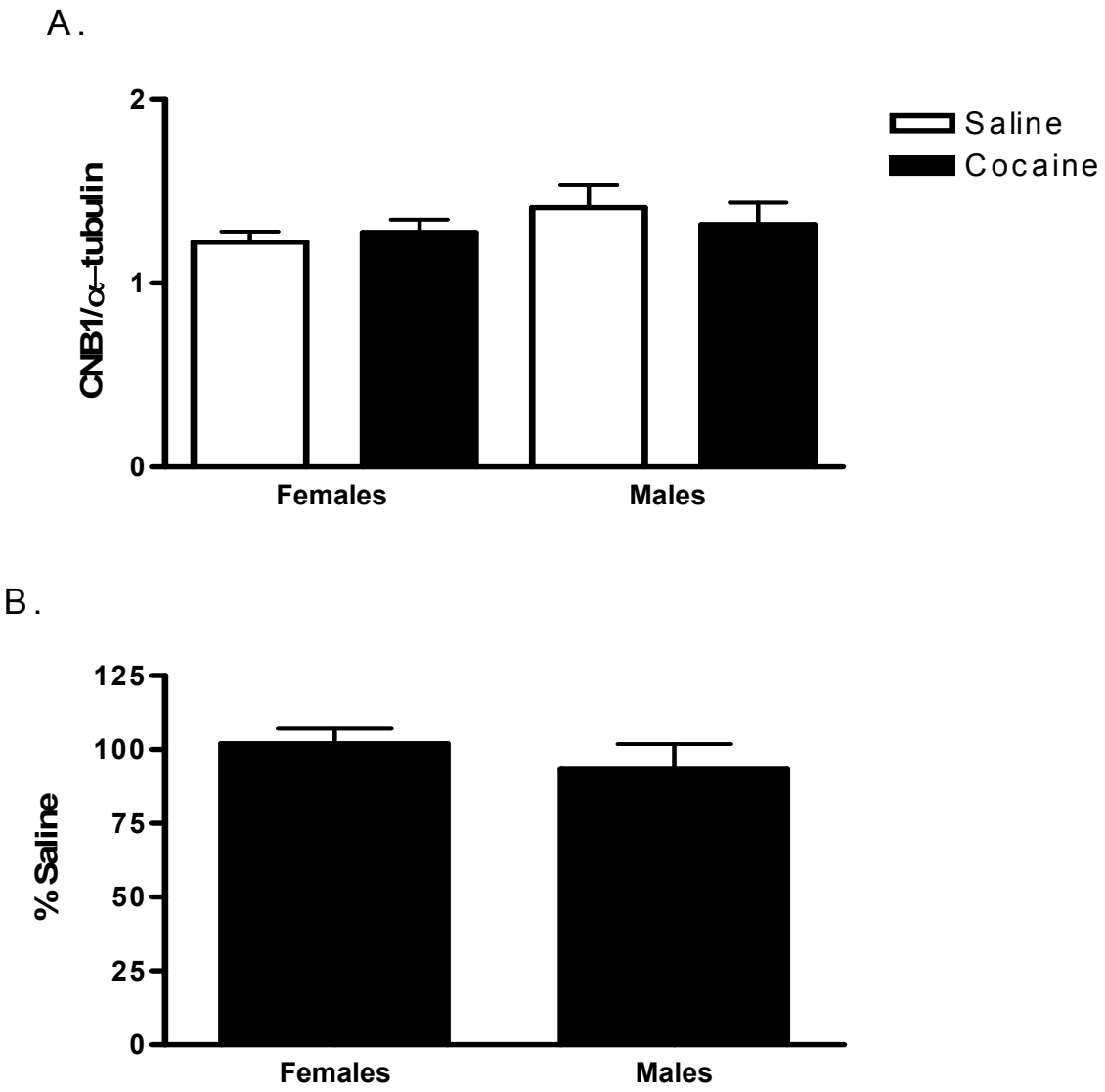


Figure 23: Males versus females in comparison before and after acute cocaine in the NAc, PP-2B (CNB1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

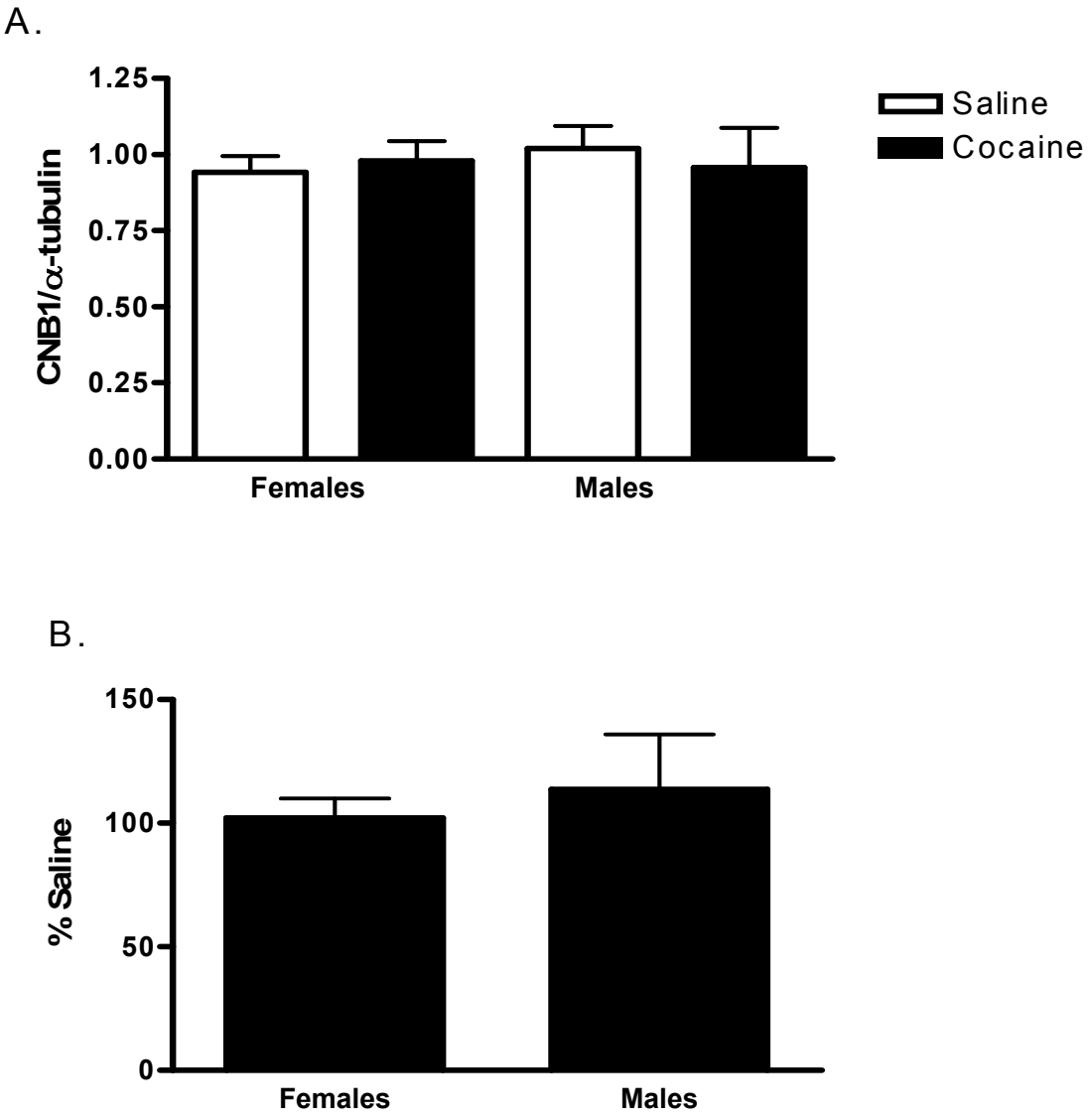


Figure 24: Males versus females in comparison before and after acute cocaine in the NAc, PP-2B (CNB1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

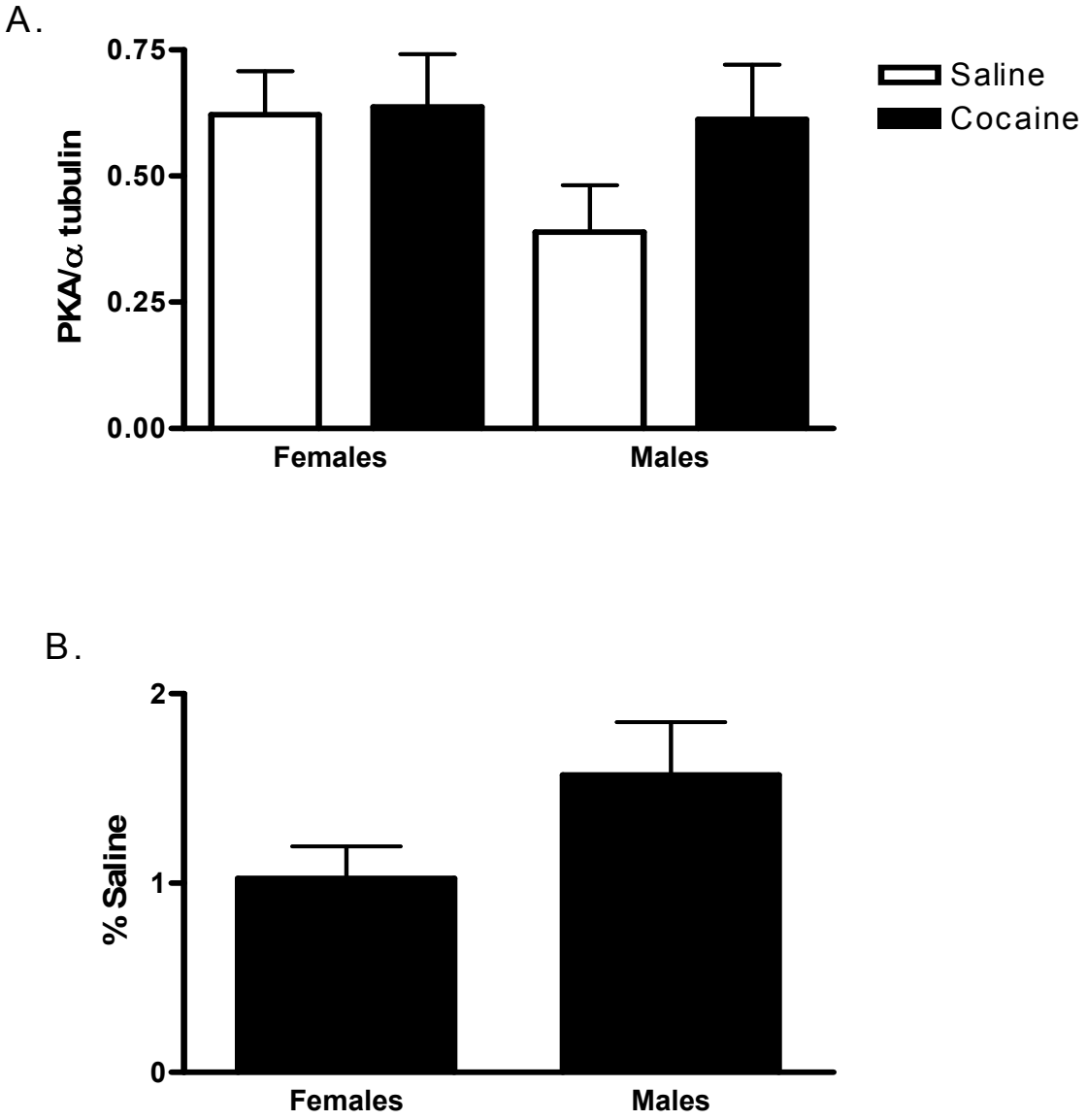


Figure 25: Males versus females in comparison before and after acute cocaine in the CPu, PKA protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

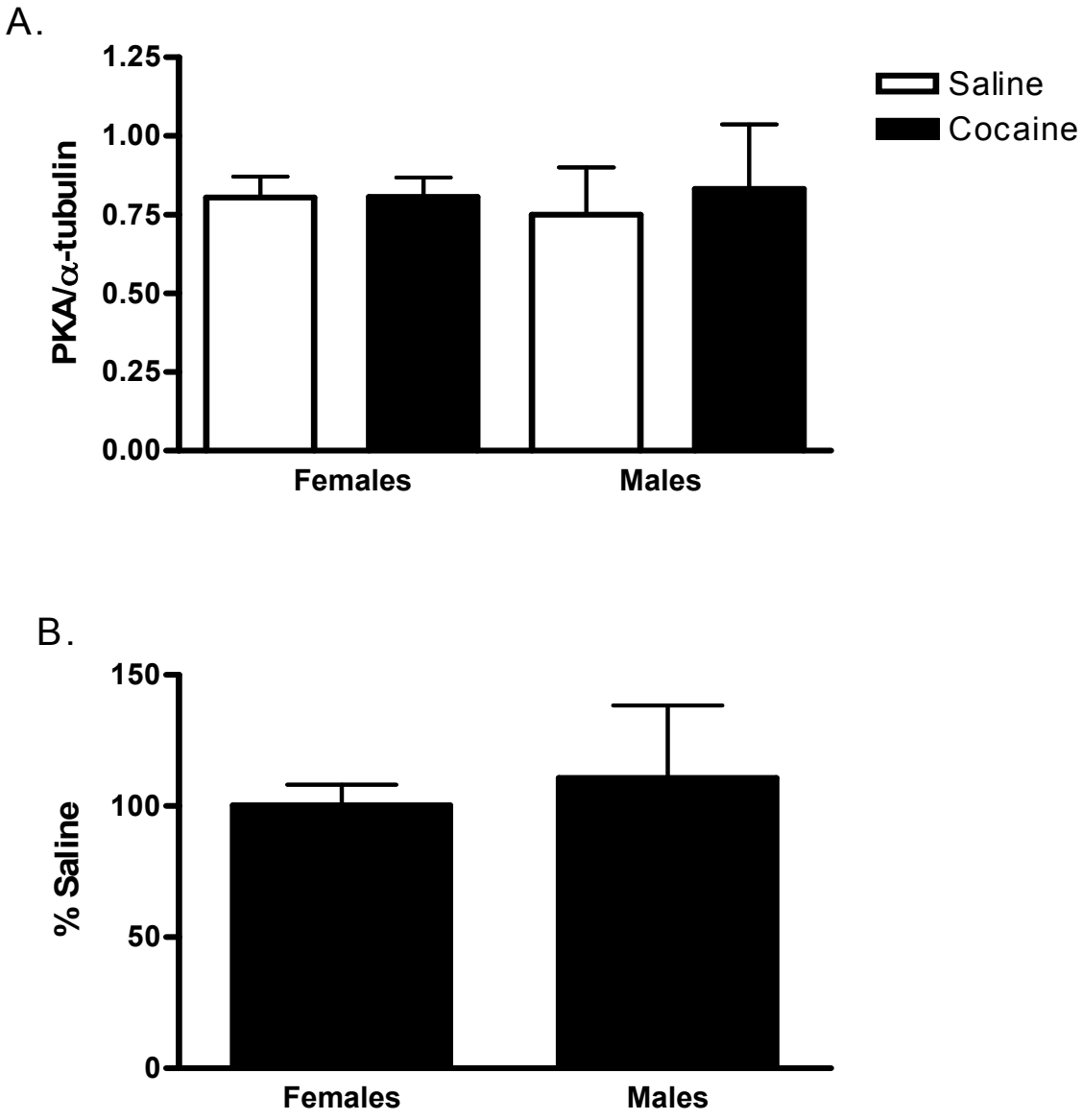


Figure 26: Males versus females in comparison before and after acute cocaine in the CPu, PKA protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

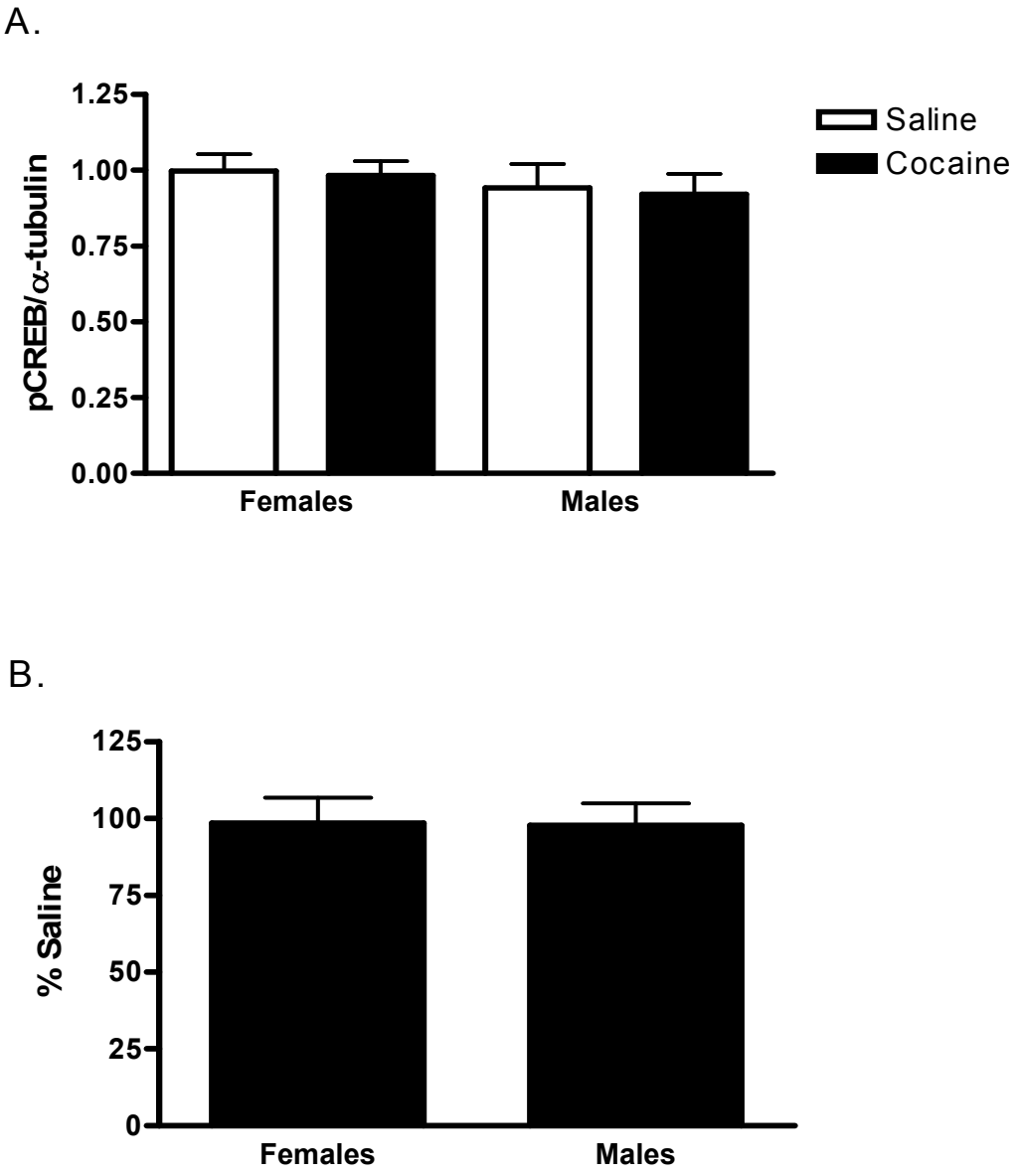


Figure 27: Males versus females in comparison before and after acute cocaine in the CPu, pCREB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

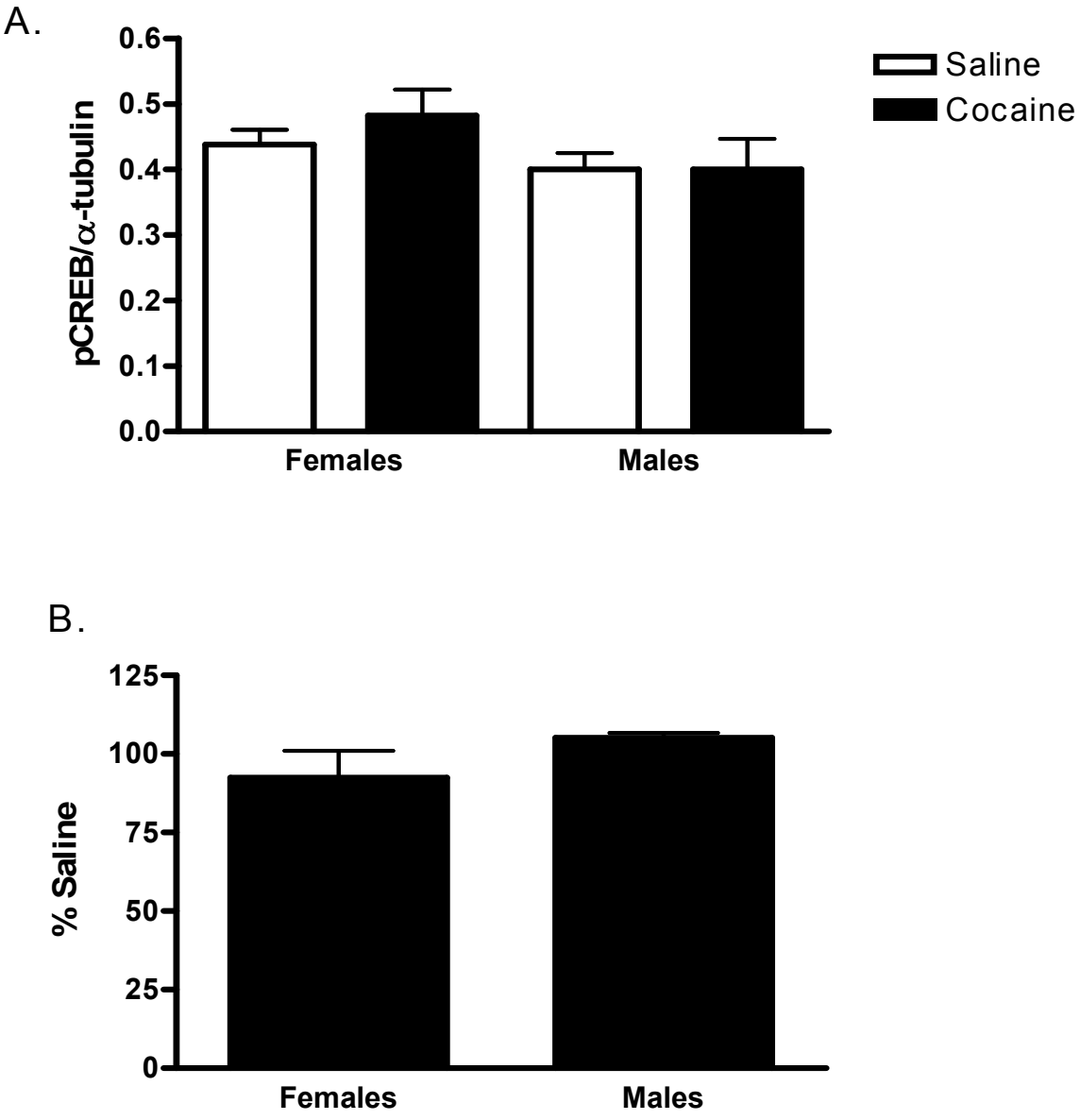


Figure 28: Males versus females in comparison before and after acute cocaine in the CPu, pCREB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

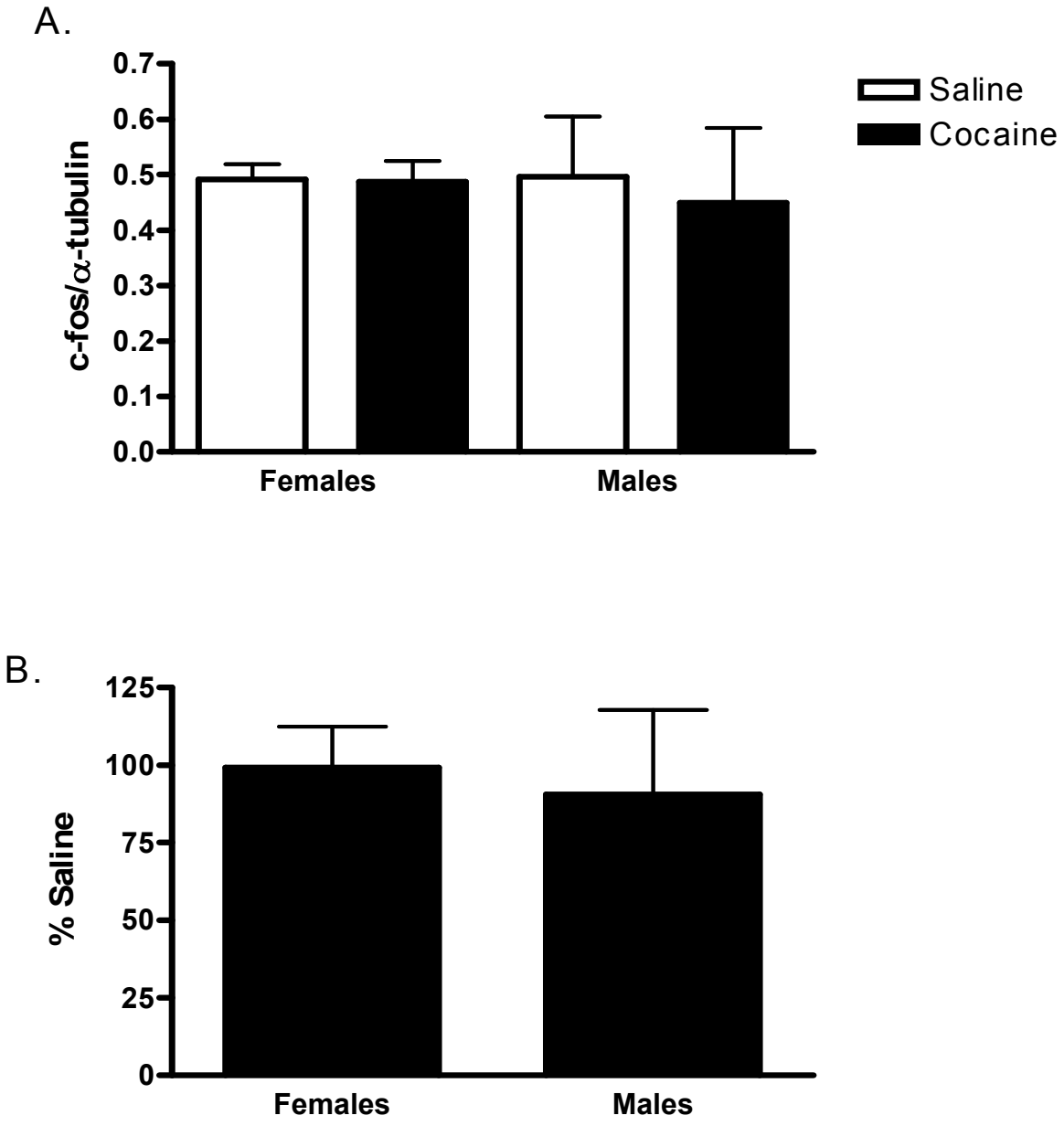


Figure 29: Males versus females in comparison before and after acute cocaine in the CPu, *c-fos* protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

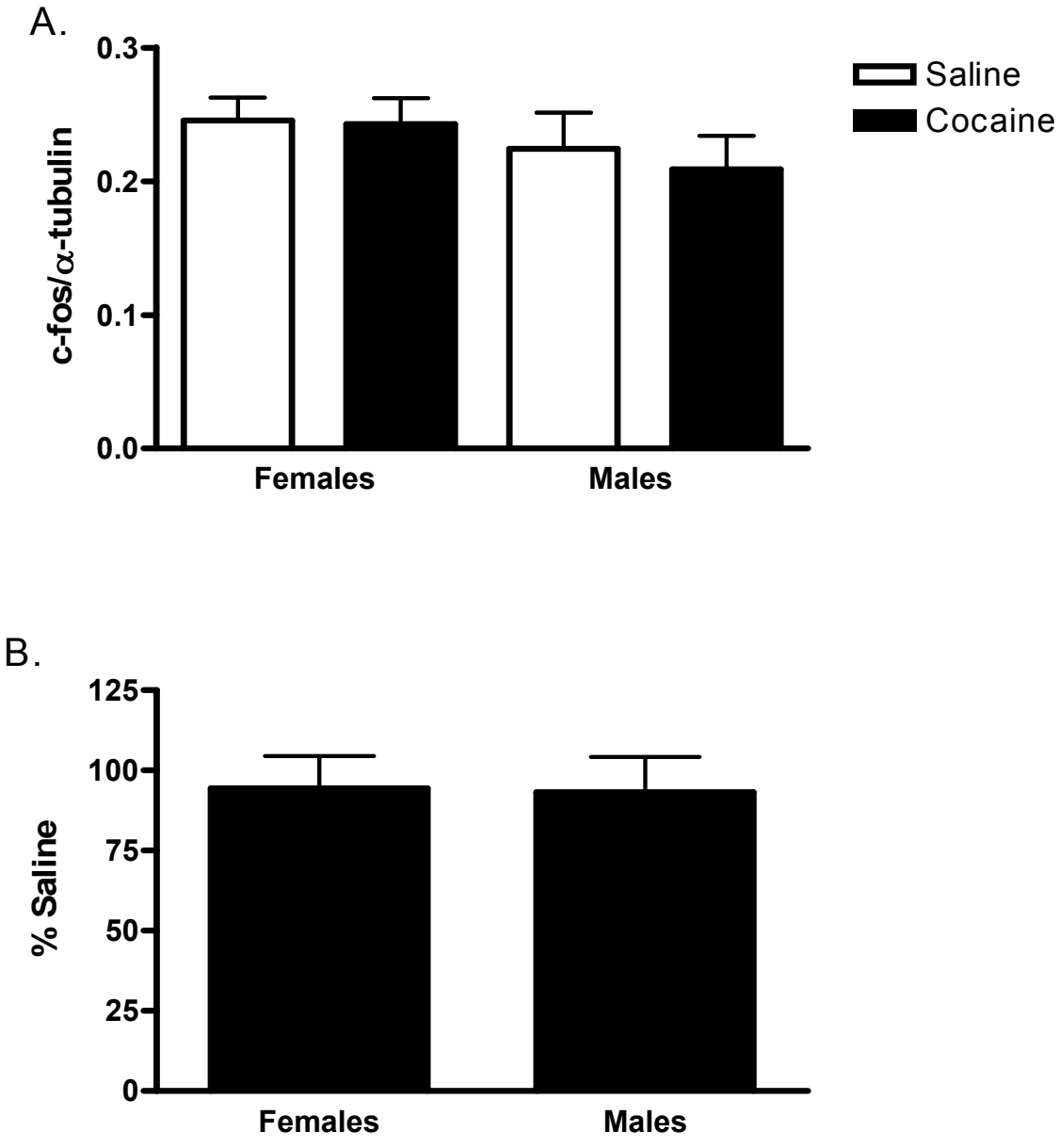


Figure 30: Males versus females in comparison before and after acute cocaine in the CPu, *c-fos* protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

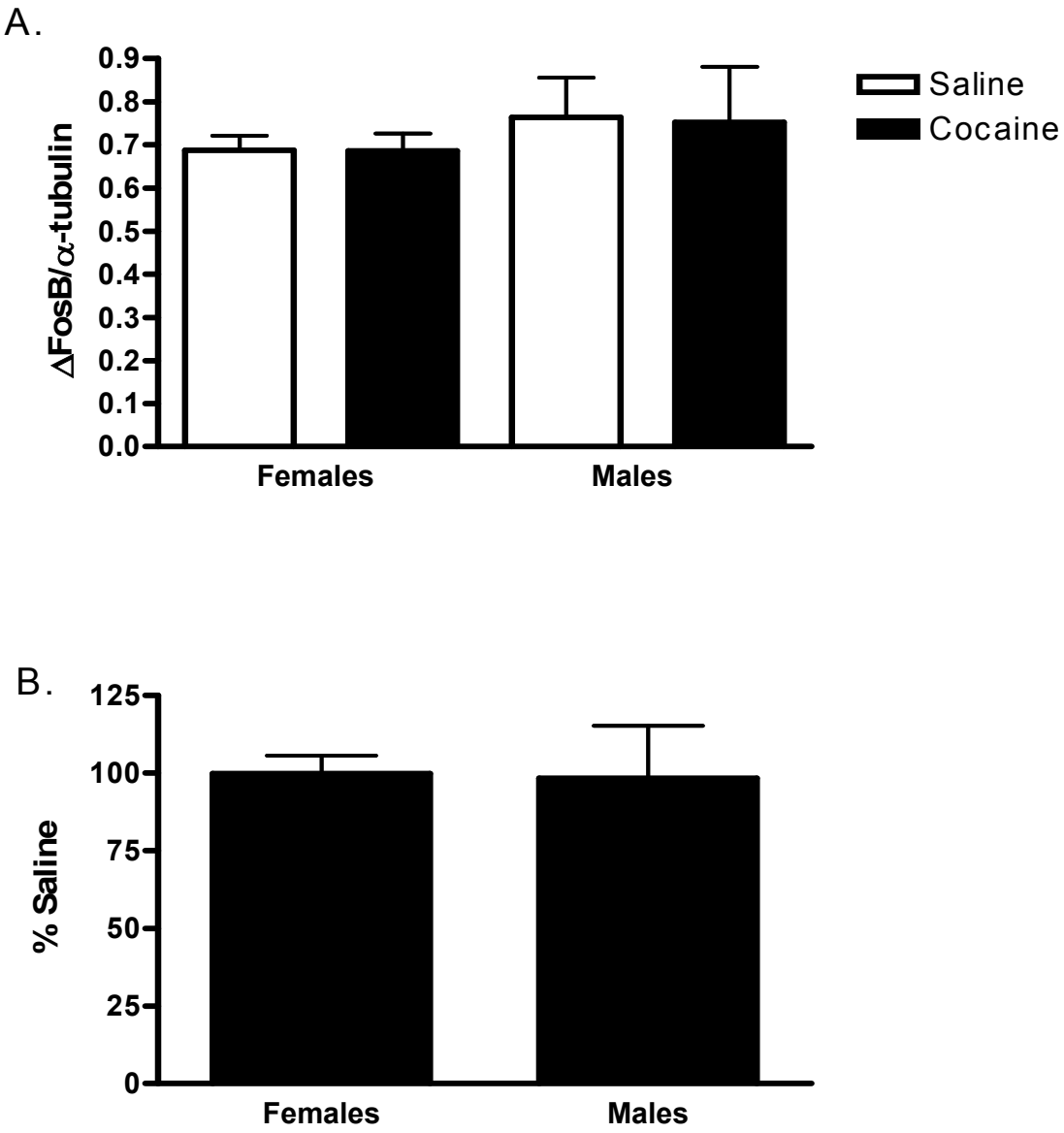


Figure 31: Males versus females in comparison before and after acute cocaine in the CPu, ΔFosB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

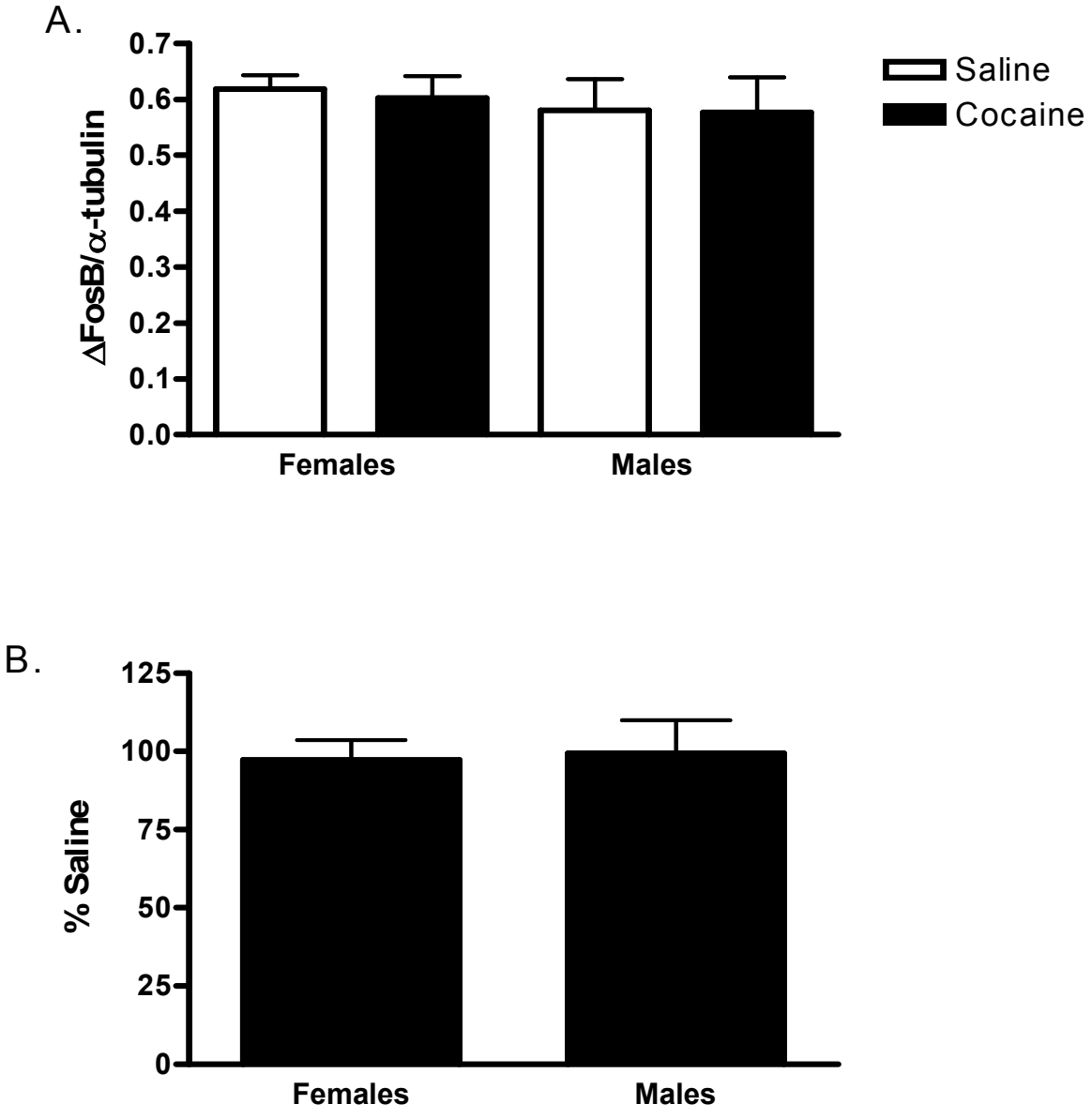


Figure 32: Males versus females in comparison before and after acute cocaine in the CPu, ΔFosB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

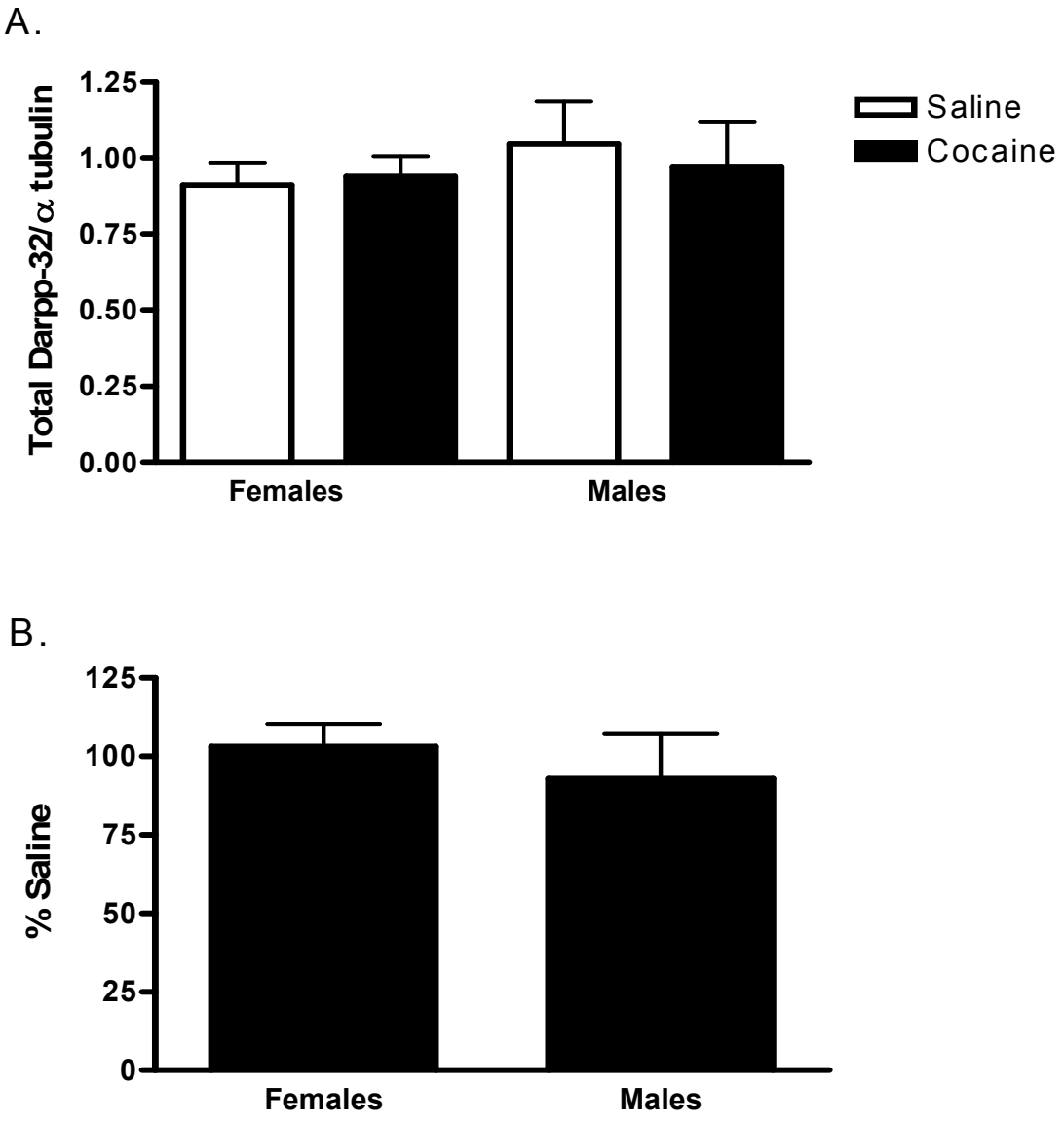


Figure 33: Males versus females in comparison before and after acute cocaine in the CPu, Total DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

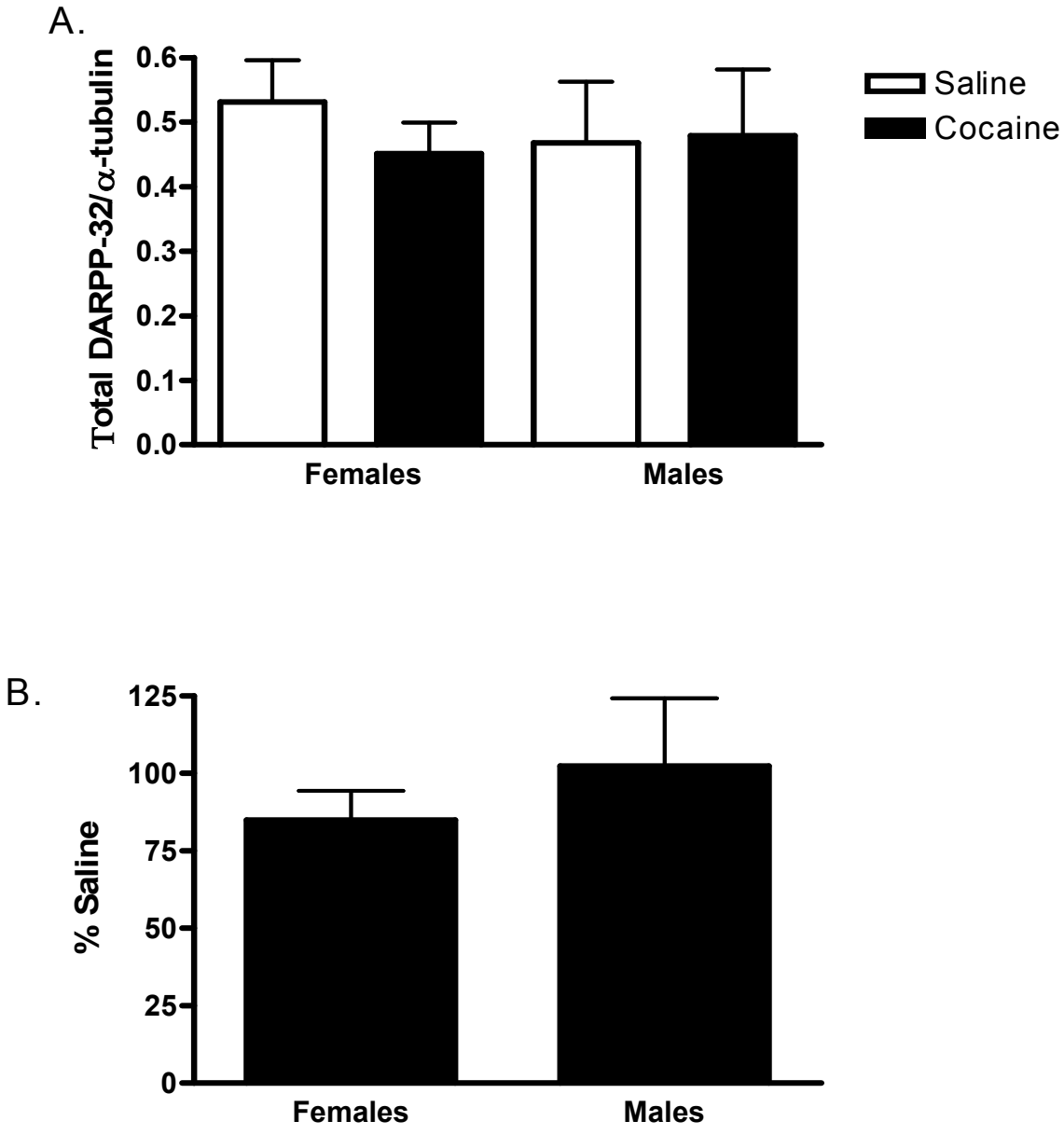


Figure 34: Males versus females in comparison before and after acute cocaine in the CPu, Total DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

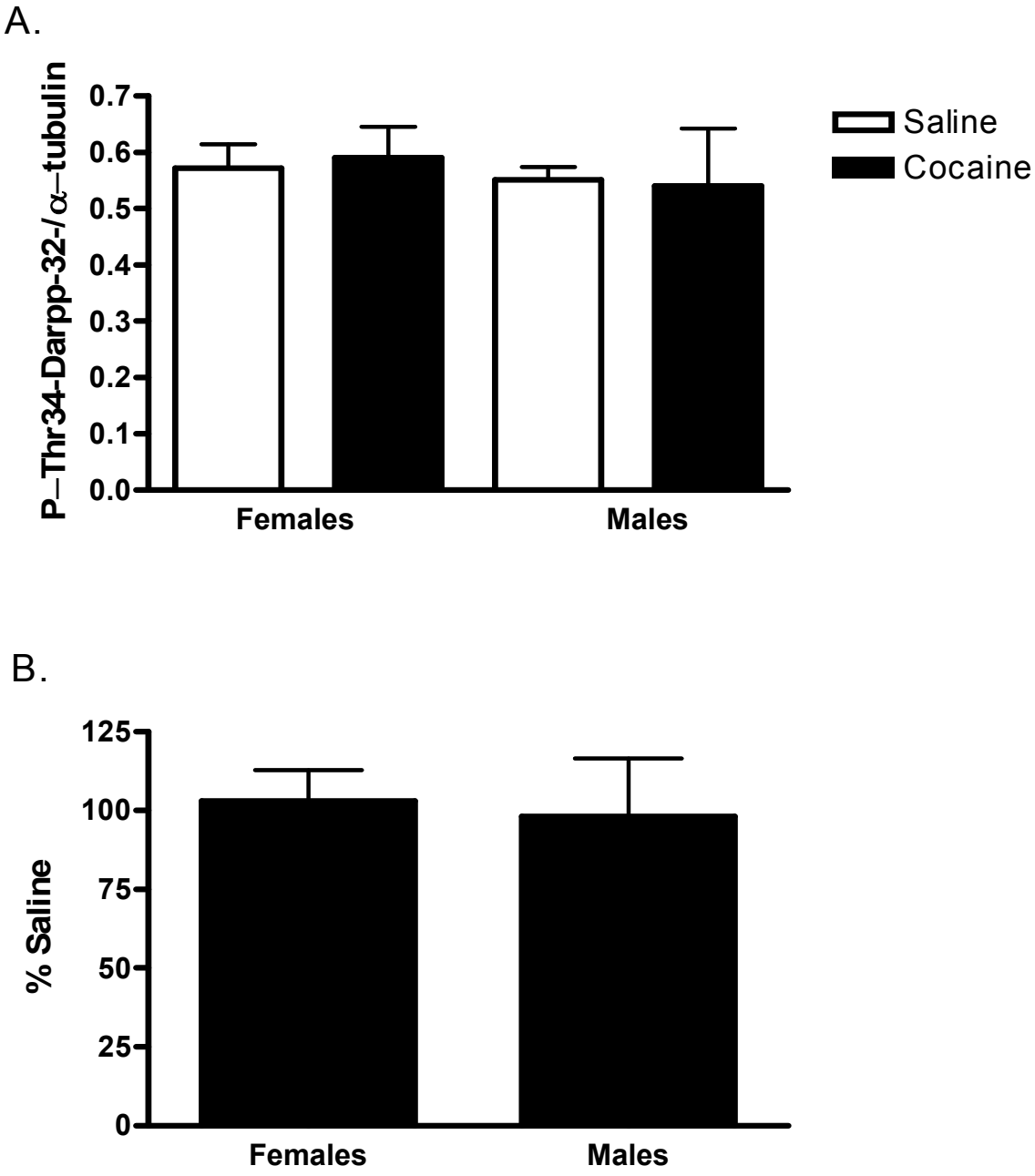


Figure 35: Males versus females in comparison before and after acute cocaine in the CPu, P-Thr-34-DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

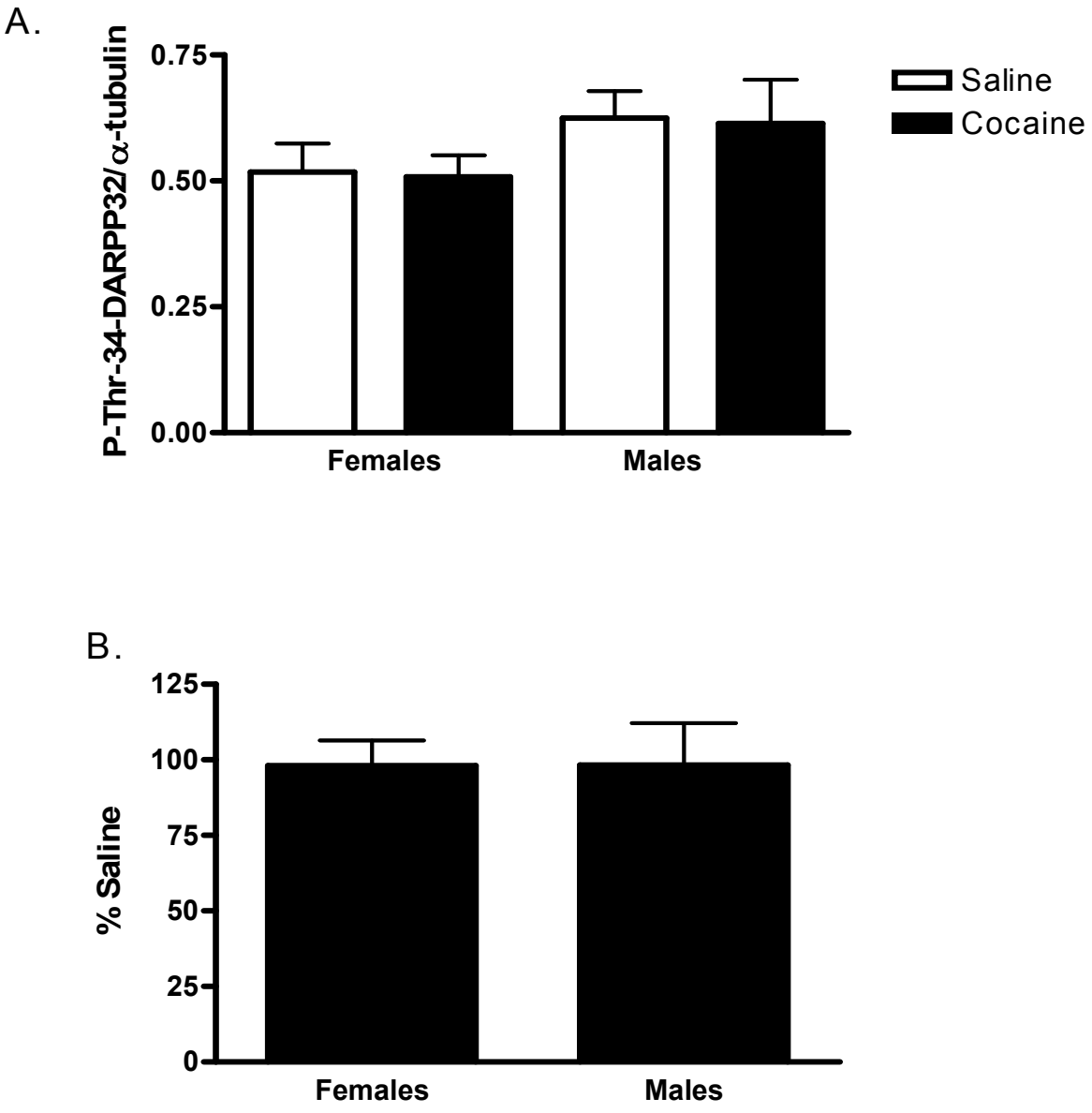


Figure 36: Males versus females in comparison before and after acute cocaine in the CPu, P-Thr-34-DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

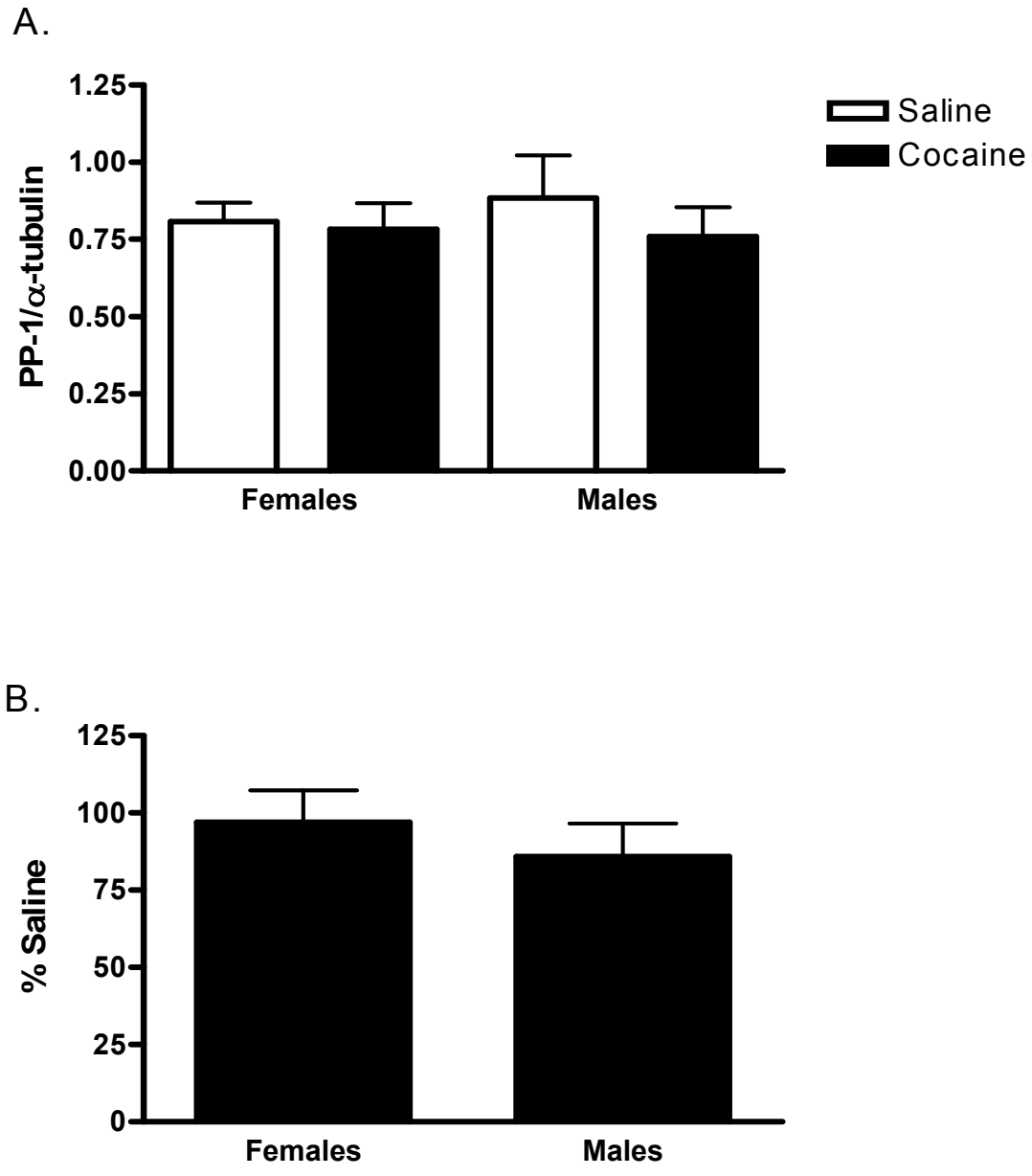


Figure 37: Males versus females in comparison before and after acute cocaine in the CPu, PP-1 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

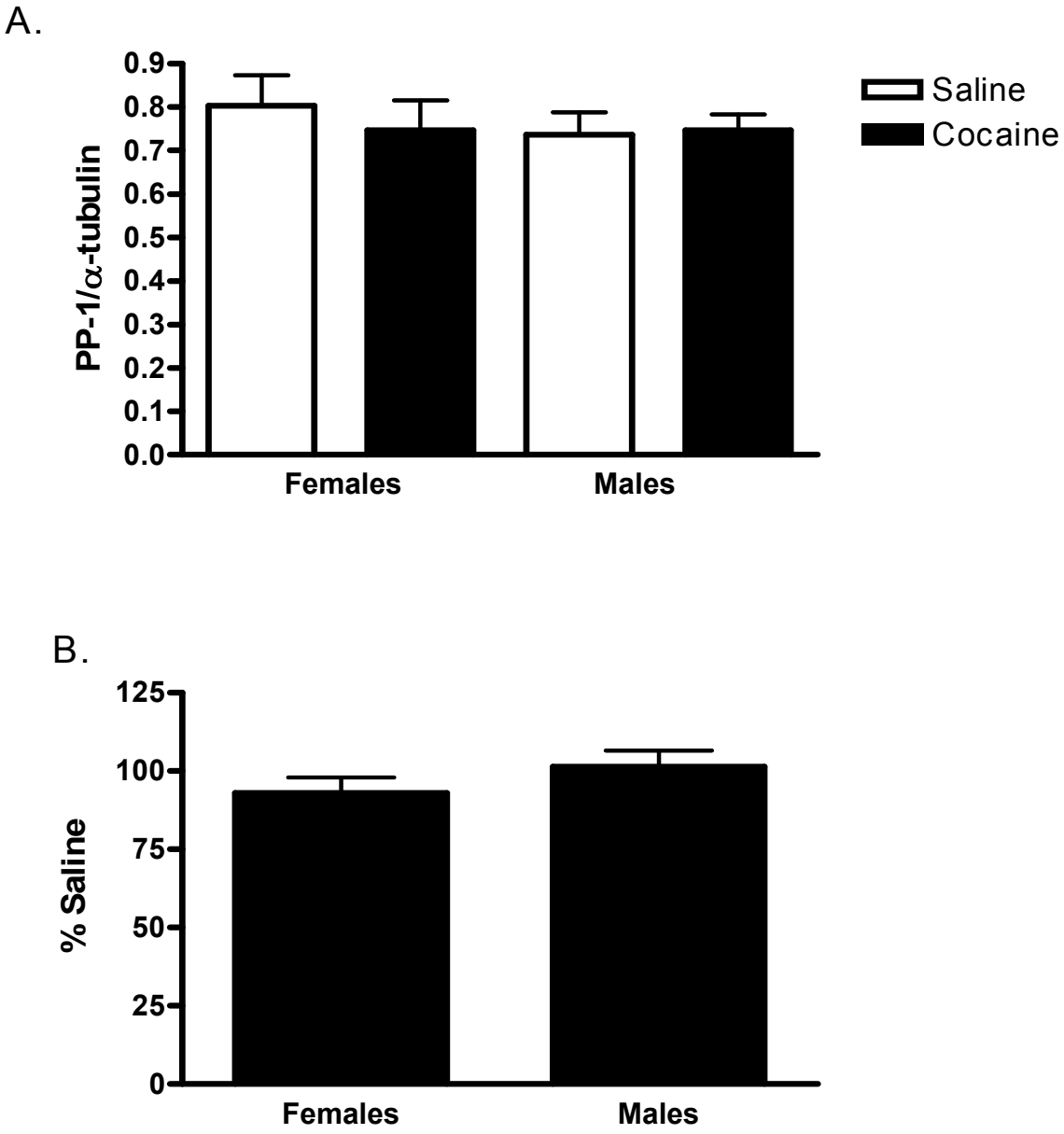


Figure 38: Males versus females in comparison before and after acute cocaine in the CPu, PP-1 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

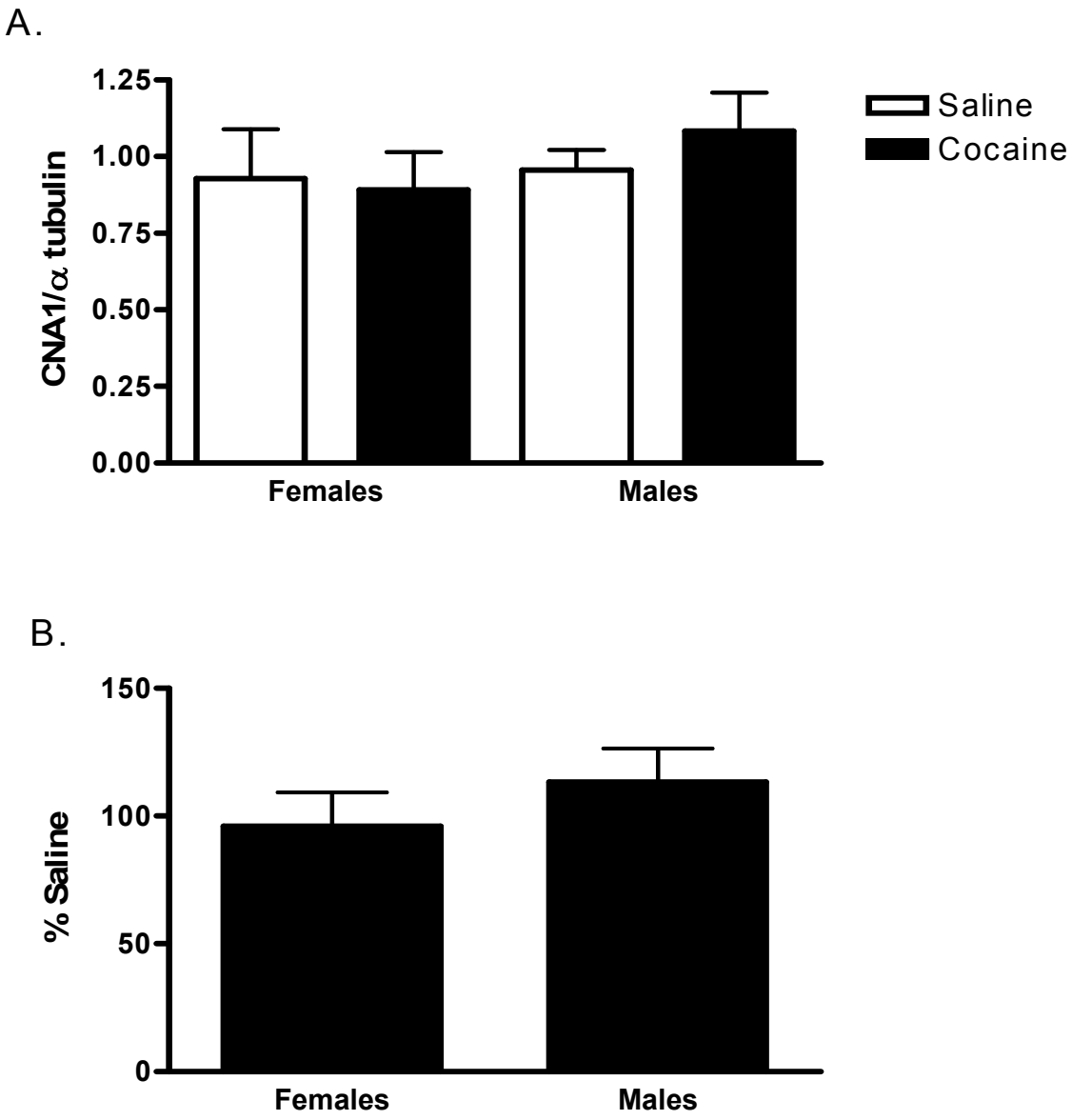


Figure 39: Males versus females in comparison before and after acute cocaine in the CPu, PP-2B (CNA1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

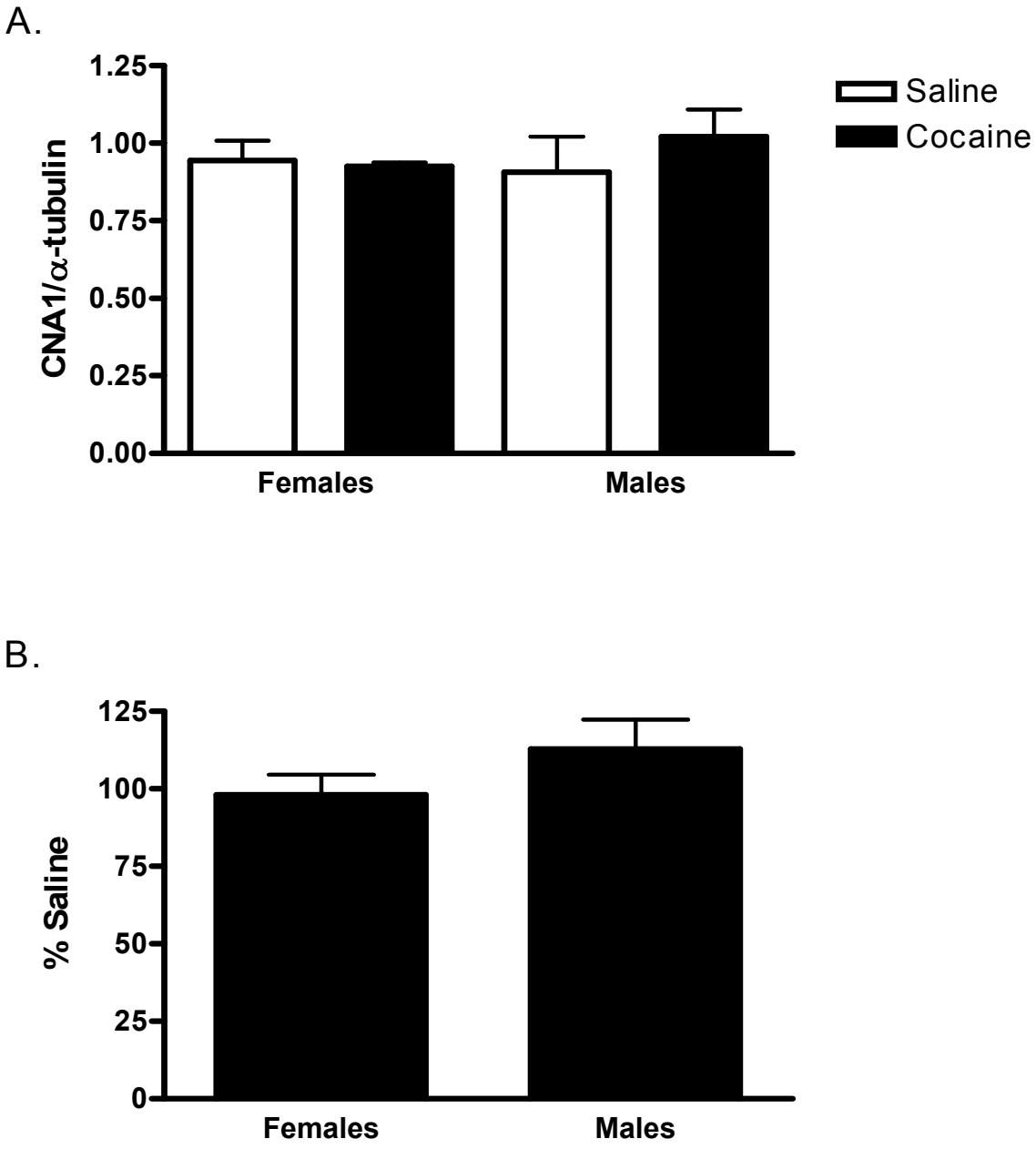


Figure 40: Males versus females in comparison before and after acute cocaine in the CPu, PP-2B (CNA1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

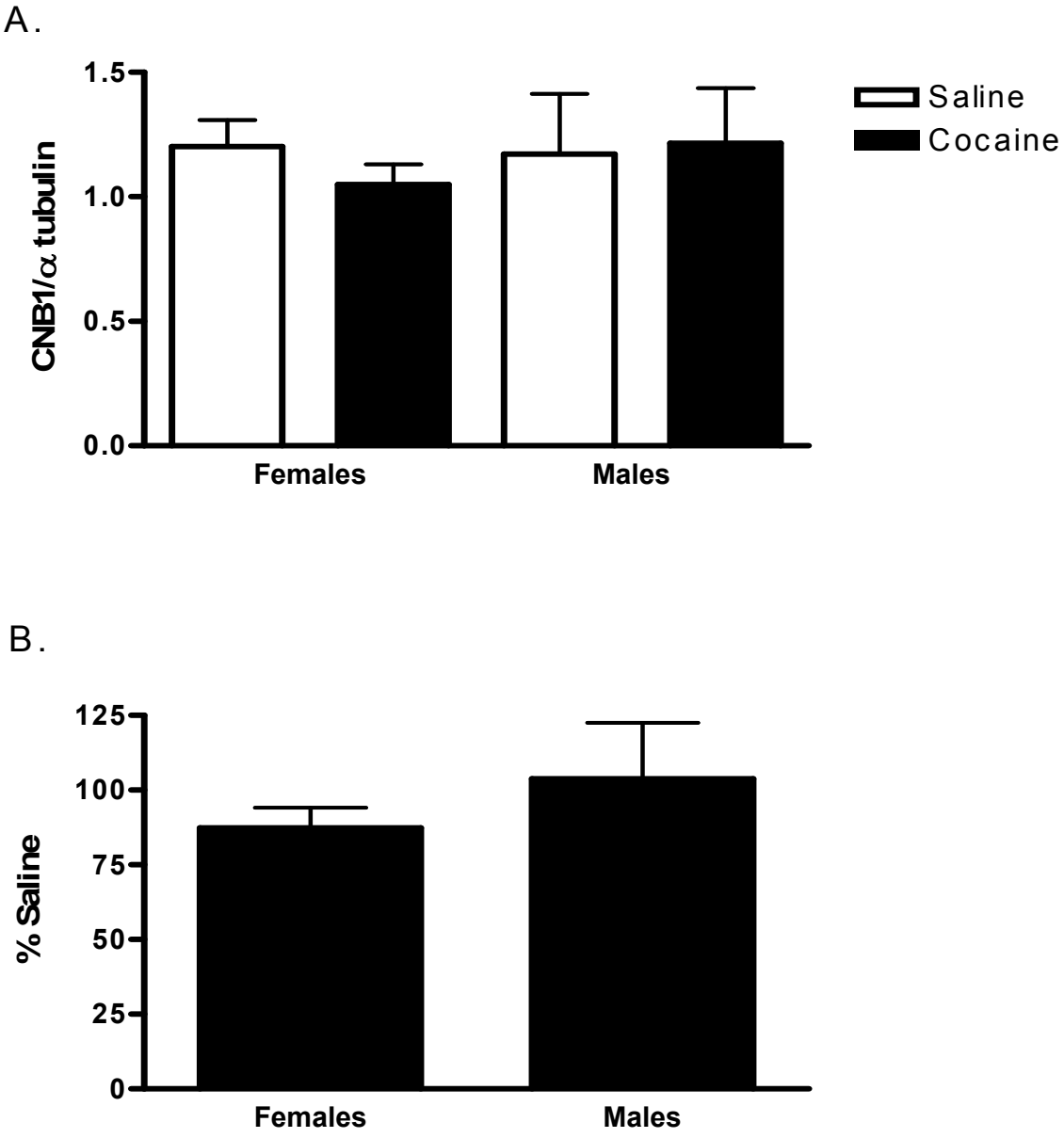


Figure 41: Males versus females in comparison before and after acute cocaine in the CPu, PP-2B (CNB1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

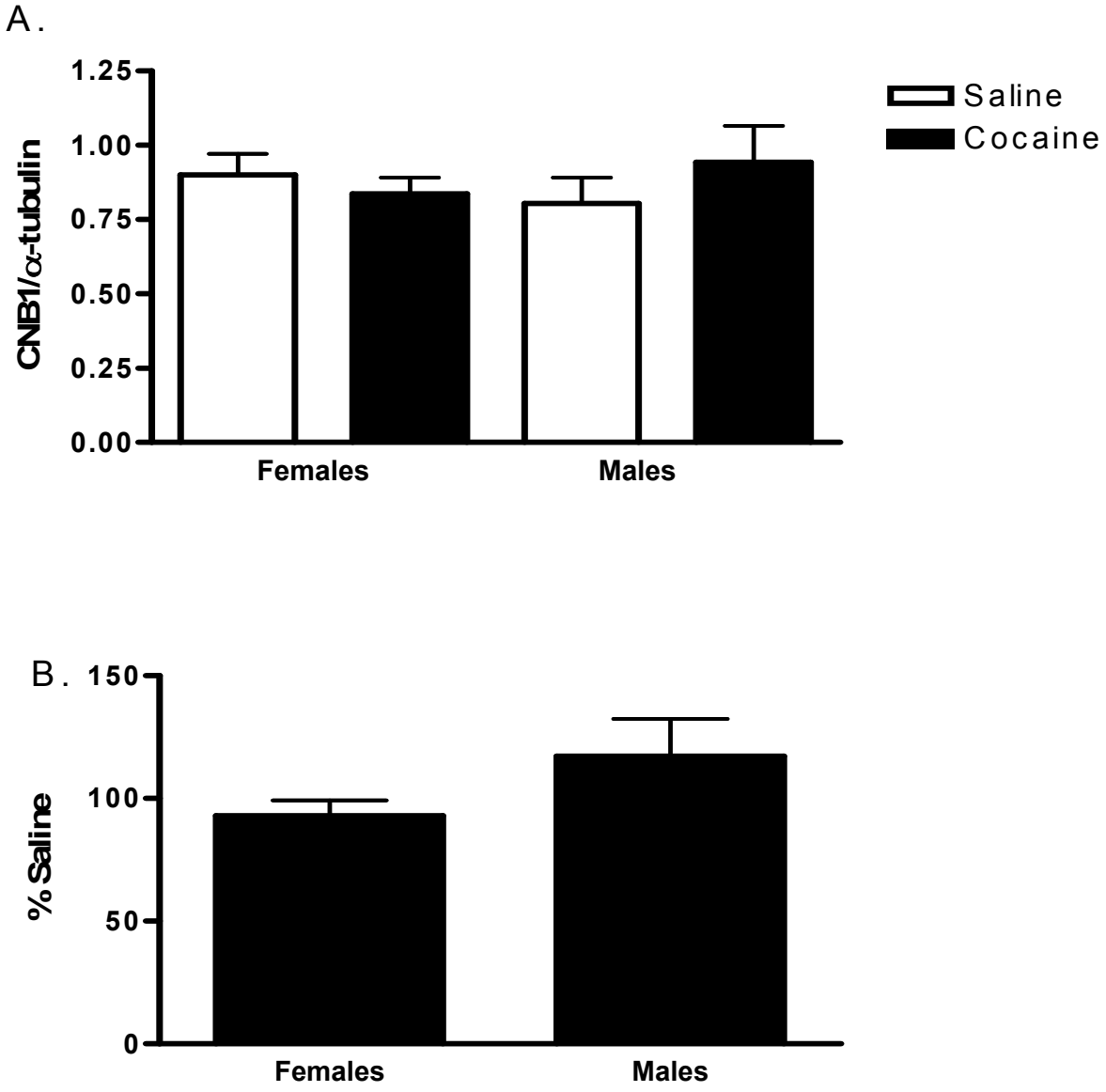


Figure 42: Males versus females in comparison before and after acute cocaine in the CPu, PP-2B (CNB1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4).

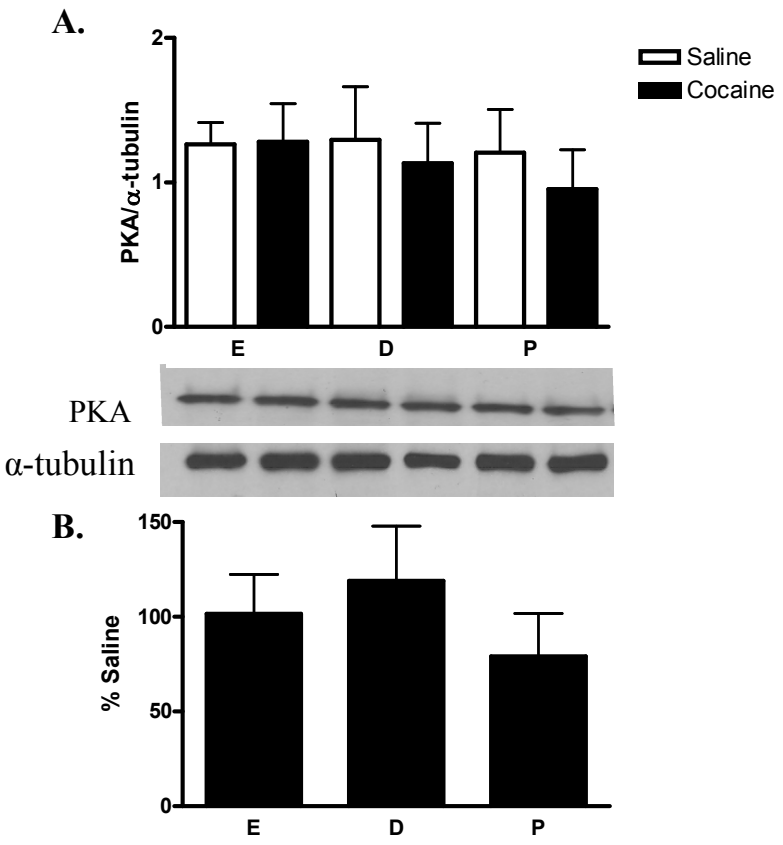


Figure 43: Estrous cycle differences in the NAc, PKA protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

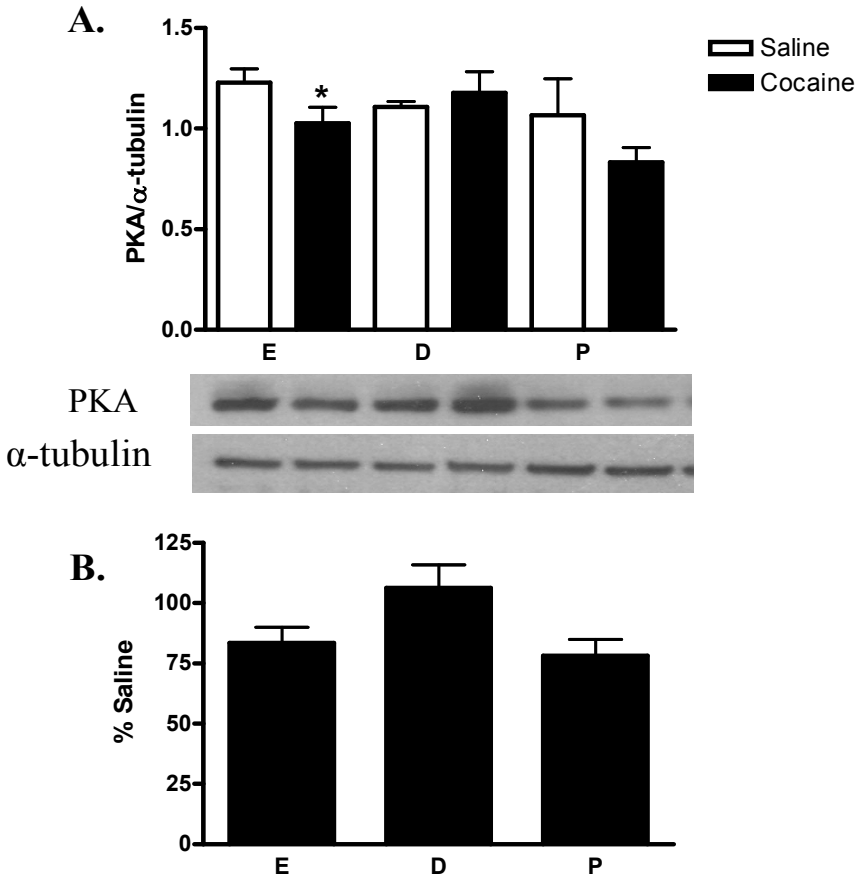


Figure 44: Estrous cycle differences in the NAc, PKA protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. * Represents statistically significant difference between saline and cocaine groups **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

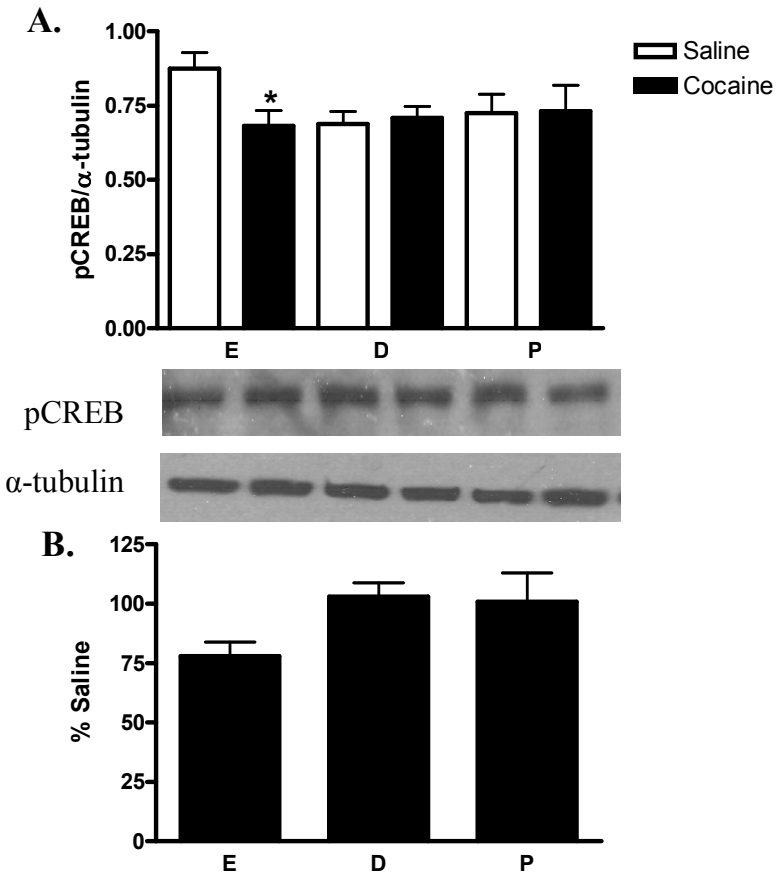


Figure 45: Estrous cycle differences in the NAc, pCREB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. * Represents significant difference between saline and cocaine groups. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

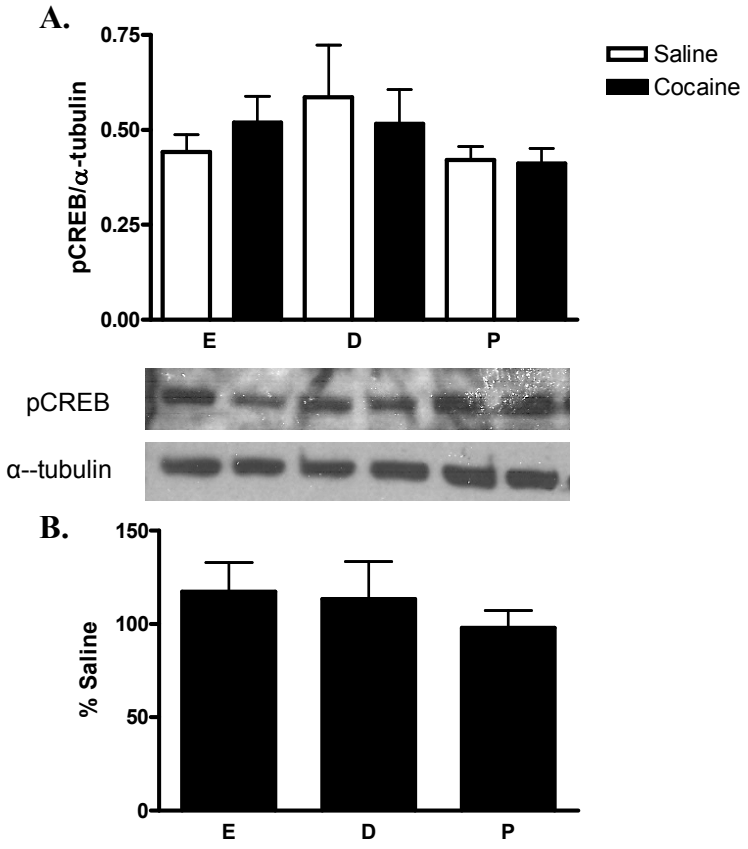


Figure 46: Estrous cycle differences in the NAc, pCREB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

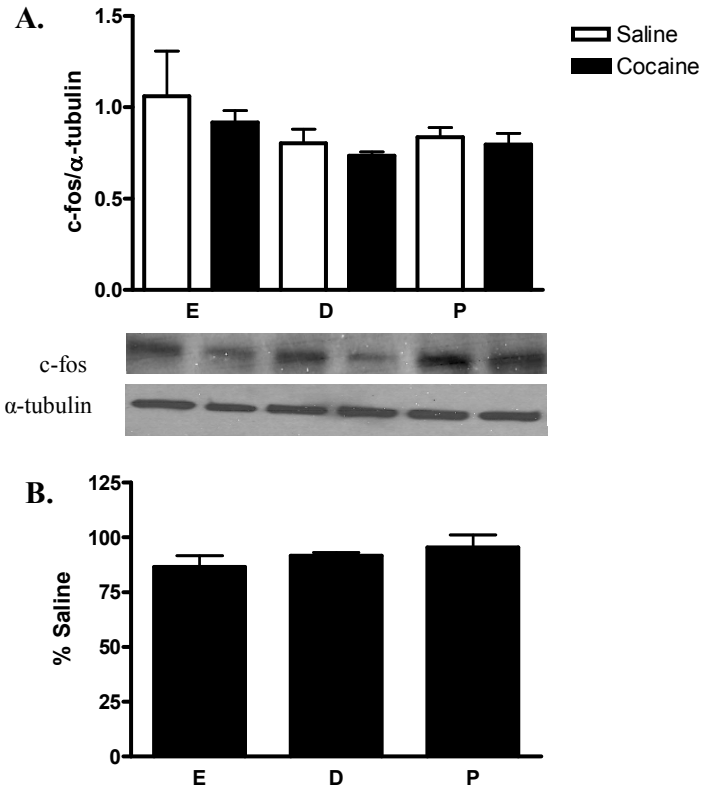


Figure 47: Estrous cycle differences in the NAc, *c-fos* protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

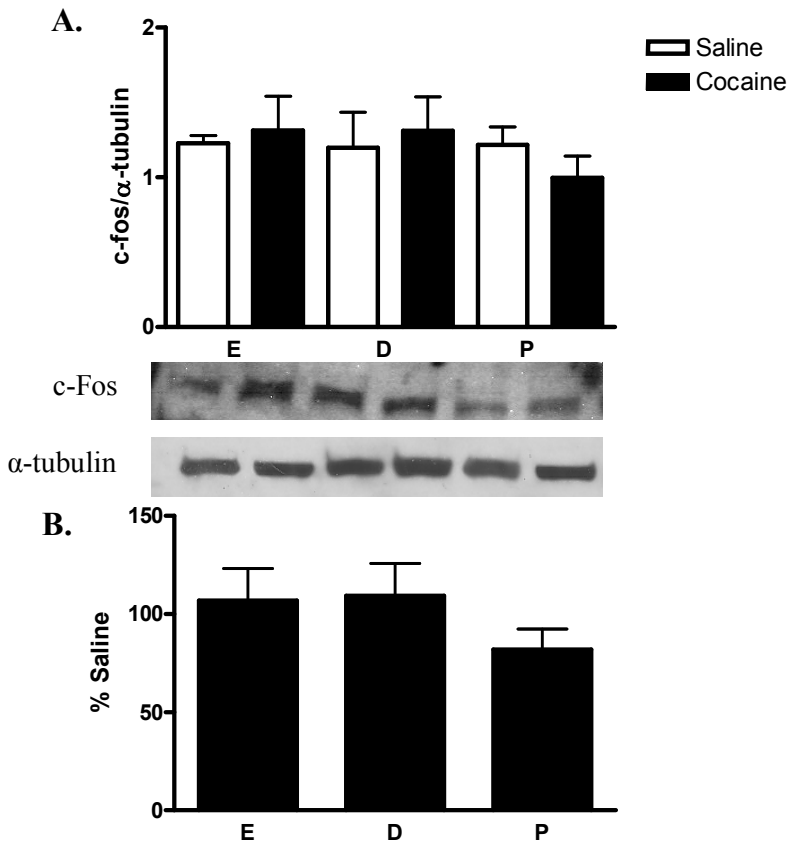


Figure 48: Estrous cycle differences in the NAc, *c-fos* protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

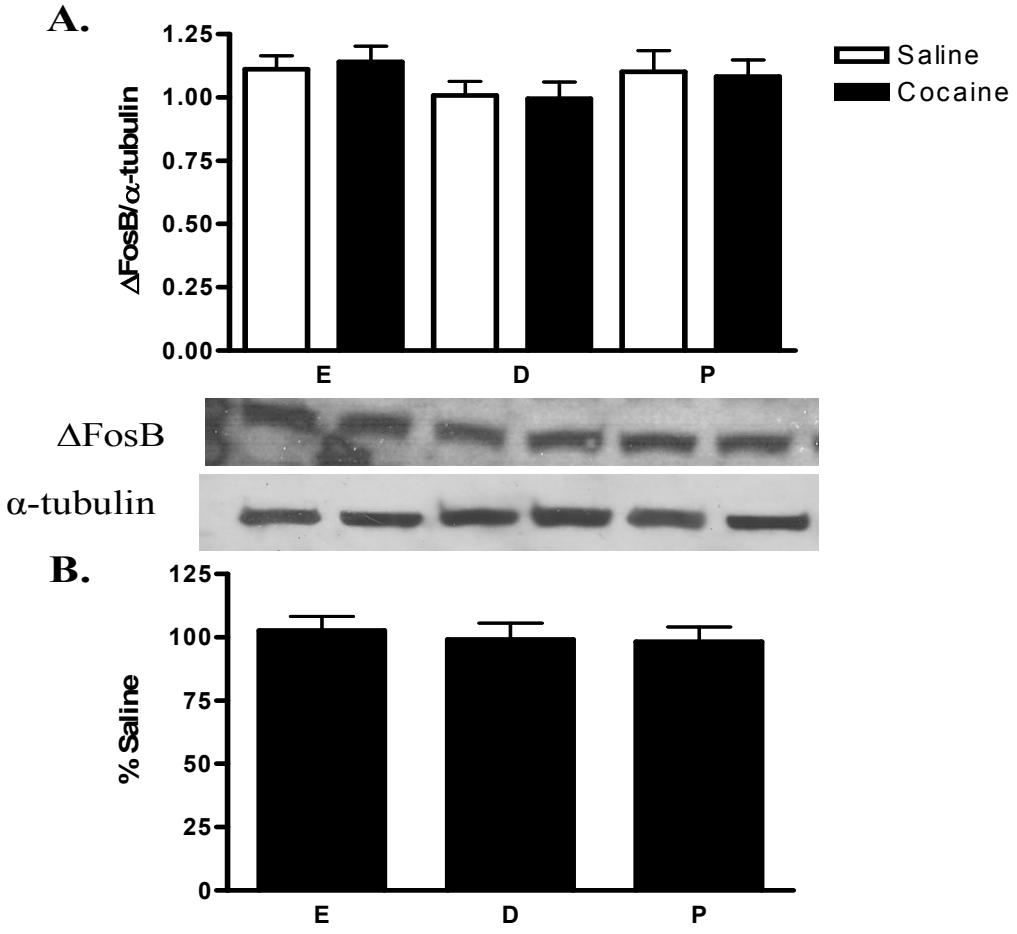


Figure 49: Estrous cycle differences in the NAc, ΔFosB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

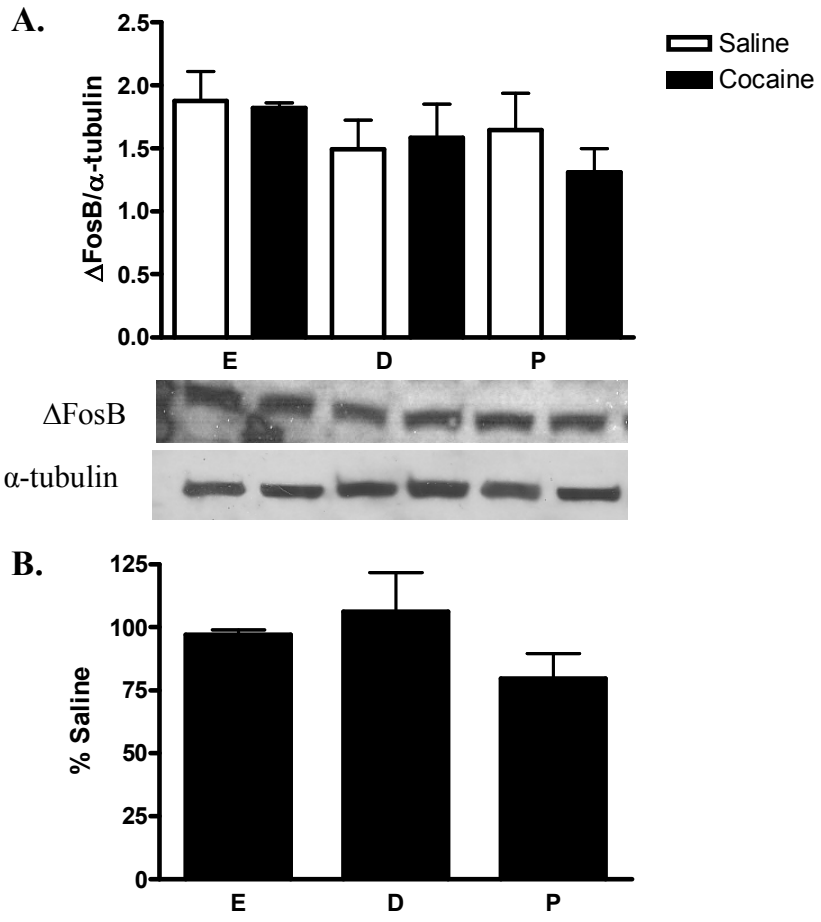


Figure 50: Estrous cycle differences in the NAc, ΔFosB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

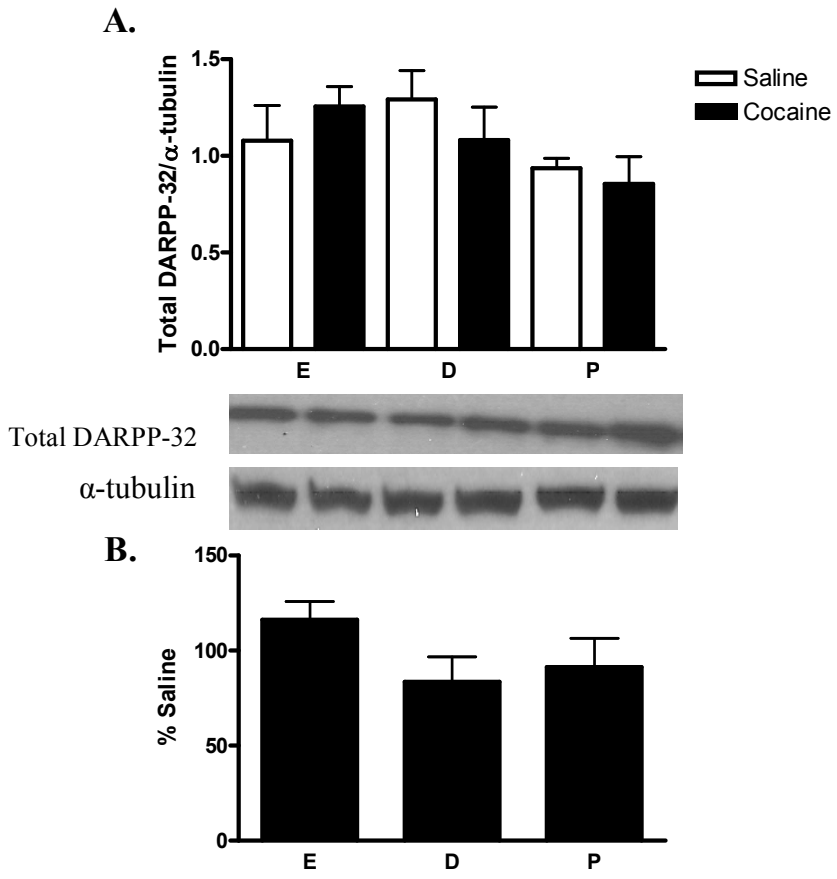


Figure 51: Estrous cycle differences in the NAc, Total DARPP-32 protein levels 15 min after saline (white bars) or protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

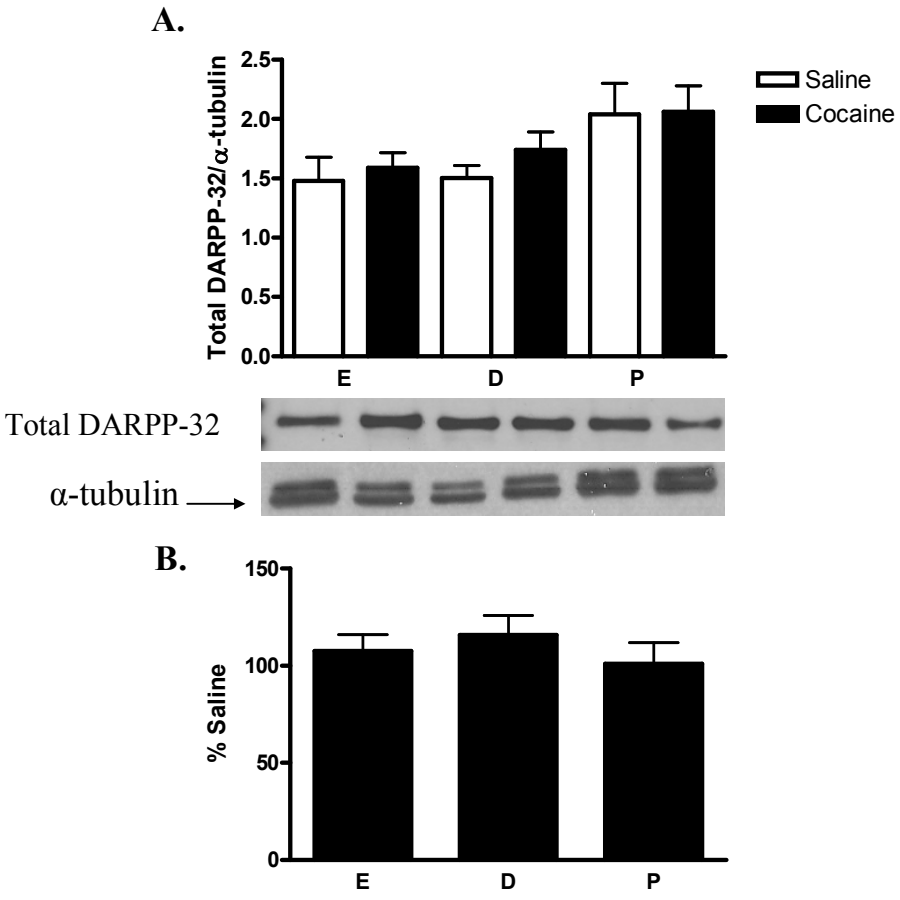


Figure 52: Estrous cycle differences in the NAc, Total DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

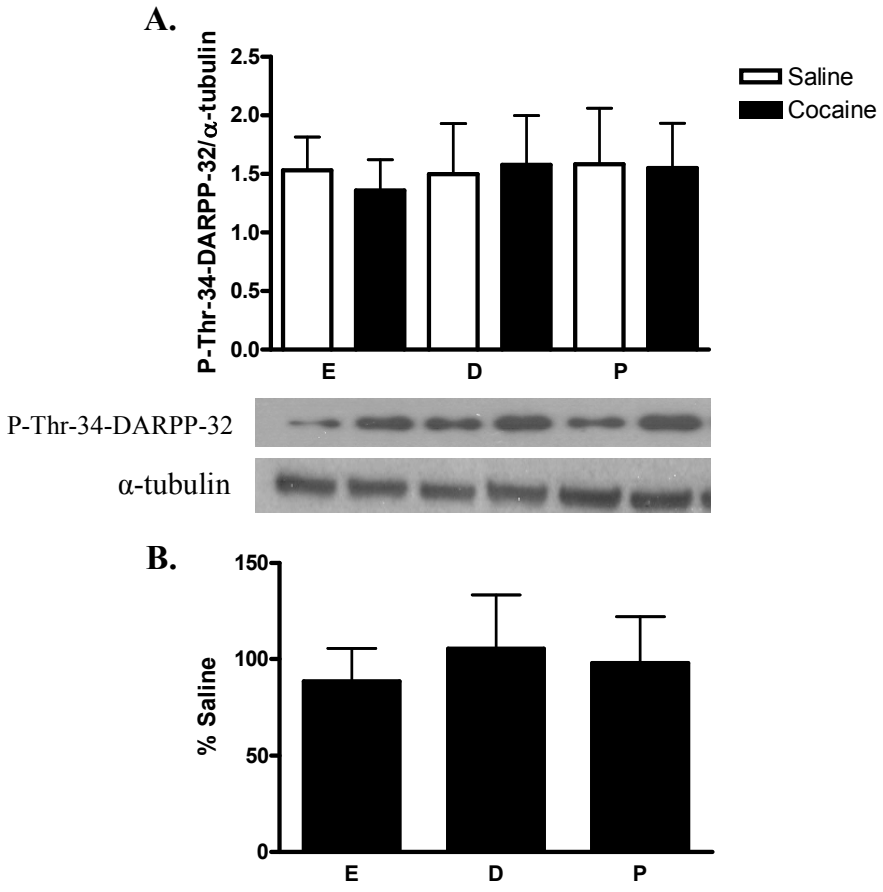


Figure 53: Estrous cycle differences in the NAc, P-Thr-34-DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

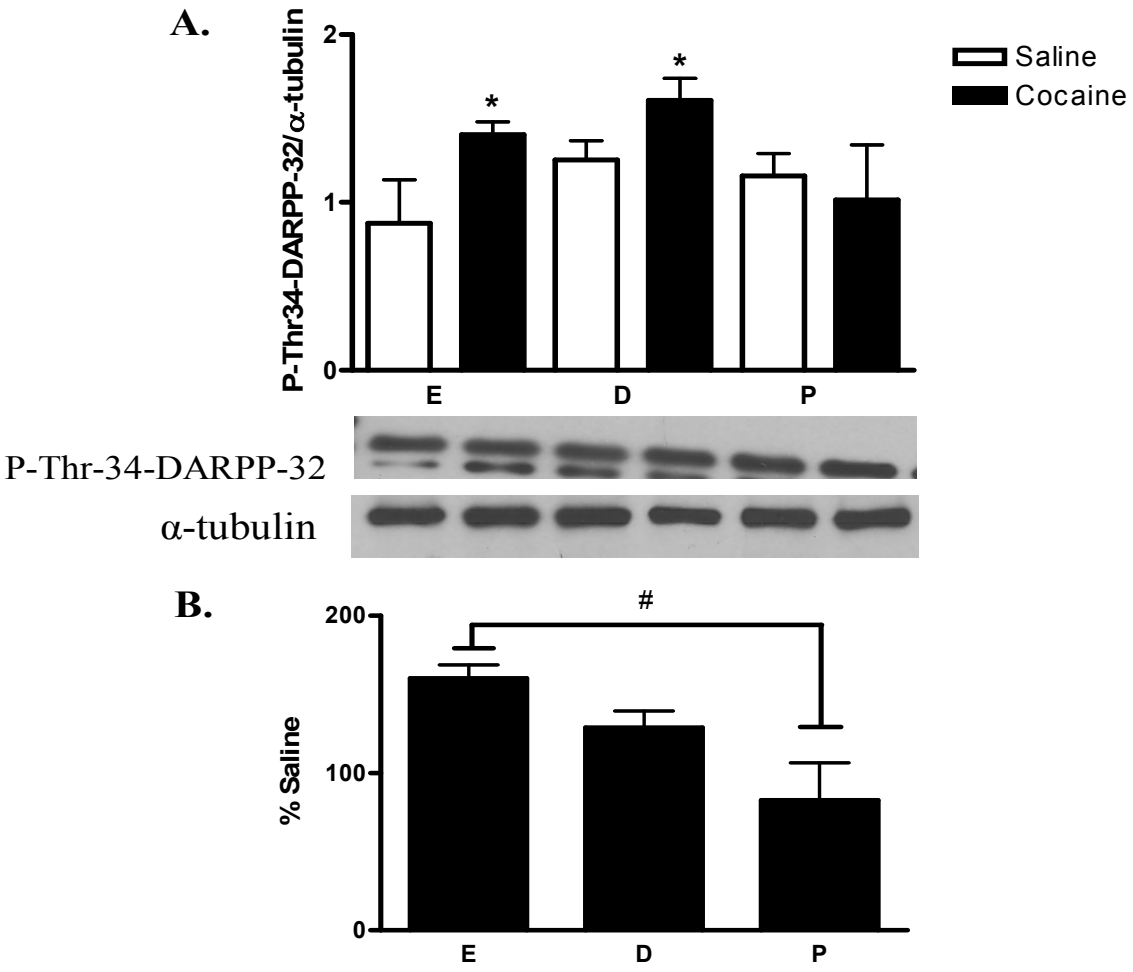


Figure 54: Estrous cycle differences in the NAc, P-Thr-34-DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels.* Represents significant difference between saline and cocaine groups. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). # Represents significant main effect; one-way ANOVA. E=Estrus, D=Diestrus, P=Proestrus.

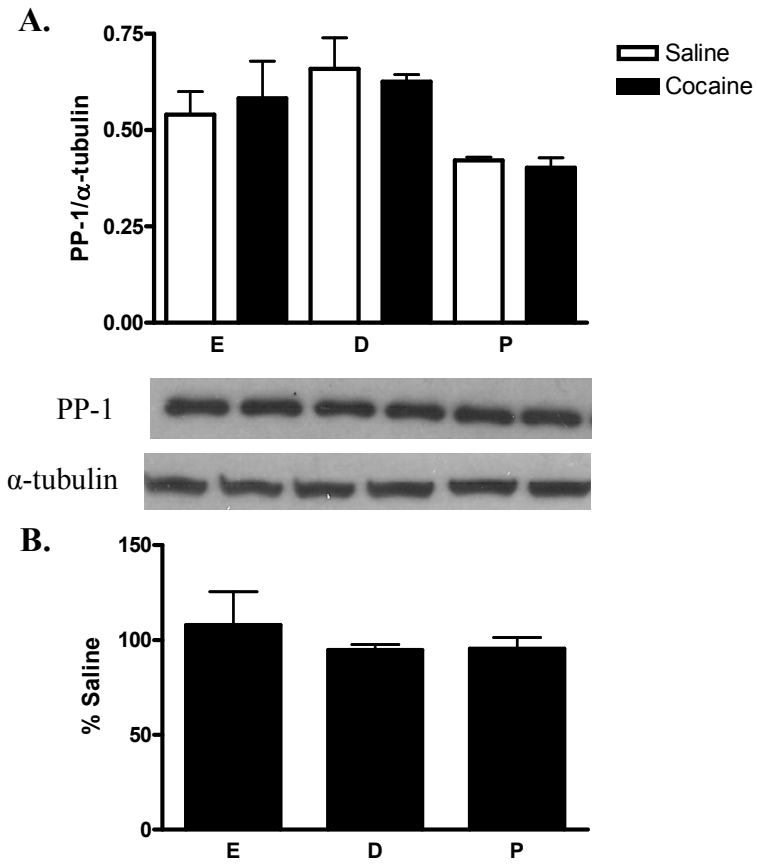


Figure 55: Estrous cycle differences in the NAc, PP-1 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

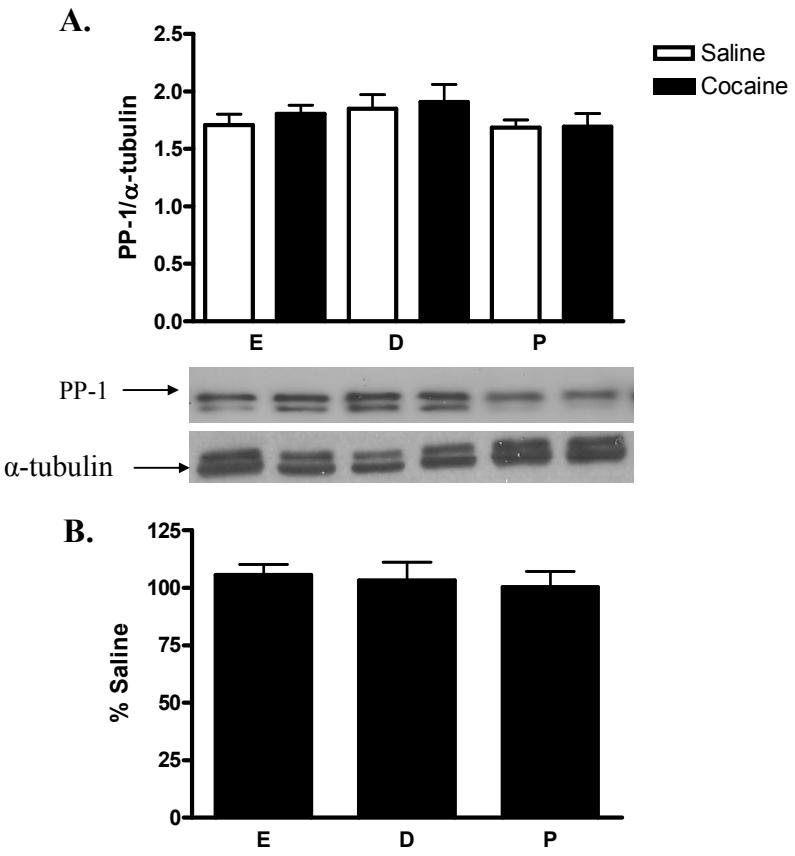


Figure 56: Estrous cycle differences in the NAc, PP-1 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

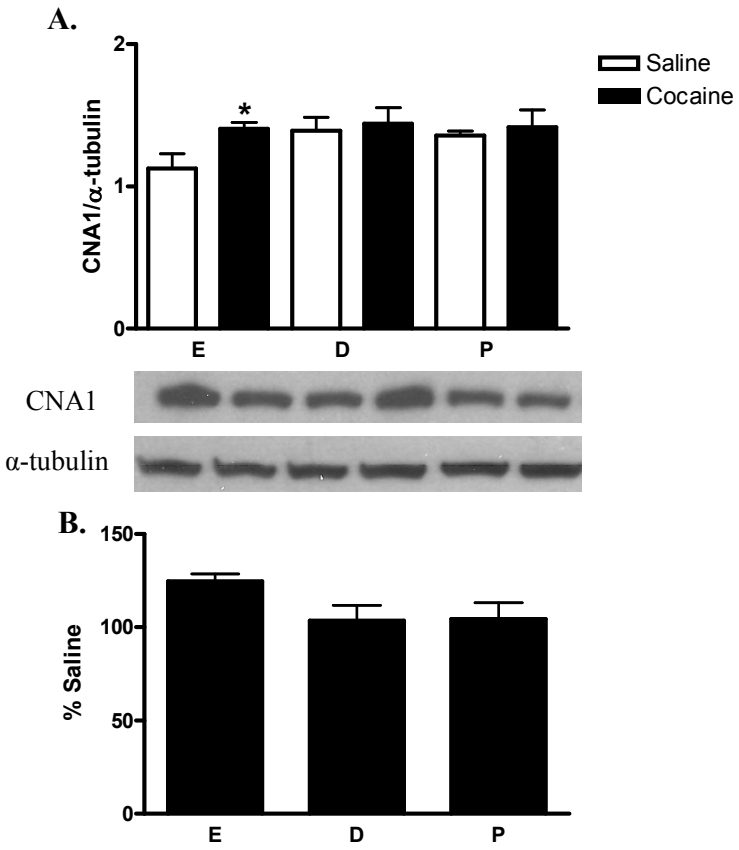


Figure 57: Estrous cycle differences in the NAc, PP-2B (CNA1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. * Represents significant difference between saline and cocaine groups. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus

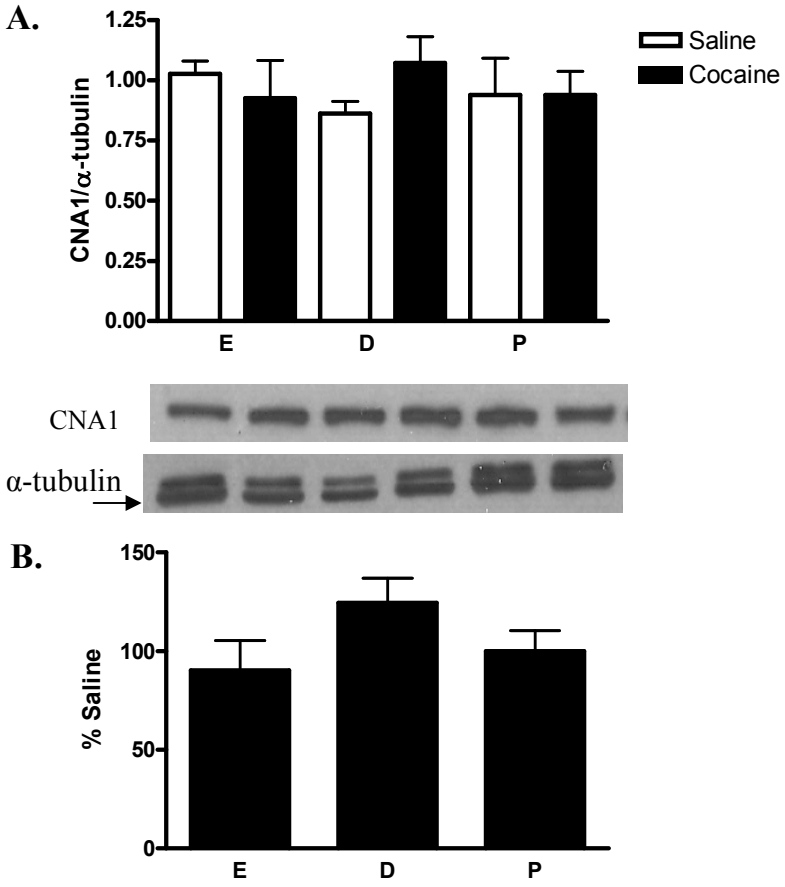


Figure 58: Estrous cycle differences in the NAc, PP-2B (CNA1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

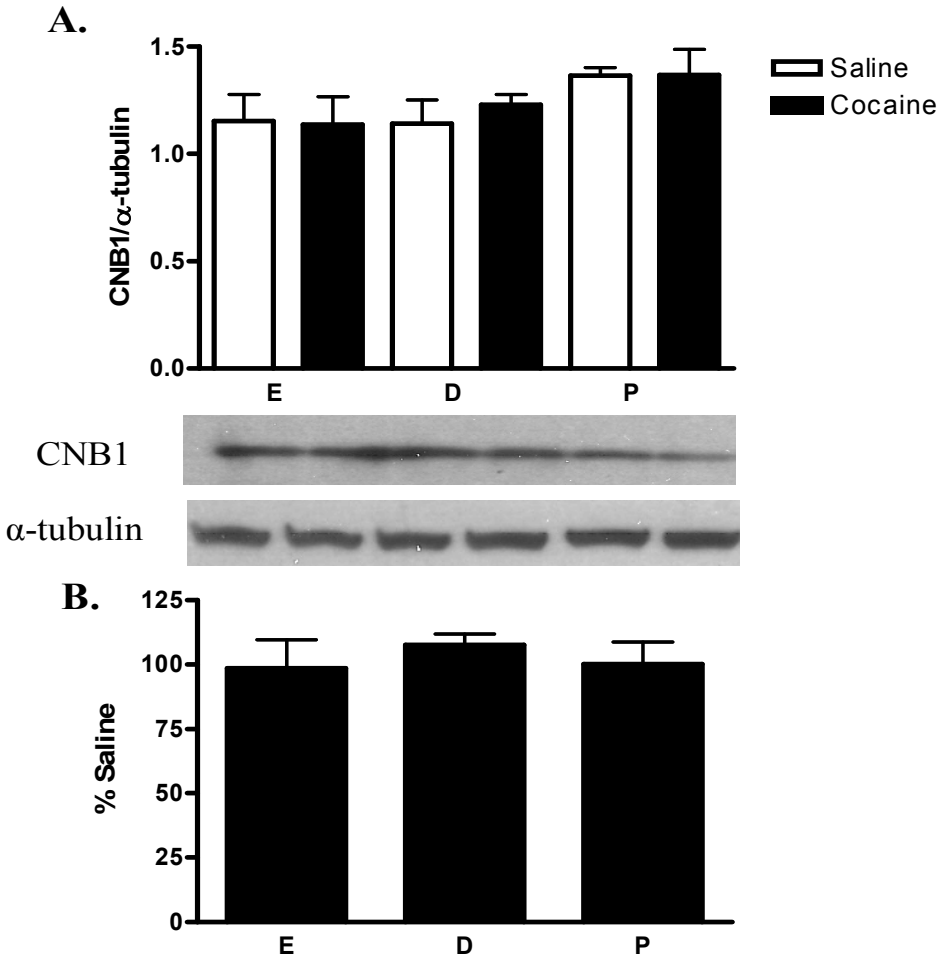


Figure 59: Estrous cycle differences in the NAc, PP-2B (CNB1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

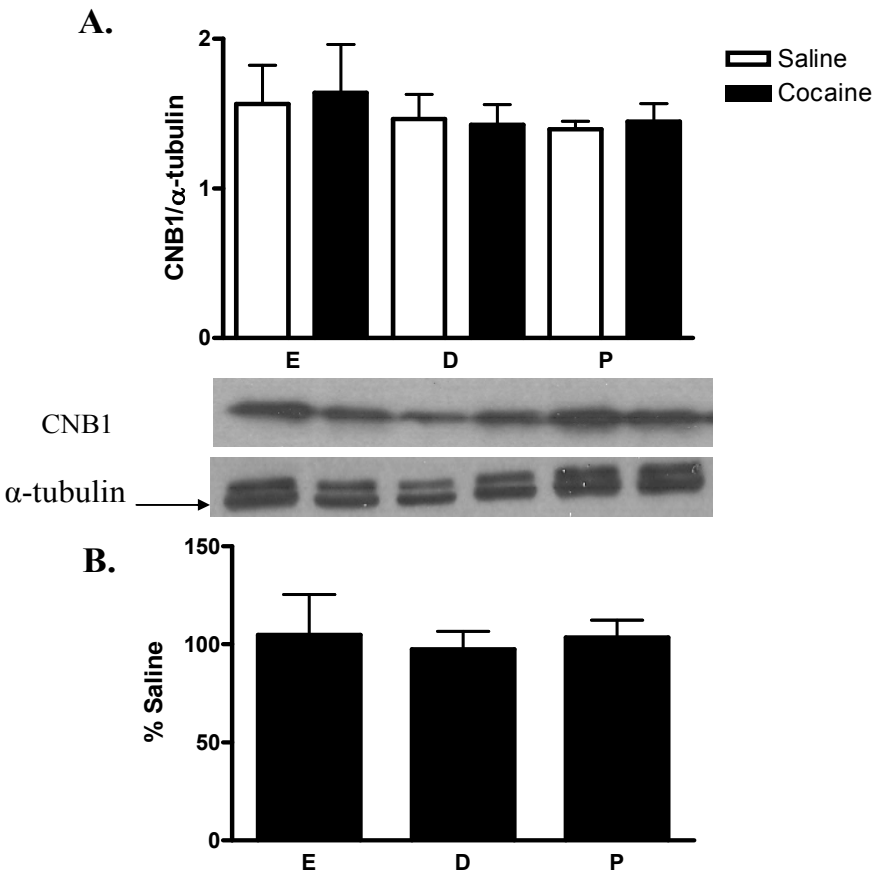


Figure 60: Estrous cycle differences in the NAc, PP-2B (CNB1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

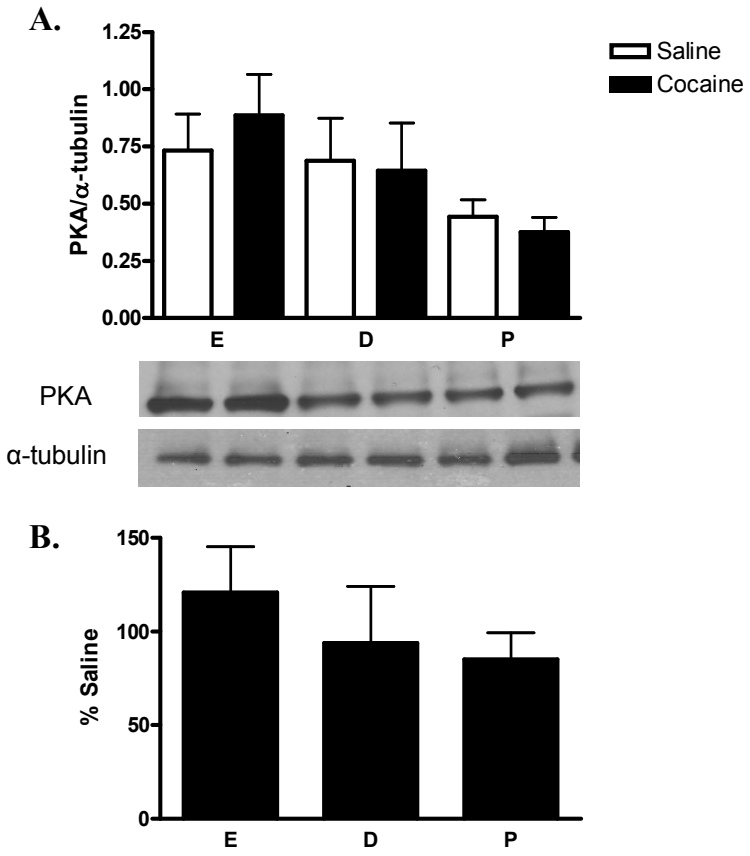


Figure 61: Estrous cycle differences in the CPu, PKA protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

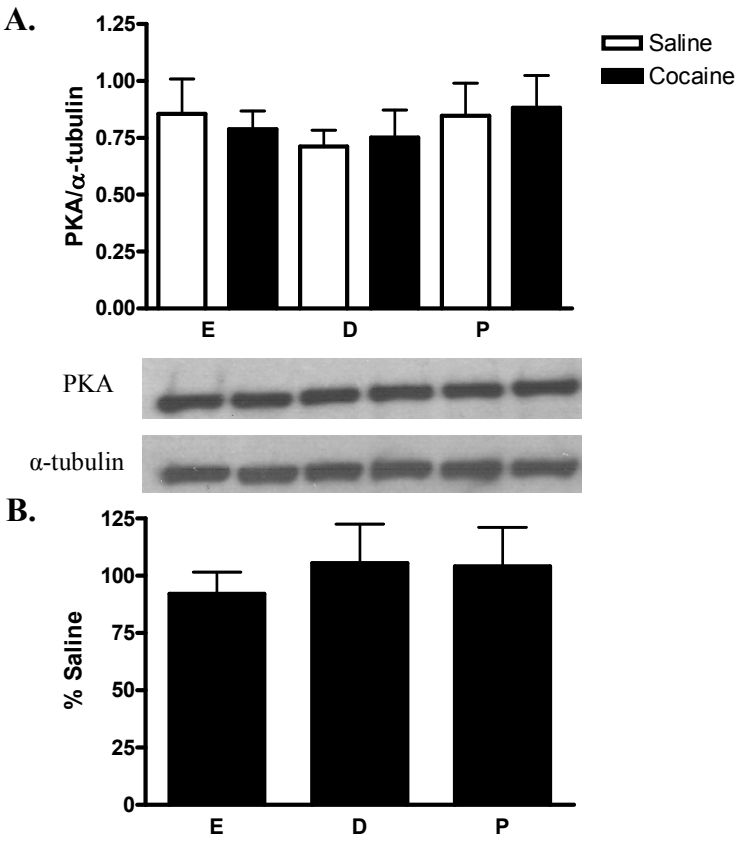


Figure 62: Estrous cycle differences in the CPu, PKA protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

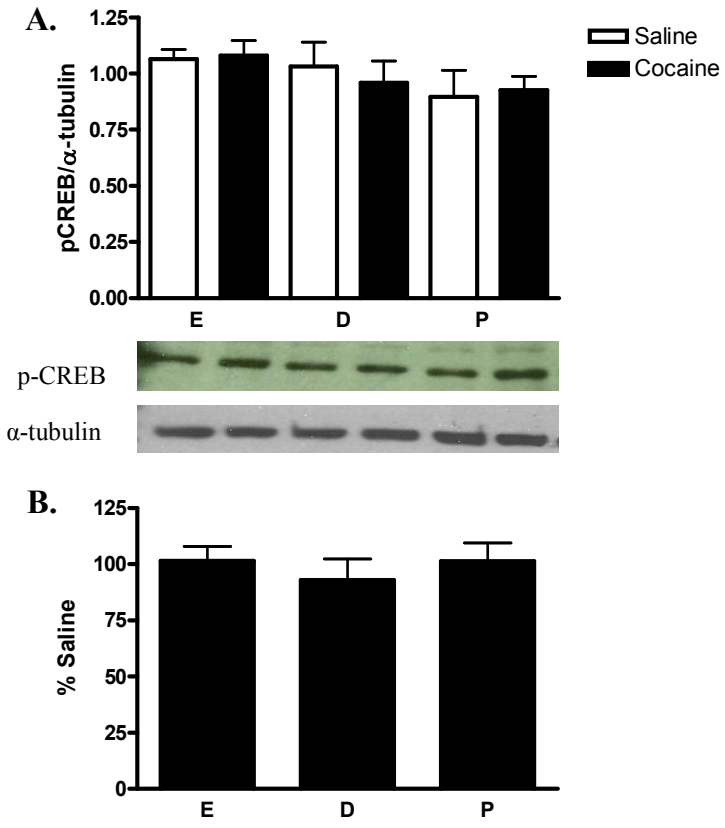


Figure 63: Estrous cycle differences in the CPu, pCREB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

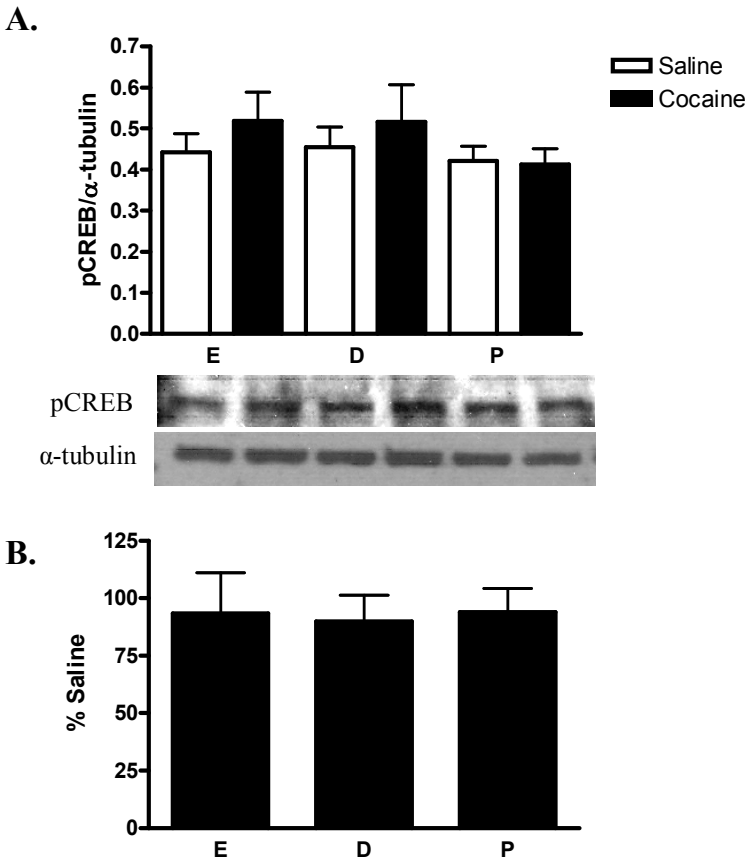


Figure 64: Estrous cycle differences in the CPu, pCREB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

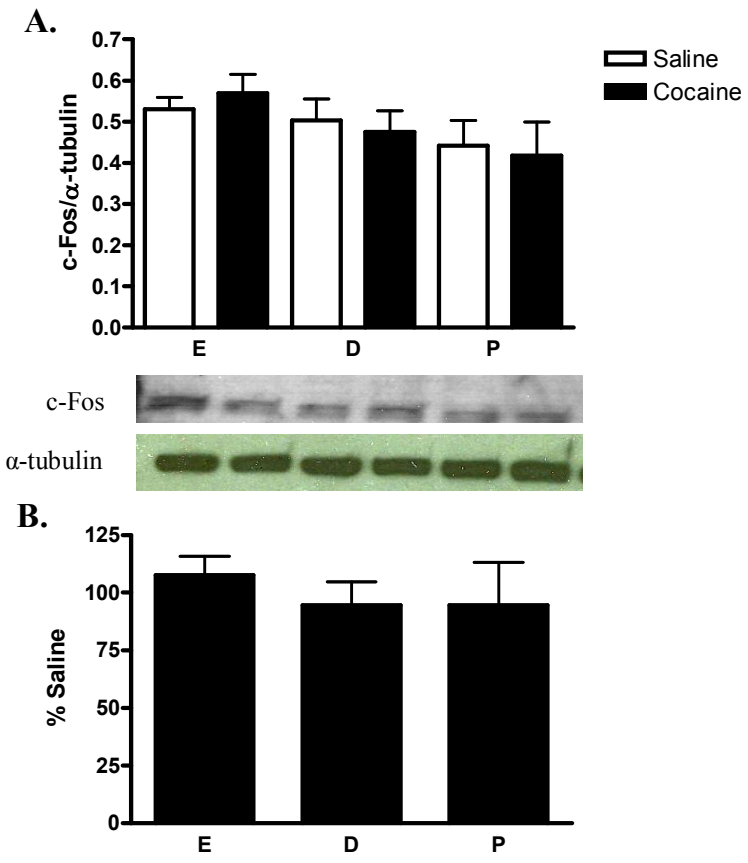


Figure 65: Estrous cycle differences in the CPu, *c-Fos* protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-Fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

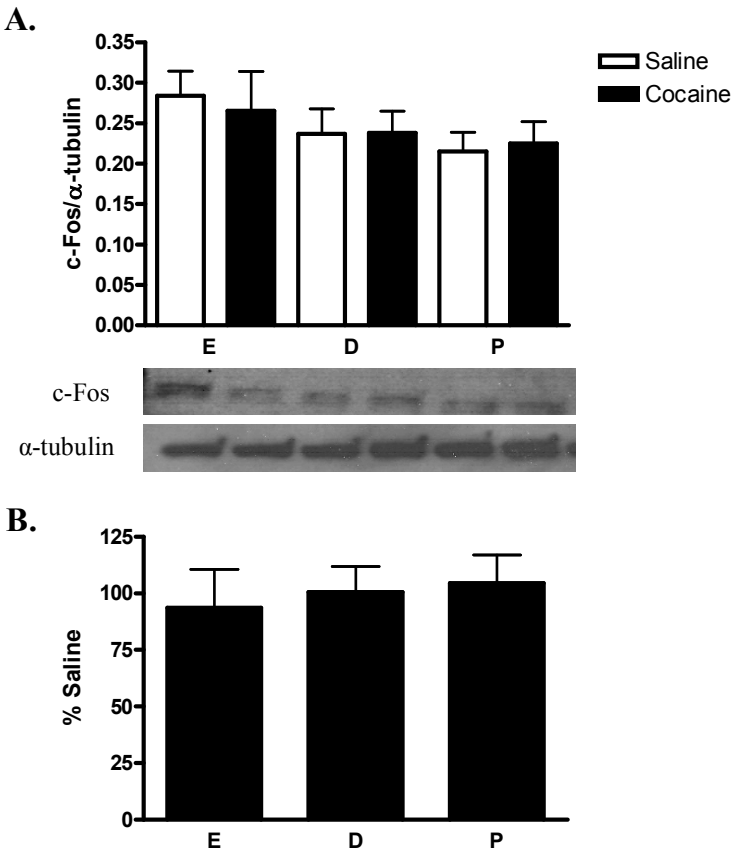


Figure 66: Estrous cycle differences in the CPu, *c-Fos* protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-Fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

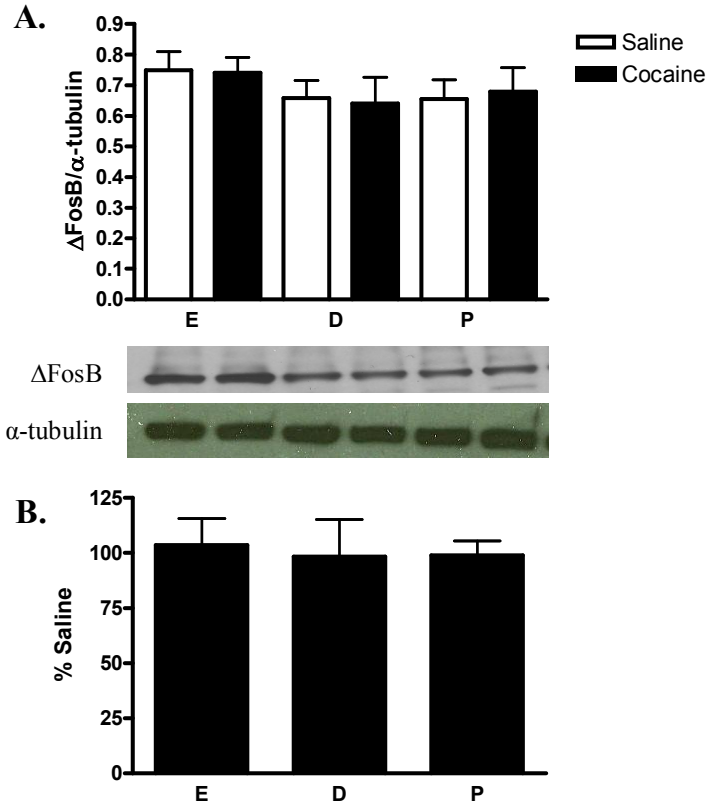


Figure 67: Estrous cycle differences in the CPu, ΔFosB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

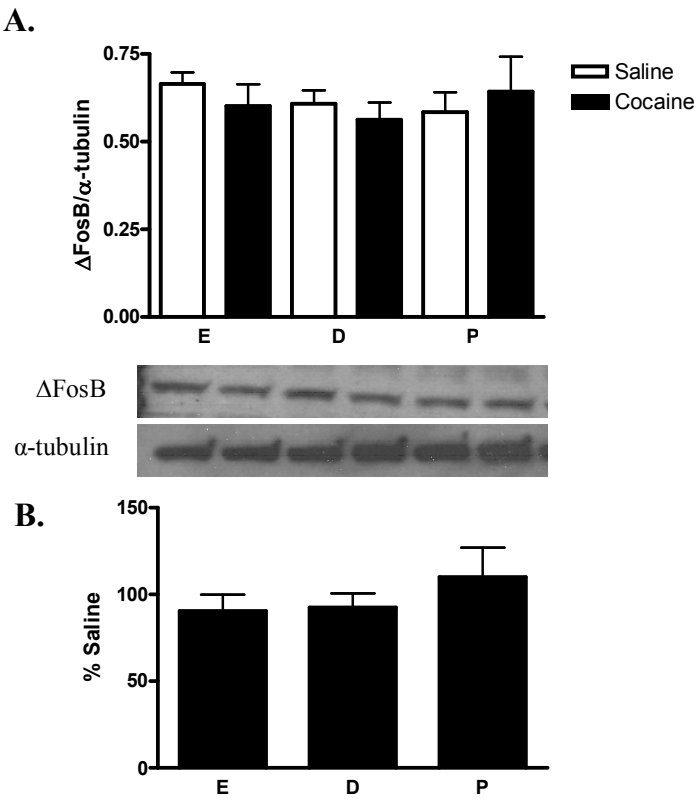


Figure 68: Estrous cycle differences in the CPu, ΔFosB protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus group, D=Diestrus group, P=Proestrus group.

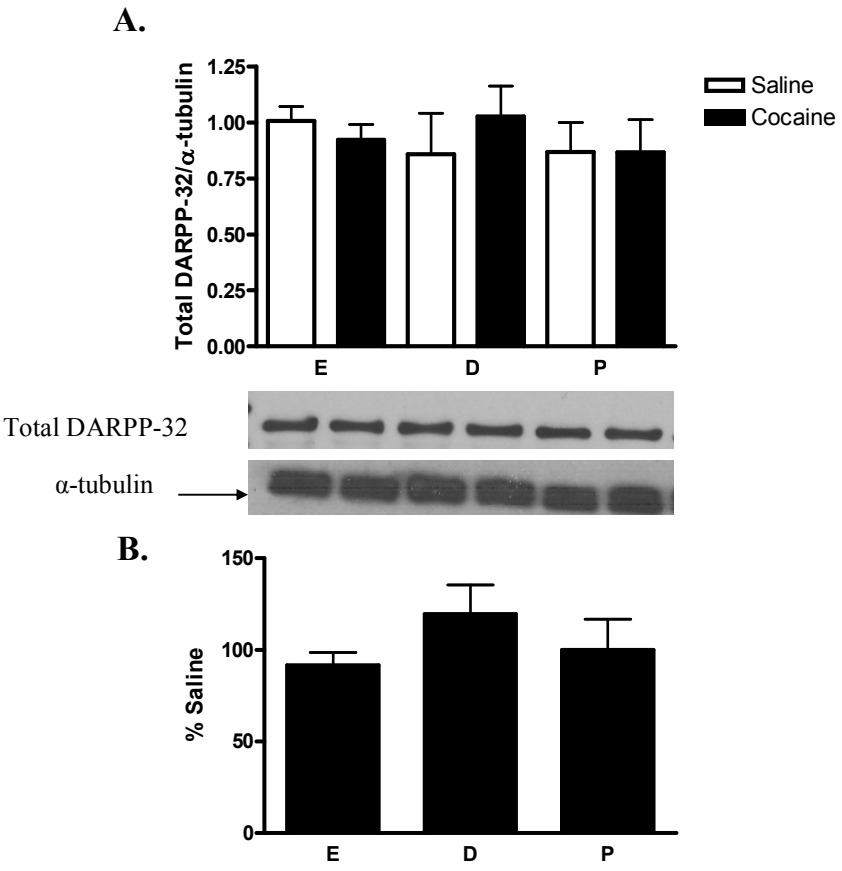


Figure 69: Estrous cycle differences in the CPu, Total DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

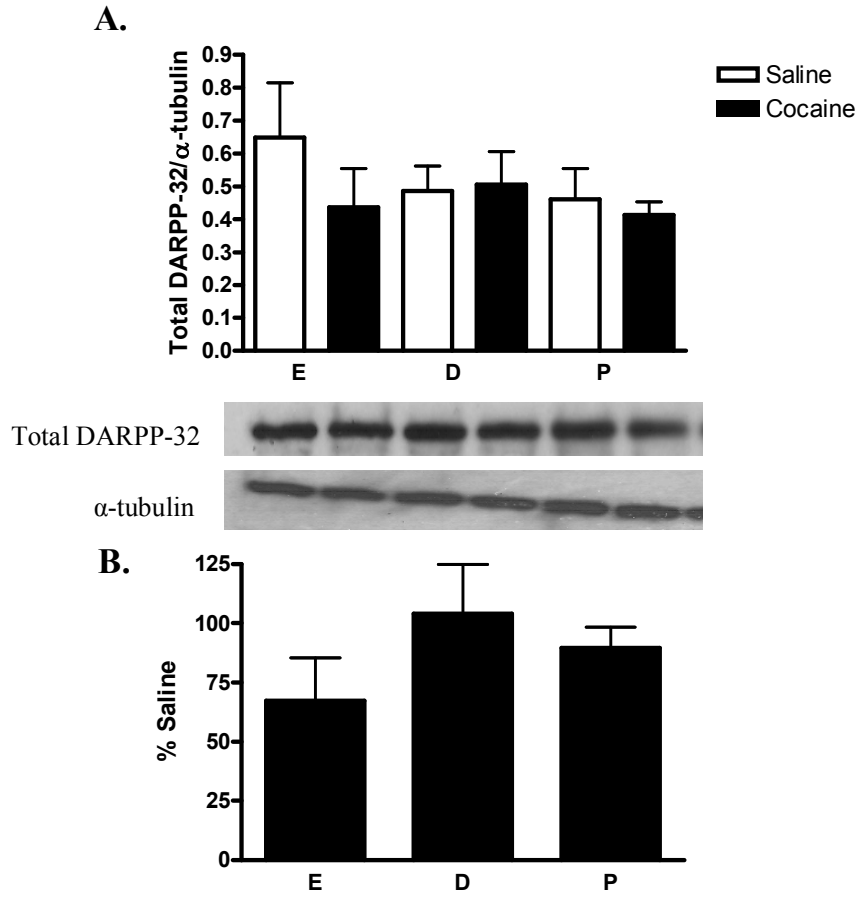


Figure 70: Estrous cycle differences in the CPu, Total DARPP-32 protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

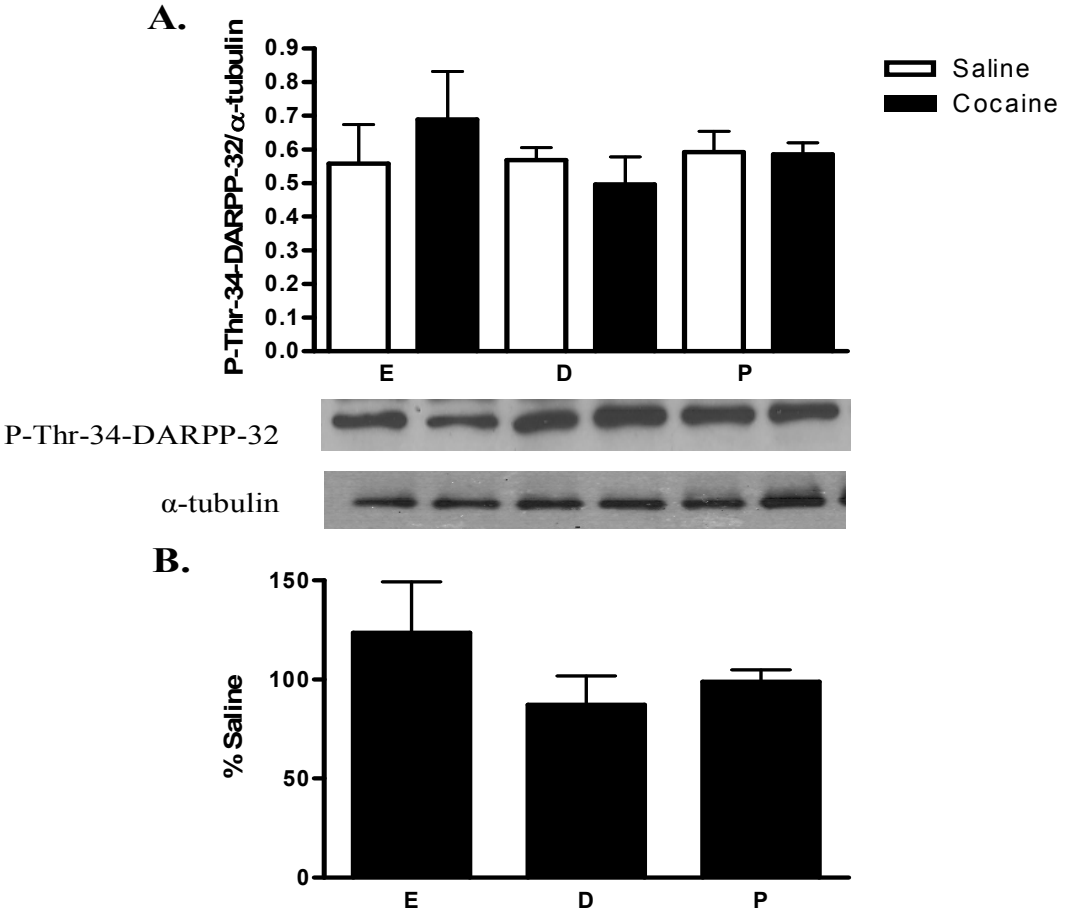


Figure 71: Estrous cycle differences in the CPu, P-Thr-34-DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

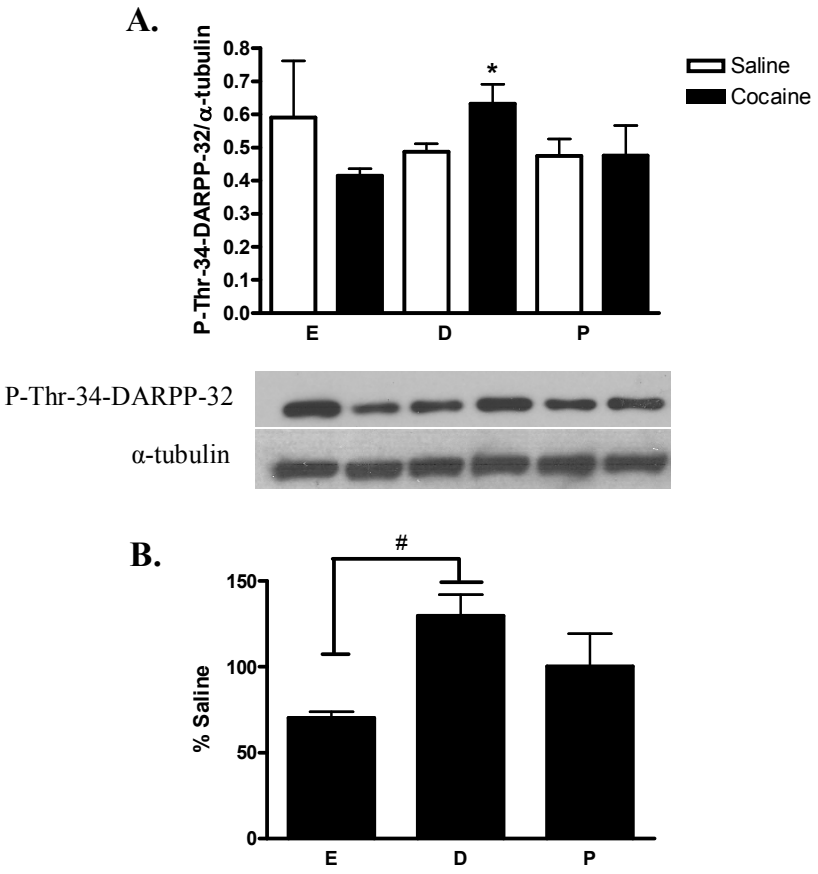


Figure 72: Estrous cycle differences in the CPu, P-Thr-34-DARPP-32 protein levels 1h min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. * Represents significant difference between saline and cocaine groups. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). # Represents significant main effect; one-way ANOVA. E=Estrus, D=Diestrus, P=Proestrus.

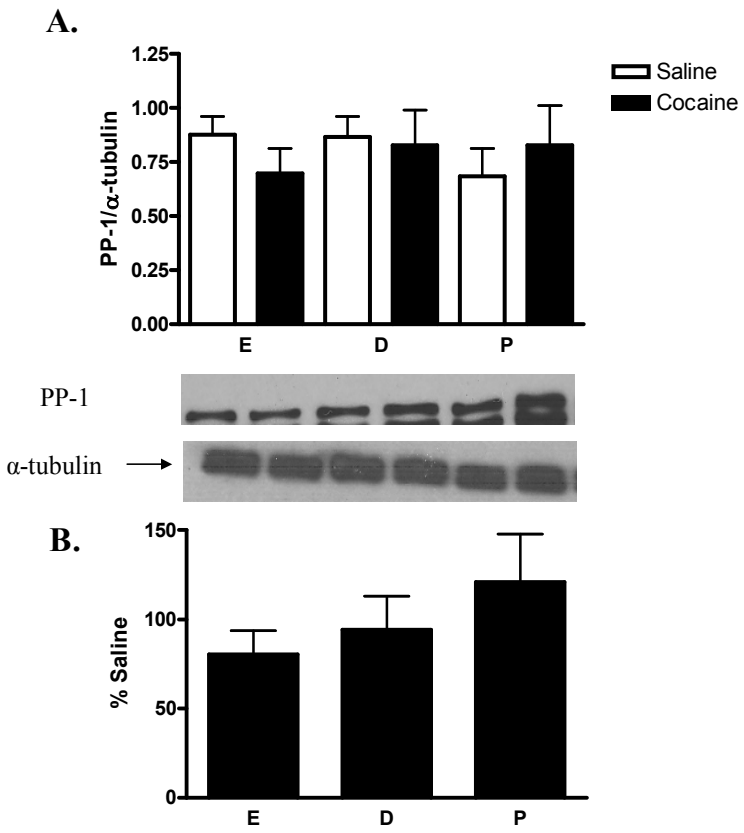


Figure 73: Estrous cycle differences in the CPu, PP-1protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

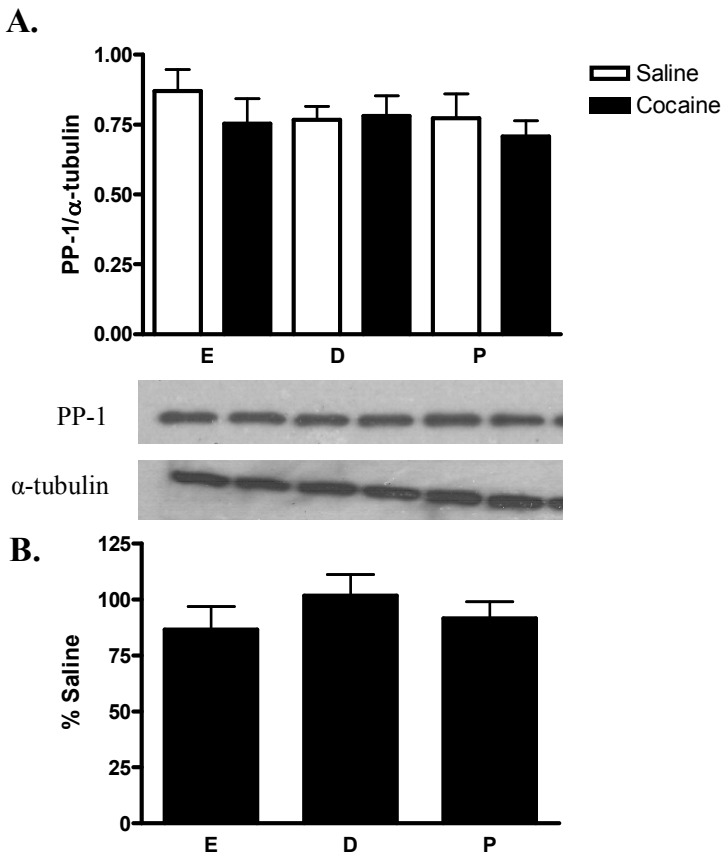


Figure 74: Estrous cycle differences in the CPu, PP-1protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

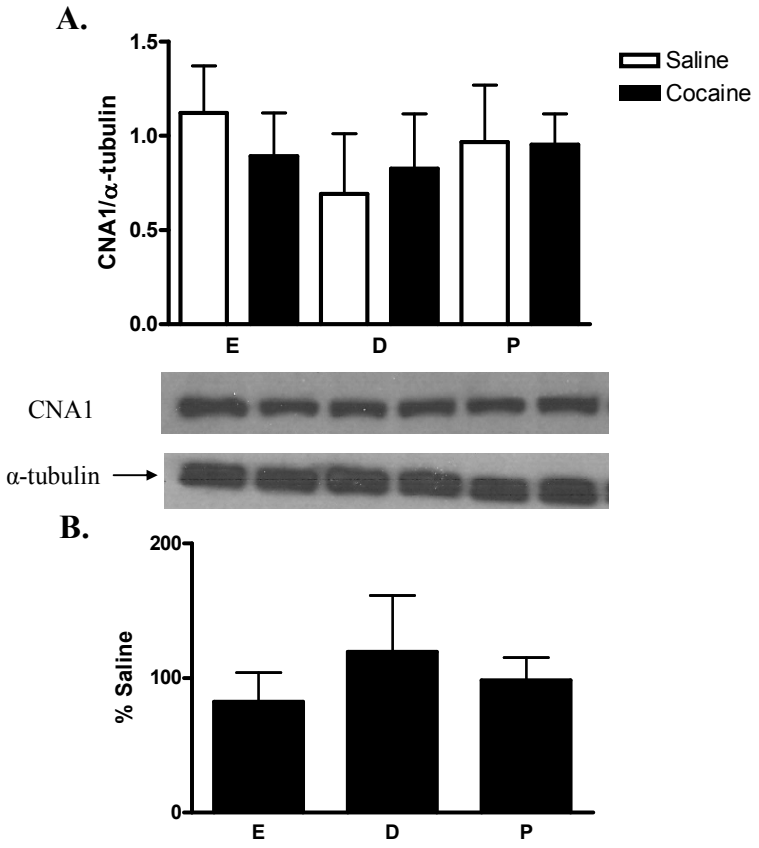


Figure 75: Estrous cycle differences in the CPu, PP-2B (CNA1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

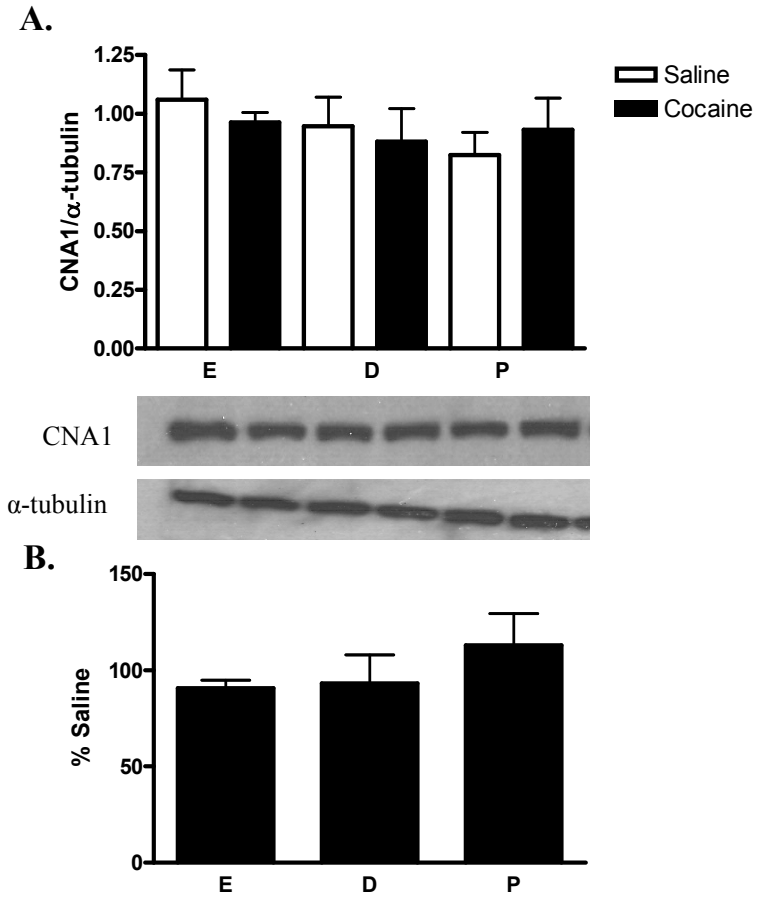


Figure 76: Estrous cycle differences in the CPu, PP-2B (CNA1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

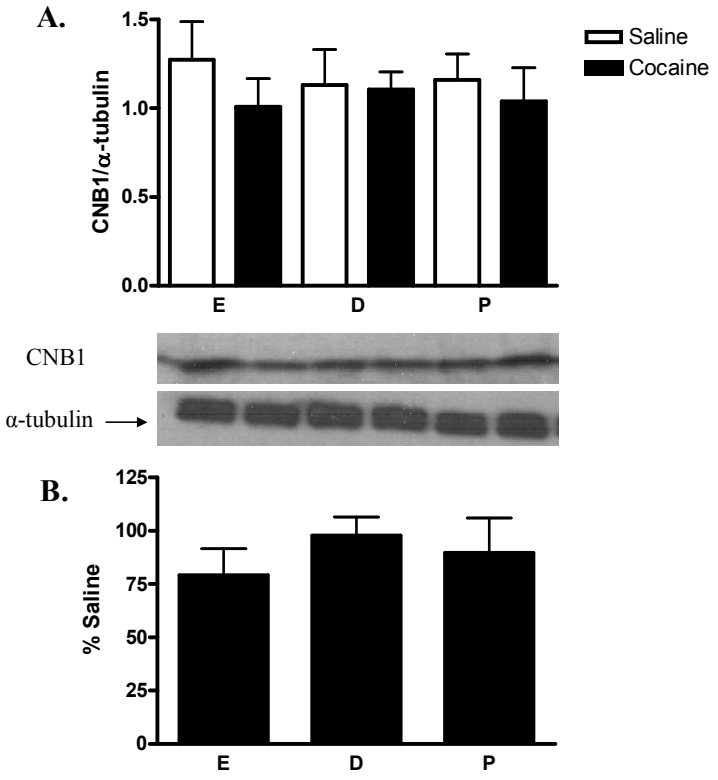


Figure 77: Estrous cycle differences in the CPu, PP-2B (CNB1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

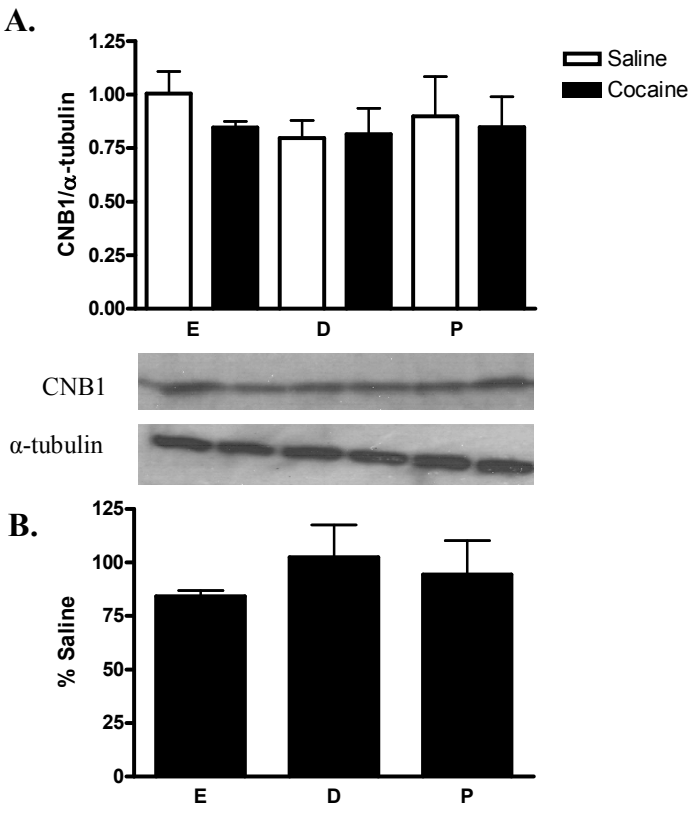


Figure 78: Estrous cycle differences in the CPu, PP-2B (CNB1) protein levels 1h after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). E=Estrus, D=Diestrus, P=Proestrus.

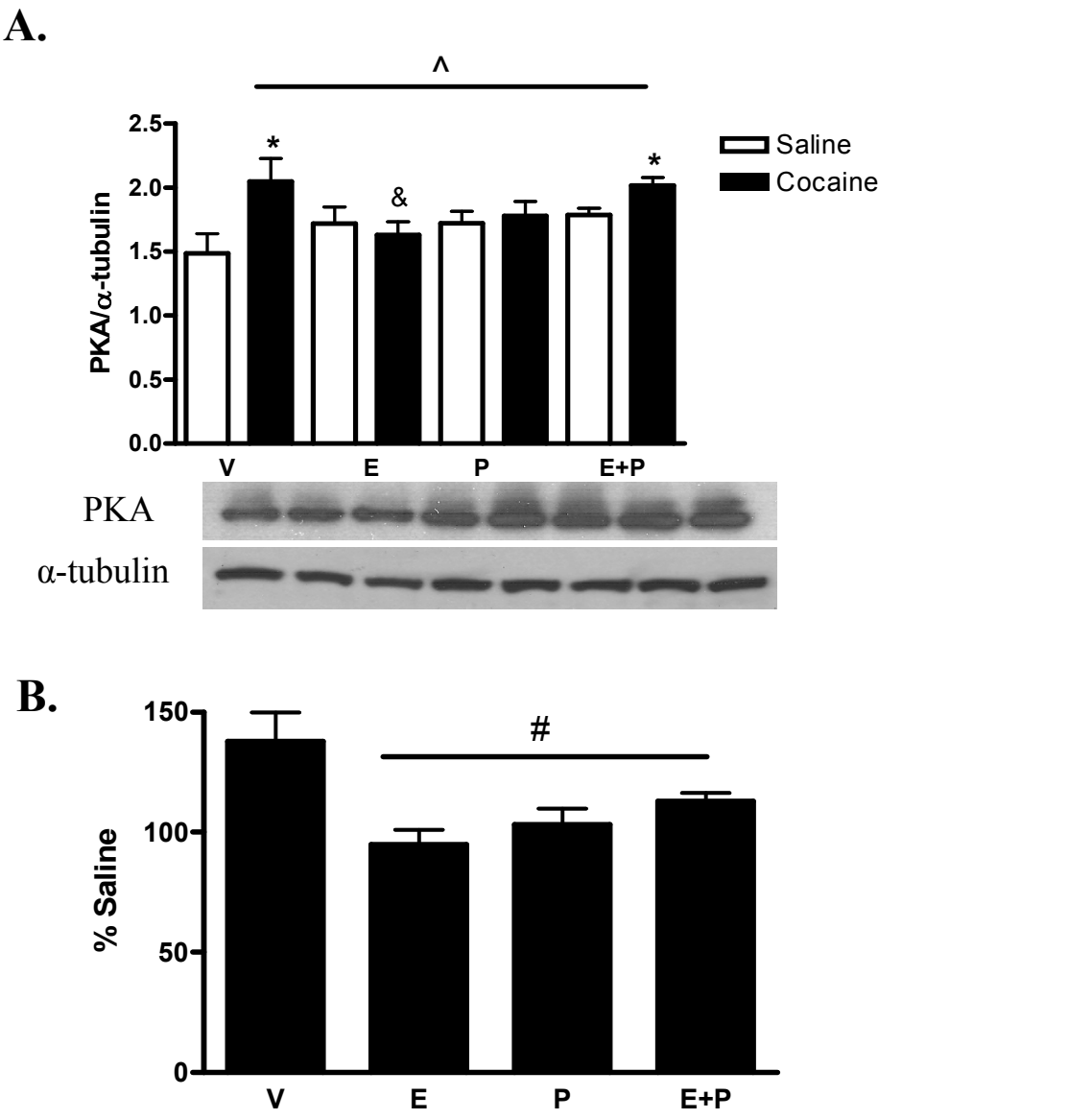


Figure 79: OVX vs hormone replacement differences in the NAc, PKA protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels.* Represents significant difference between saline and cocaine groups. ^ Represents significant drug effect; two-way ANOVA. & Represents estrus compared to vehicle- and E+P-treated cocaine groups **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). # Represents significant main effect, one-way ANOVA. V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

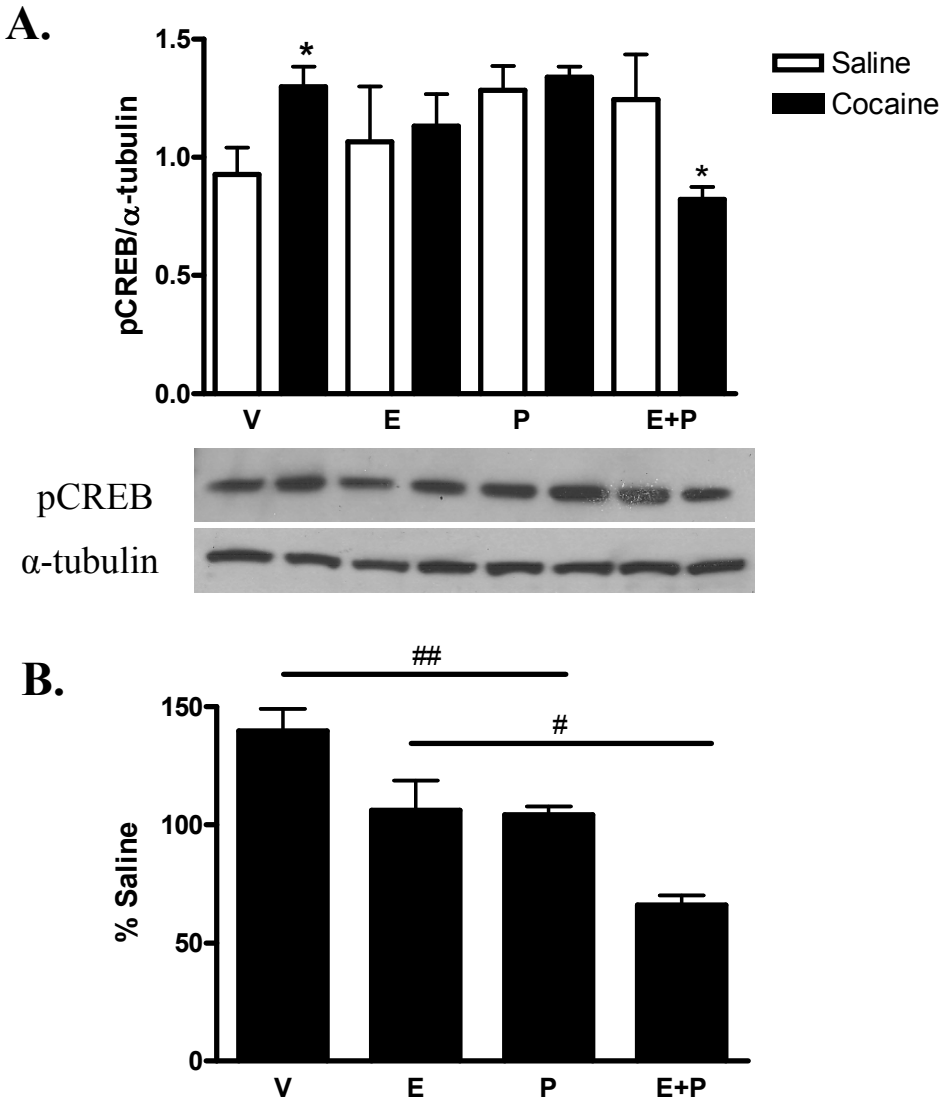


Figure 80: OVX vs hormone replacement differences in the NAc, pCREB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. * Represents significant difference between saline and cocaine groups. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). # Represents significant main effect between vehicle and hormone replacement groups, one-way ANOVA. ## Represents significant main effect E+P compared to other groups; one-way ANOVA. V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

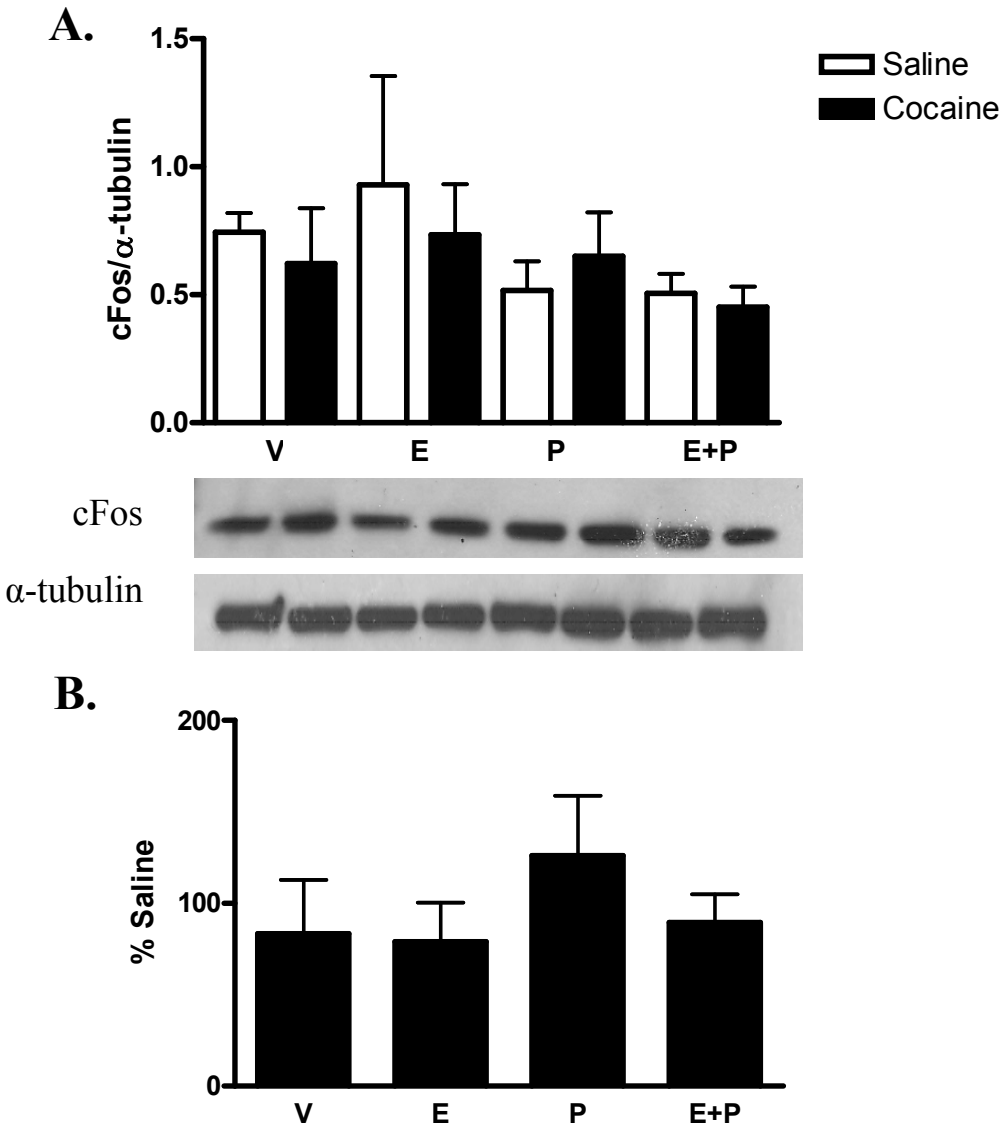


Figure 81: OVX vs hormone replacement differences in the NAc, *c-Fos* protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-Fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

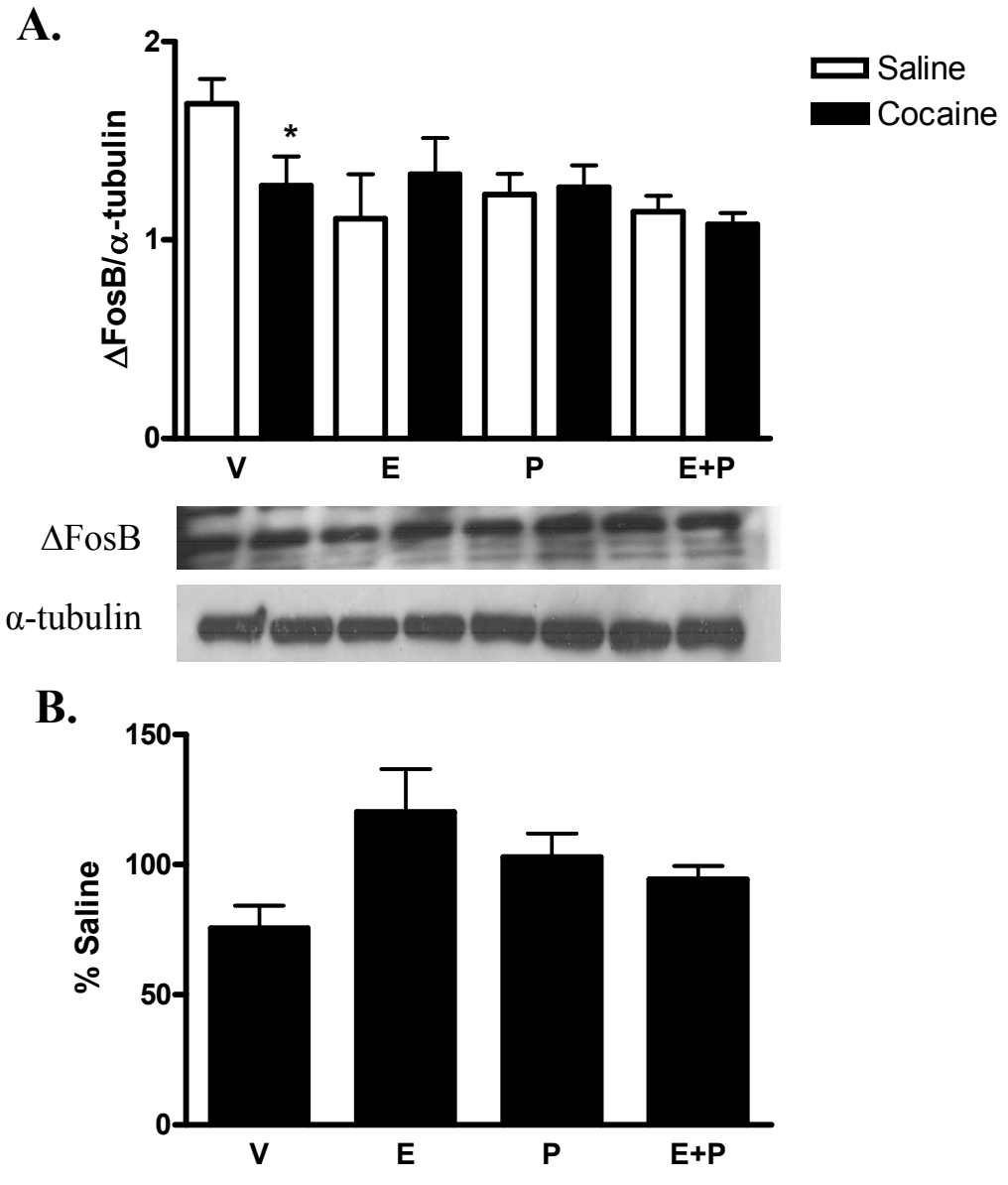


Figure 82: OVX vs hormone replacement differences in the NAc, ΔFosB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

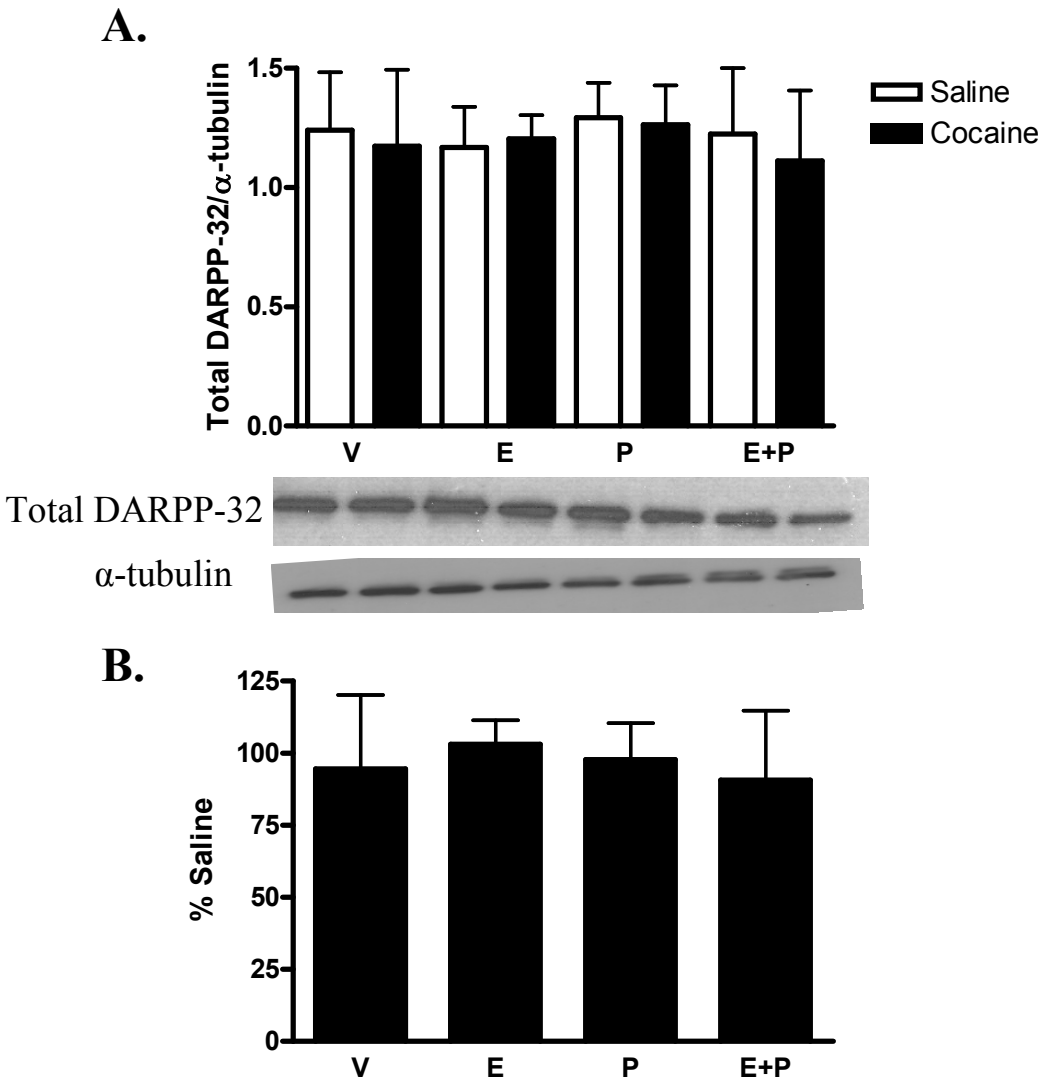


Figure 83: OVX vs hormone replacement differences in the NAc, Total DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

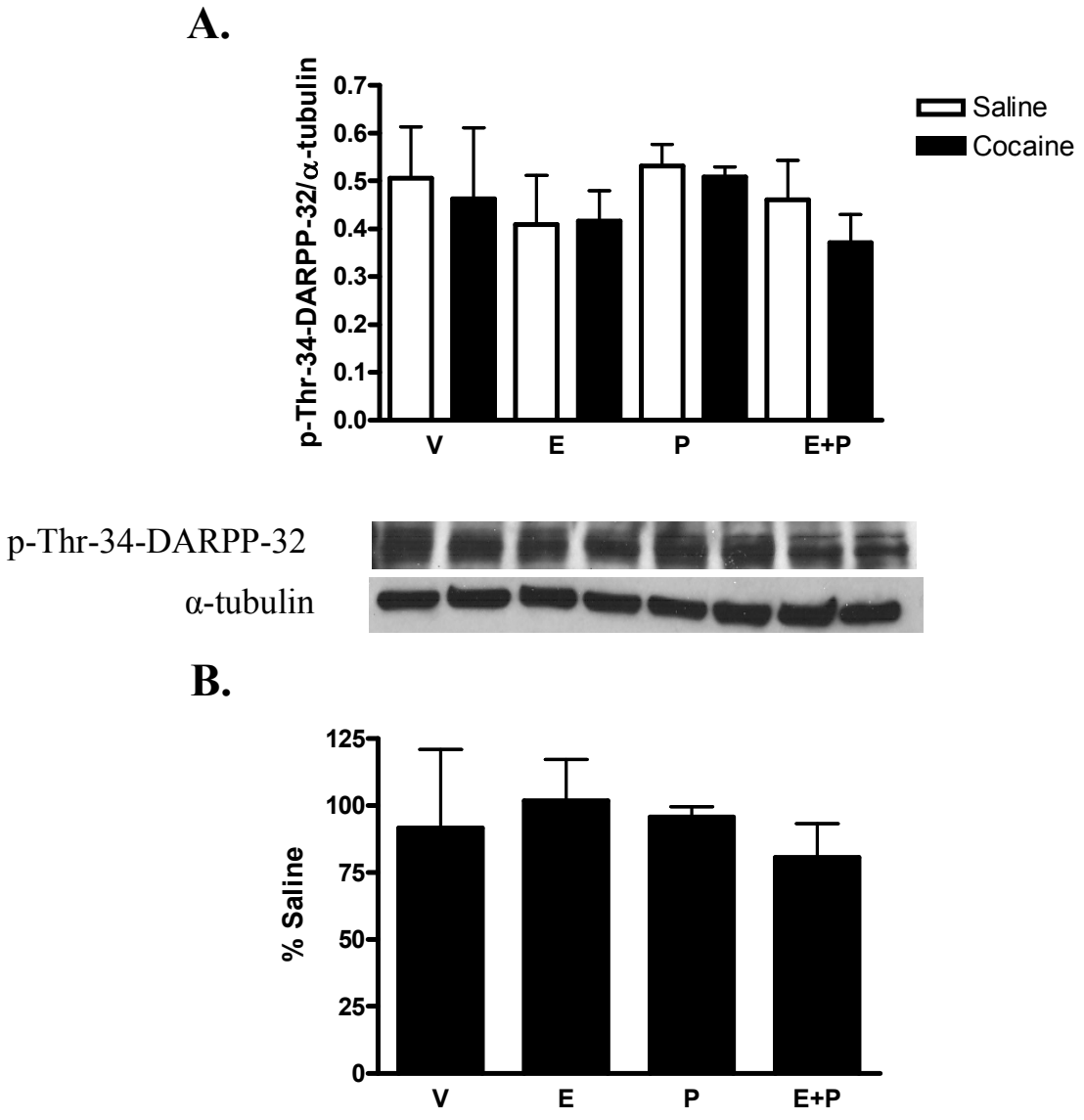


Figure 84: OVX vs hormone replacement differences in the NAc, P-Thr-34-DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

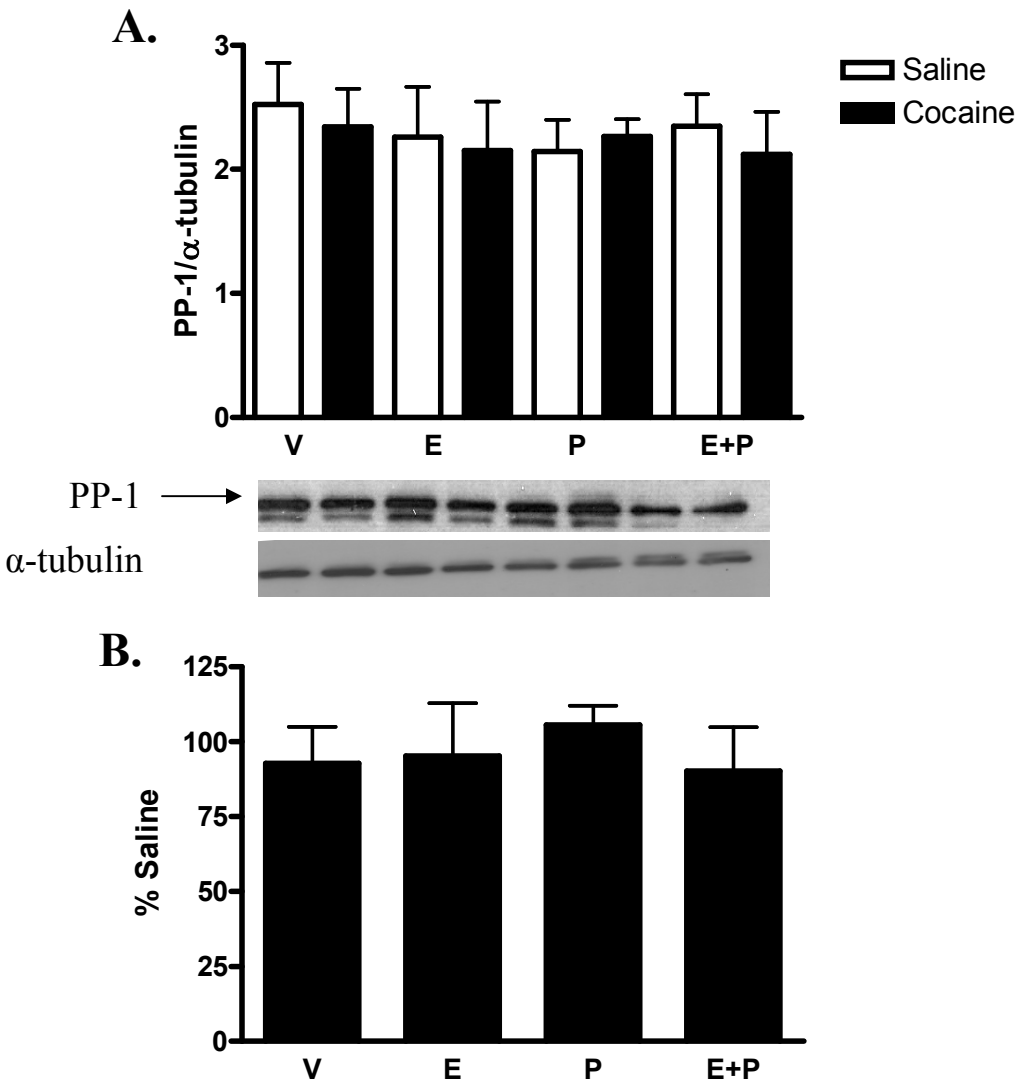


Figure 85: OVX vs hormone replacement differences in the NAc, PP-1 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

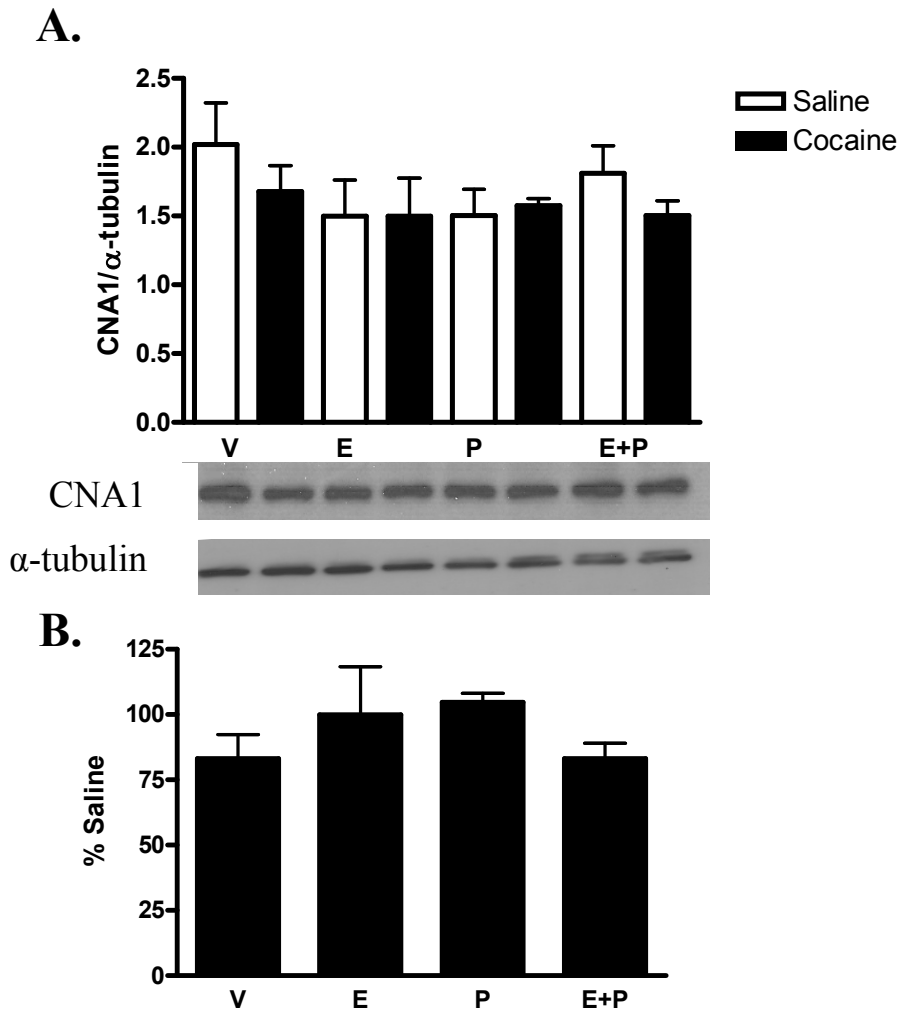


Figure 86: OVX vs hormone replacement differences in the NAc, PP-2B (CNA1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

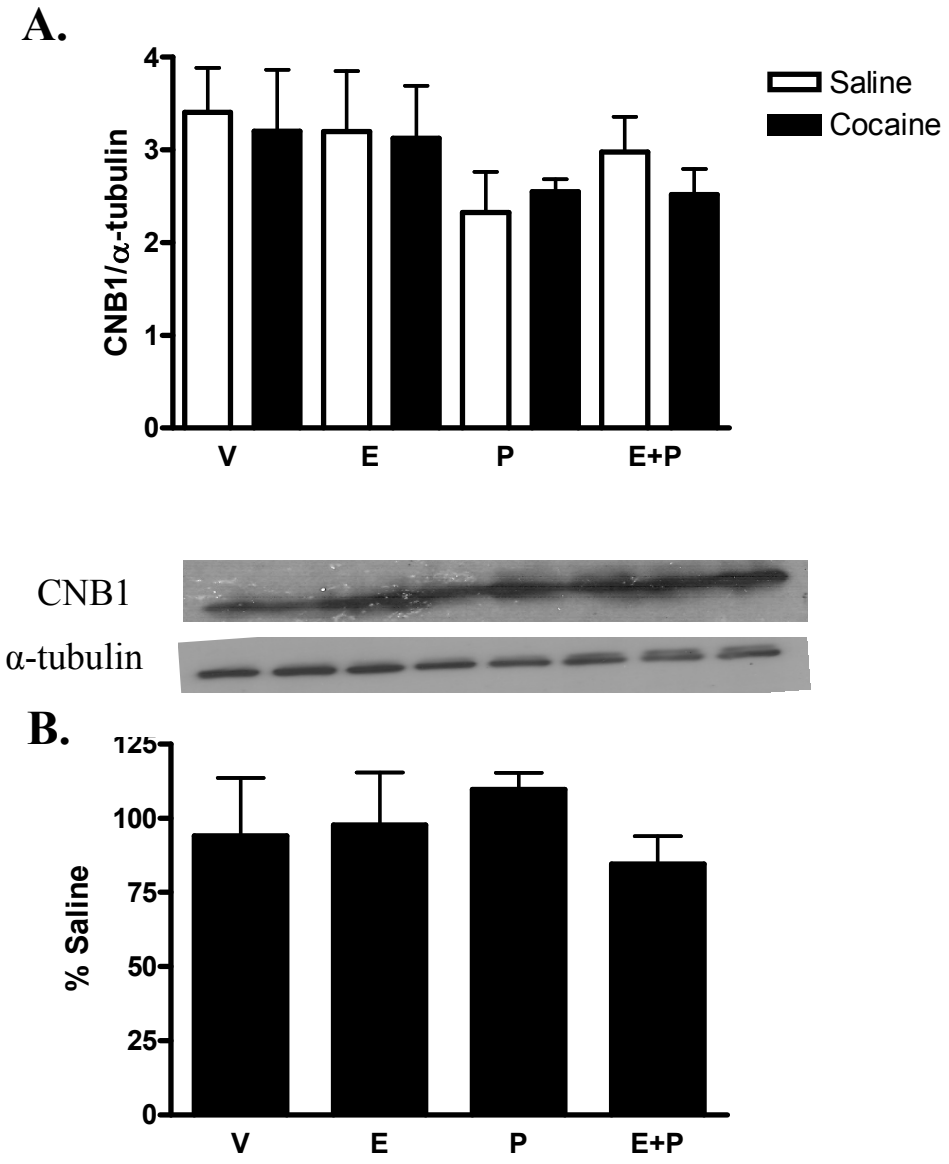


Figure 87: OVX vs hormone replacement differences in the NAc, PP-2B (CNB1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

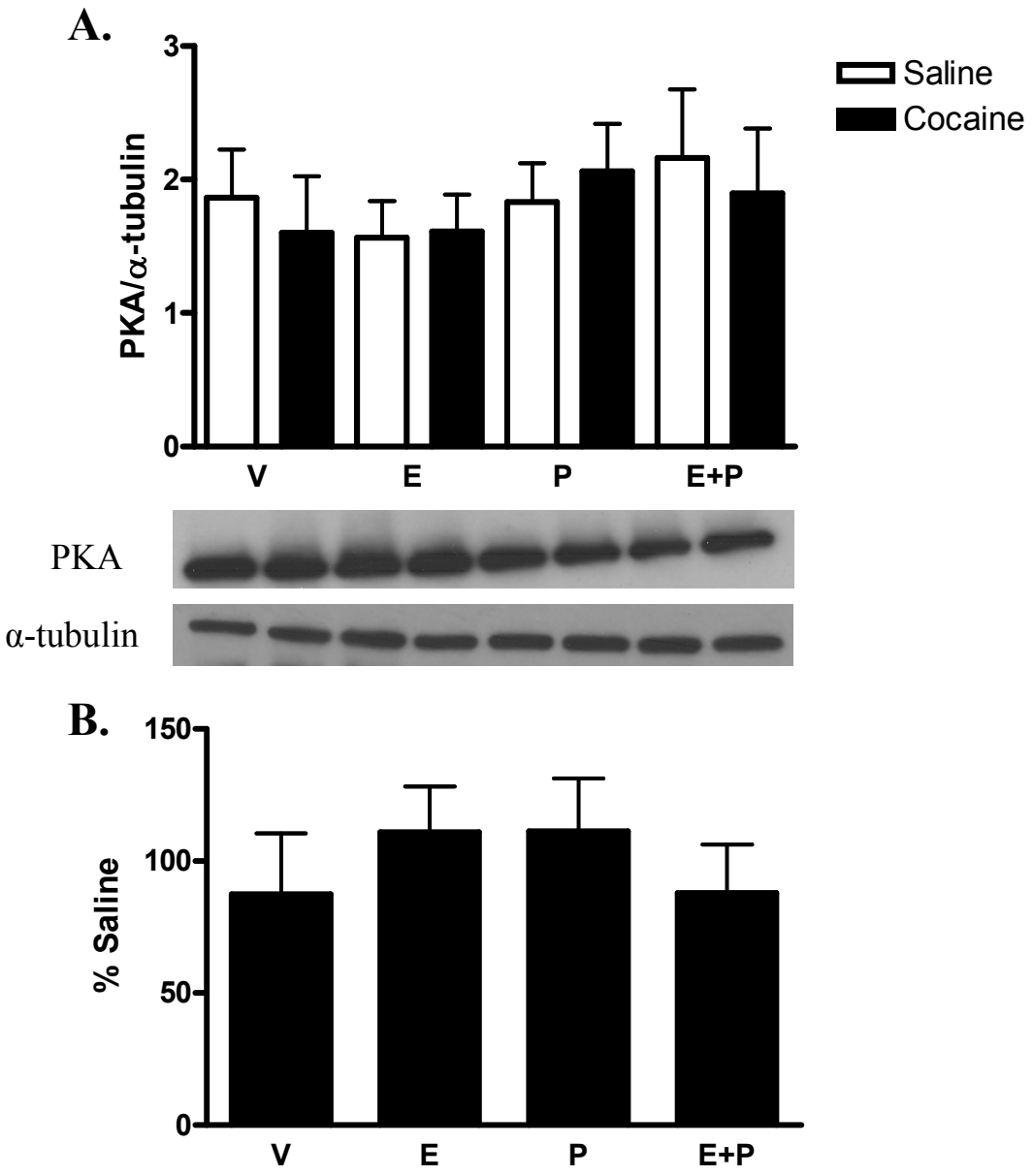


Figure 88: OVX vs hormone replacement differences in the CPu, PKA protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PKA protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

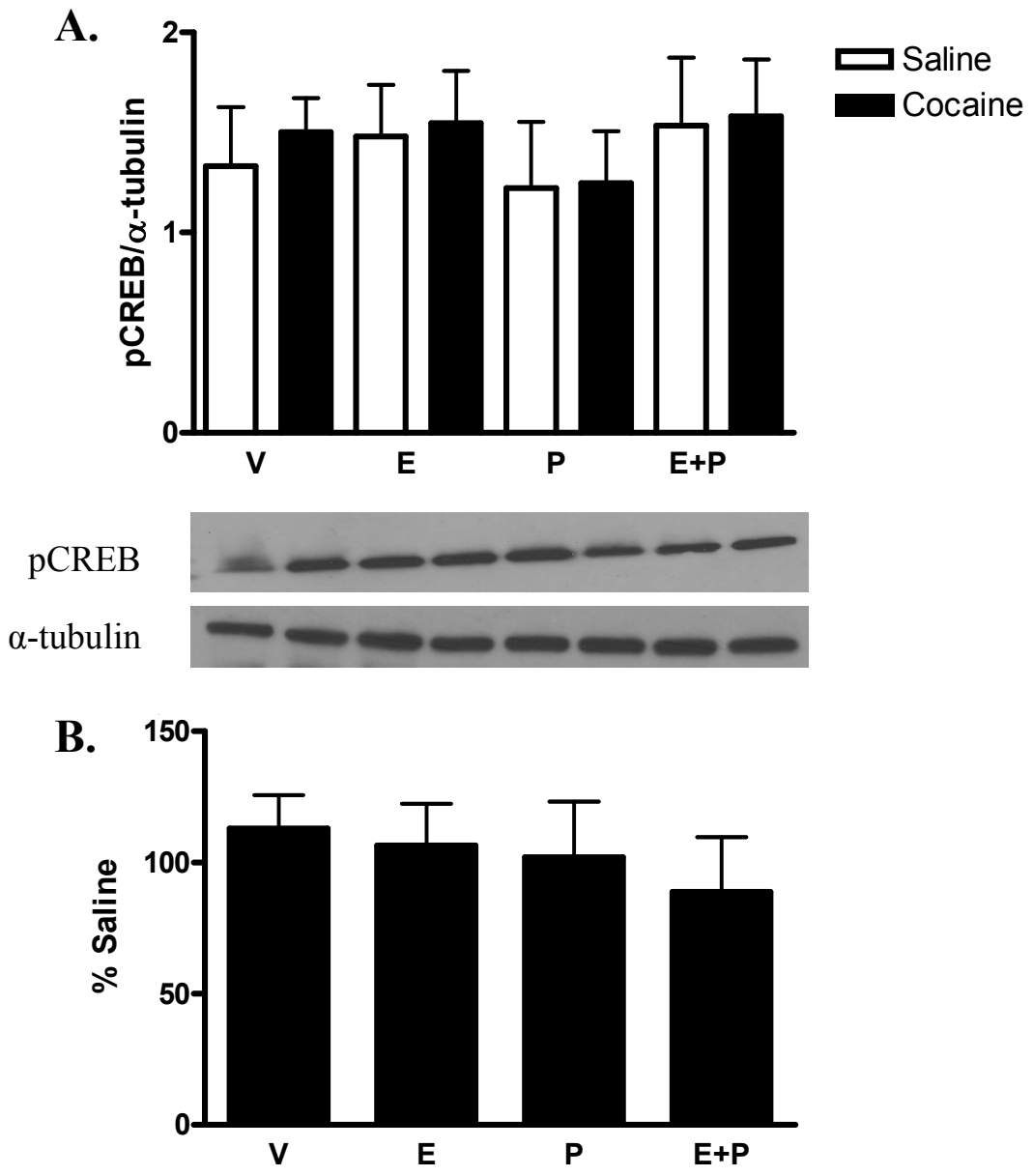


Figure 89: OVX vs hormone replacement differences in the CPu, pCREB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of pCREB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

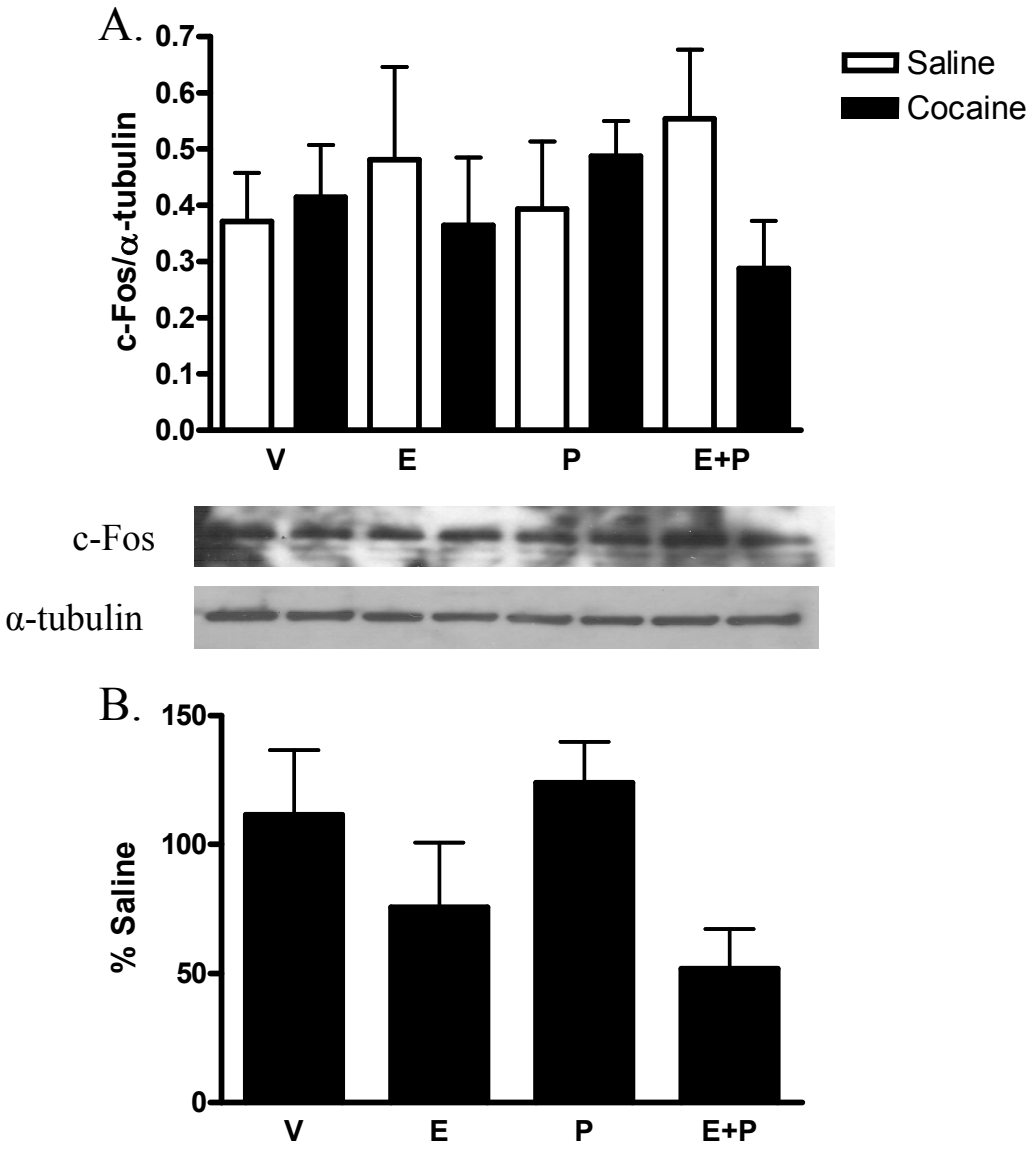


Figure 90: OVX vs hormone replacement differences in the CPu, *c-Fos* protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of *c-Fos* protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

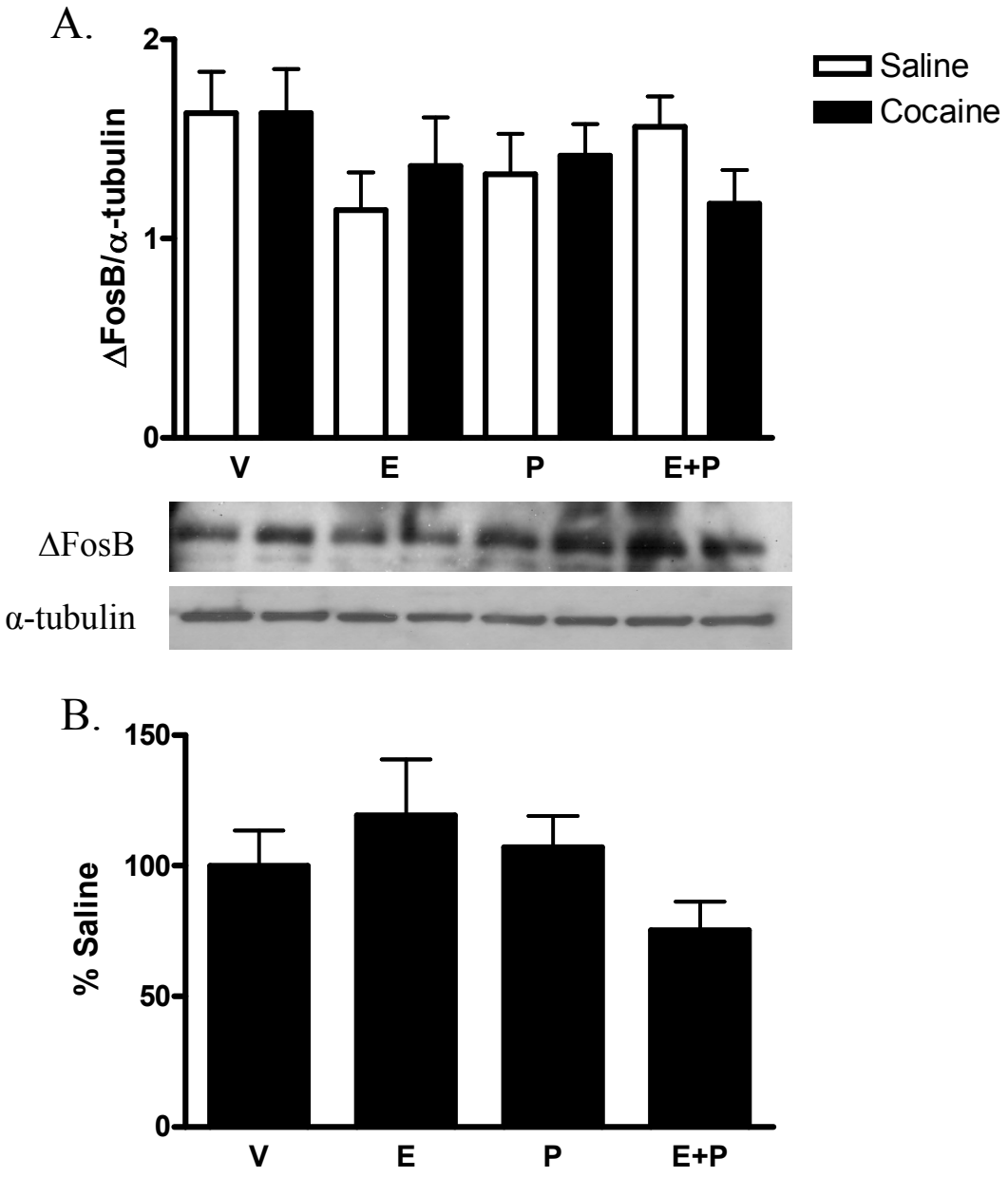


Figure 91: OVX vs hormone replacement differences in the CPu, ΔFosB protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of ΔFosB protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

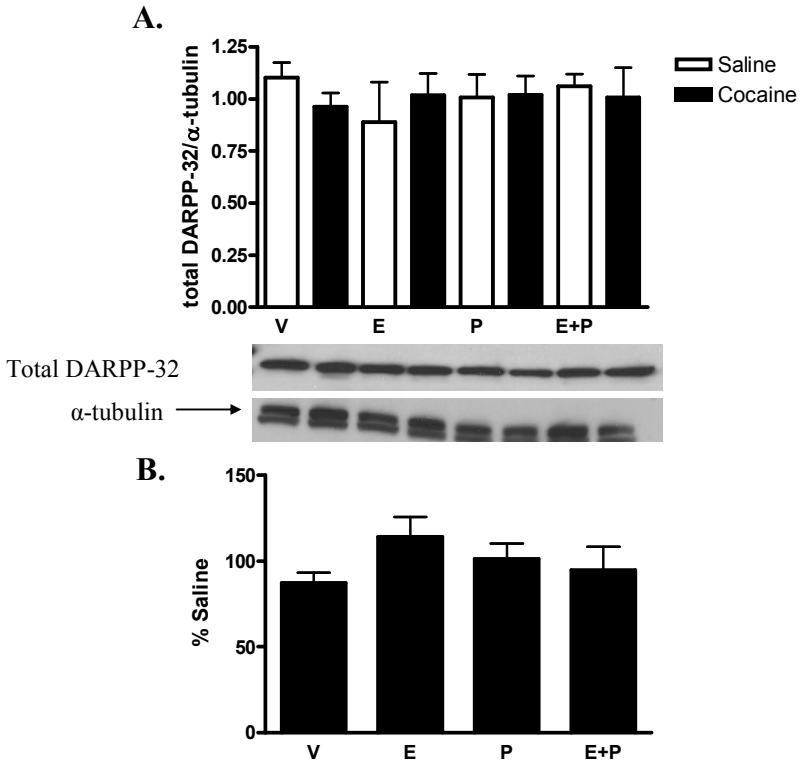


Figure 92: OVX vs hormone replacement differences in the CPu, Total DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of Total DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

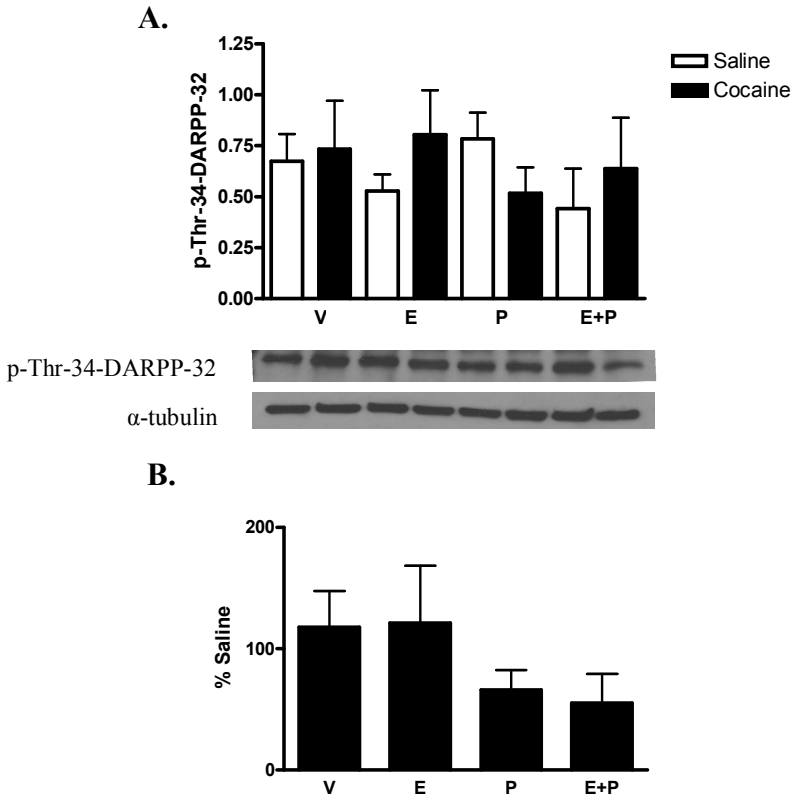


Figure 93: OVX vs hormone replacement differences in the CPu, P-Thr-34-DARPP-32 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of P-Thr-34-DARPP-32 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

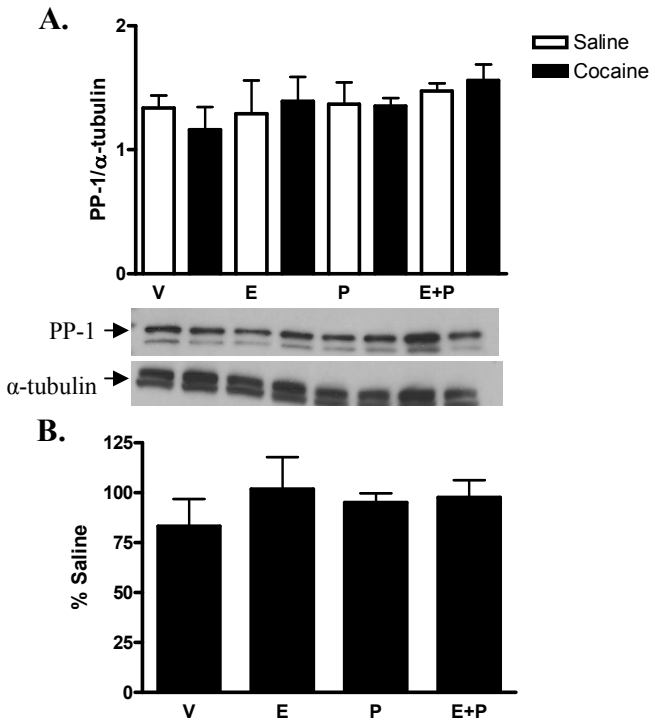


Figure 94: OVX vs hormone replacement differences in the CPu, PP-1 protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-1 protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

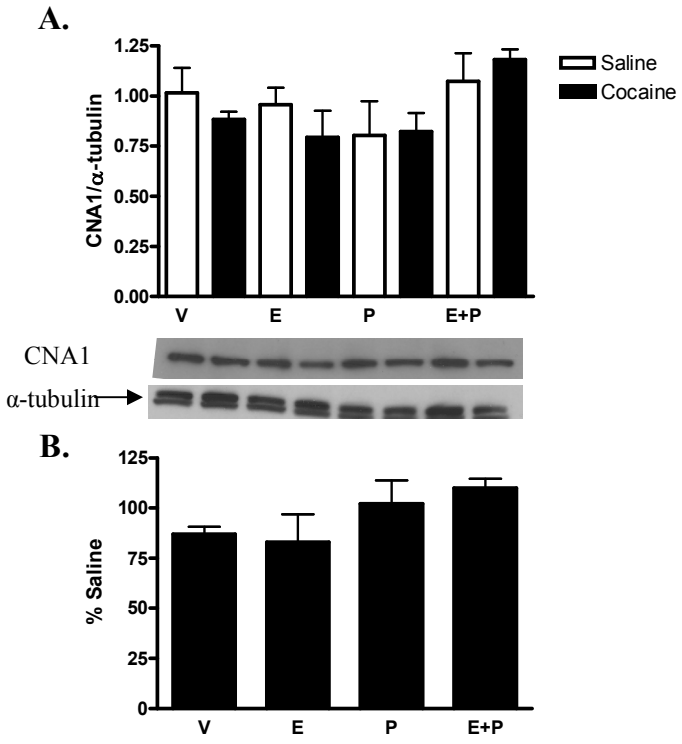


Figure 95: OVX vs hormone replacement differences in the CPu, PP-2B (CNA1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNA1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration.

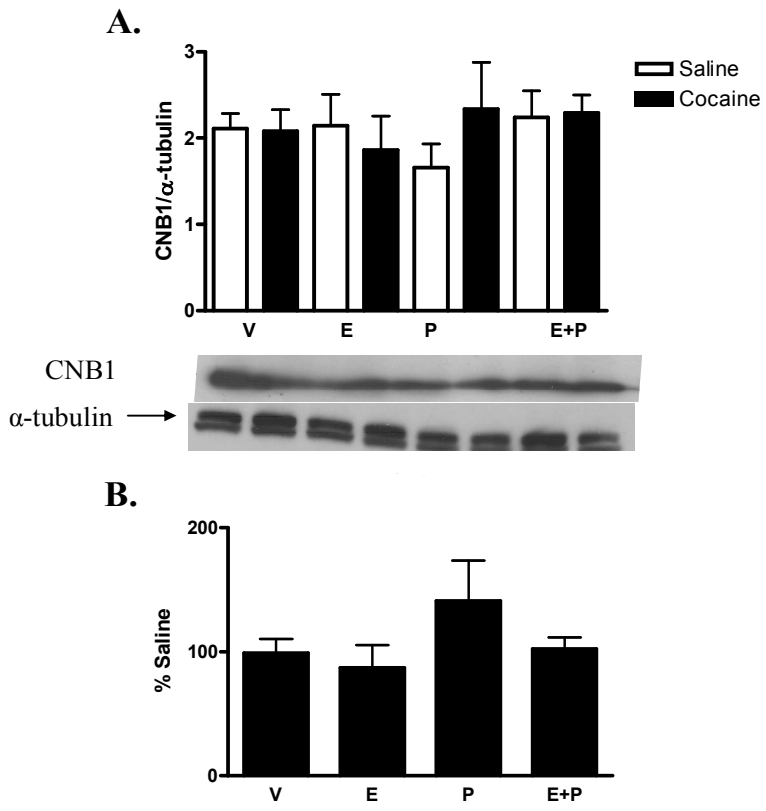


Figure 96: OVX vs hormone replacement differences in the CPu, PP-2B (CNB1) protein levels 15 min after saline (white bars) or cocaine administration (black bars). **(A)** Data are presented as mean ±SEM of PP-2B (CNB1) protein levels normalized to α-tubulin levels. **(B)** Data are presented as mean of % saline; cocaine animal/respective saline average (n=4). V=Vehicle group, E=Estrogen administration. P=Progesterone administration, E+P=Estrogen and Progesterone co-administration

Table IV: Estrous cycle effects on the ratio of phosphorylation of DARPP-32 before and after cocaine administration

Area	Time	Stage	Saline	Cocaine
NAc	15 min	Estrus	1.49±.25	1.10±.21
		Diestrus	1.21±.41	1.63±.58
		Proestrus	1.63±.40	2.01±.55
	1 hour	Estrus	.62±.17	.91±.11
		Diestrus	.86±.13	.95±.12
		Proestrus	.59±.09	.49±.15
CPu	15 min	Estrus	.57±.13	.79±.21
		Diestrus	.76±.18	.52±.12
		Proestrus	.74±.14	.72±.11
	1hour	Estrus	.98±.31	1.12±.24
		Diestrus	1.09±.20	1.44±.37
		Proestrus	1.15±.23	1.18±.21

Table V: Effects of hormone replacement on the ratio of phosphorylation of DARPP-32 before and after cocaine administration

Area	Treatment	Saline	Cocaine
NAc	Veh	.41±.04	.53±.21
	E	.34±.07	.37±.09
	P	.45±.05	.46±.06
	E+P	.47±.04	.54±.22
CPu	Veh	.60±.08	.81±.30
	E	.84±.39	.59±.16
	P	.79±.13	.53±.15
	E+P	.74±.18	.41±.15

Table VI: Summary Table 1: Sex differences in PKA-mediated pathways after cocaine administration

		Males		Females		Sex Difference	
Time	Proteins	NAc	CPu	NAc	CPu	NAc	CPu
15 min	Total DARPP-32	-	-	-	-	-	-
	P-Thr-34-DARPP-32	-	-	-	-	-	-
	PP-1	↓	-	-	-	-	-
	PP-2B (CNA1)	-	-	-	-	-	-
	PP-2B (CNB1)	-	-	-	-	-	-
	PKA	-	-	-	-	-	-
	pCREB	-	-	-	-	-	-
	c-Fos	-	-	-	-	-	-
	ΔFosB	-	-	-	-	-	-
		Males		Females		Sex Difference	
	Proteins	NAc	CPu	NAc	CPu	NAc	CPu
1h	Total DARPP-32	-	-	-	-	-	-
	P-Thr-34-DARPP-32	-	-	-	-	♂↓	-
	PP-1	-	-	-	-	-	-
	PP-2B (CNA1)	-	-	-	-	-	-
	PP-2B (CNB1)	-	-	-	-	-	-
	PKA	-	-	-	-	♂↑	-
	pCREB	-	-	-	-	-	-
	c-Fos	-	-	-	-	-	-
	ΔFosB	-	-	-	-	-	-

Table Legend:

↑= increased compared to saline

↓= decreased compared to saline

♂↓= decreased compared to females

♂↑= increased compared to females

Table VII: Summary Table 2: Estrous cycle differences in PKA-mediated pathways after cocaine administration

		Estrus		Diestrus		Proestrus		E Cycle Difference	
Time	Proteins	NAc	CPu	NAc	CPu	NAc	CPu	NAc	CPu
15 min	Total DARPP-32	-	-	-	-	-	-	-	-
	P-Thr-34-DARPP-32	-	-	-	-	-	-	-	-
	PP-1	-	-	-	-	-	-	-	-
	PP-2B (CNA1)	↑	-	-	-	-	-	-	-
	PP-2B (CNB1)	-	-	-	-	-	-	-	-
	PKA	↓	-	-	-	-	-	-	-
	pCREB	↓	-	-	-	-	-	-	-
	c-Fos	-	-	-	-	-	-	-	-
	ΔFosB	-	-	-	-	-	-	-	-
		Estrus		Diestrus		Proestrus		E Cycle Difference	
	Proteins	NAc	CPu	NAc	CPu	NAc	CPu	NAc	CPu
1h	Total DARPP-32	-	-	-	-	-	-	-	-
	P-Thr-34-DARPP-32	↑	-	↑	↑	-	-	**E↑P**	**D↑E**
	PP-1	-	-	-	-	-	-	-	-
	PP-2B (CNA1)	-	-	-	-	-	-	-	-
	PP-2B (CNB1)	-	-	-	-	-	-	-	-
	PKA	-	-	-	-	-	-	-	-
	pCREB	-	-	-	-	-	-	-	-
	c-Fos	-	-	-	-	-	-	-	-
	ΔFosB	-	-	-	-	-	-	-	-

<u>Table Legend</u>:
↑= increased compared to saline
↓= decreased compared to saline
E↑P= Estrus is higher than Proestrus
D↑E= Diestrus is higher than Estrus

Table VIII: Summary Table 3: OVX and hormone replacement differences in PKA-mediated pathways after cocaine administration

15 min	Vehicle		E		P		E+P		HR effects	
Proteins	NAc	CPu	NAc	CPu	NAc	CPu	NAc	CPu	NAc	CPu
Total DARPP-32	-	-	-	-	-	-	-	-	-	-
P-Thr-34-DARPP-32	-	-	-	-	-	-	-	-	-	-
PP-1	-	-	-	-	-	-	-	-	-	-
PP-2B CNA1	-	-	-	-	-	-	-	-	-	-
PP-2B CNB1	-	-	-	-	-	-	-	-	-	-
PKA	↑	-	-	-	-	-	↑	-	**V ↑**	-
pCREB	↑	-	-	-	-	-	↓	-	**V ↑ E+P↓**	-
c-Fos	-	-	-	-	-	-	-	-	-	-
ΔFosB	↓	-	-	-	-	-	-	-	-	-

Table Legend:

↑= increased compared to saline

↓= decreased compared to saline

V↑ = Vehicle is higher compared to E, P, and E+P groups

E+P↓= E+P groups is lower compared to Veh, E, and P groups

Discussion

Current literature demonstrates females have higher cocaine-induced reward associations and locomotor responses than males that is in part, mediated by hormonal fluctuations during the estrous cycle. However, it is unknown whether gender and estrous cycle differences in the behavioral responses to cocaine are mediated through differential protein activation in signal transduction pathways. Our results extend current knowledge in that they demonstrated cocaine-induced differences in two PKA-mediated pathways known to affect the response to cocaine: DARPP-32/PP-1/PP2B and PKA/CREB/Fos pathways.

PKA/CREB/Fos pathway was differentially affected by sex, estrous cycle, and hormone replacement after acute cocaine administration

A wide body of literatures suggests D1 receptor activation mediates many of the behavioral effects of cocaine. At the cellular level, D1 receptor activation increases cAMP production and PKA activation, resulting in subsequent activation of downstream PKA-mediated intracellular signal transduction cascades [204]. In male rats, pharmacological activation of PKA enhanced cocaine-induced behavioral responding [124;171]. Moreover, a previous study demonstrated pharmacological inhibition of PKA decreased cocaine self-administration, indicating an increase in the rewarding effects of cocaine, such that less cocaine is necessary to maintain the same effect [172]. A recent study by our group, observed sex differences in PKA protein levels in the NAc; female rats had higher basal PKA protein levels than male rats [133].

In this study, in the NAc, 1h after cocaine administration, we demonstrated higher PKA protein levels of male rats compared to female rats; suggesting inherent sex

differences in PKA-mediated intracellular mechanisms in response to cocaine. Previous studies have demonstrated sex differences in cocaine's rewarding/reinforcing effects; females acquired cocaine-self administration faster and with lower doses of cocaine than do male rats [112;114;167]. Because lower PKA has been associated with increases in rewarding effects, we postulate that lower PKA protein levels in females after acute cocaine administration may underlie the higher cocaine-induced rewarding effects in females.

In the NAc, 15 min after cocaine administration, when analyzed according to the estrous cycle, we report PKA protein levels decrease in estrus females when compared to their saline controls. Roberts (1989) observed that cocaine self-administration increases in estrus females compared to other stages of the cycle [162]. Additionally, cocaine-induced locomotor activity is higher in estrus compared to other stages of the cycle [156;173]. Consistent with our theory, the lower PKA levels during estrous may underlie higher rewarding effects of this psychostimulant. This further demonstrates intrinsic differences in the regulation of the PKA pathway during the reproductive cycle. Both estrogen and progesterone levels are augmented in estrus females; therefore, we postulate that higher levels of ovarian hormones attenuate PKA activation after acute cocaine administration, suggesting greater rewarding effects during estrus than during other stages of the cycle.

We further demonstrated that PKA protein levels in the NAc increased in OVX rats pretreated with vehicle and co-administration of estrogen plus progesterone, when compared to their saline controls, but not after pretreatment with estrogen or progesterone alone. However, when normalized to their respective saline controls, vehicle-treated females had higher PKA protein levels than estrogen-, progesterone, and estrogen +

progesterone- treated groups. Kantak et al (2007) demonstrated estrus females self-administer less than males, whereas, Roberts et al 1989, demonstrated estrus females have increased motivation to self-administer cocaine compared to rats in other stages of the cycle [97;162]. Taken as a whole, these data suggest the presence of estrogen, progesterone, and/or co-administration of estrogen + progesterone attenuated PKA protein activation as compared to vehicle. A previous report demonstrated acute estrogen replacement decreased cocaine self-administration in OVX females when compared to controls [74]. Thus, steroid hormone replacement may differentially inhibit cocaine-induced activation of PKA protein levels in female rats.

Co-administration of estrogen + progesterone in OVX rats has been reported to increase, inhibit or cause no affect on cocaine-induced behavioral activity [147;155;173;181]. For example, co-administration of estrogen + progesterone increases the magnitude of CPP, and potentiated brain stimulation reward in OVX rats [19;166]. However, Sircar and Kim (1999), found no effect on cocaine-induced behaviors using similar hormonal replacement paradigms [181]. On the countrary, after a "binge" pattern of cocaine administration (3 injections, 1 h apart), a bimodal interaction between E+P was observed; co-administration of E+P inhibited cocaine-induced locomotor activity after the first injection of cocaine, whereas co-administration of E+P augmented these same behaviors subsequent to the second and third injections [155]. Thus, estrogen priming + progesterone may have transiently increased PKA activation in OVX+E+P females after a single cocaine injection; however, subsequent cocaine injections may result in a different pattern of PKA activation. Discrepancies in the results after single cocaine + E + P administration may reside in differences in administration paradigms, and/or temporal interactions between estrogen and progesterone.

Overall, our study demonstrated higher PKA protein levels in males than females, and higher PKA protein levels in the vehicle group, compared to E, P, and E+P groups in OVX females after single cocaine injection. Our results support the postulate that fluctuations of estrogen and progesterone during the estrous cycle may render estrus females more vulnerable than females during other stages of the cycle to develop the rewarding and reinforcing effects of cocaine. Taken together, the influence of ovarian hormones on activation of PKA-mediated transduction pathways may be critical for initiating neuroadaptive changes that underlie the sex and estrous cycle differences in behavioral aspects of addiction.

In the NAc, drug-induced activation of CREB has been implicated in decreasing an individual's sensitivity to subsequent drug exposure [134]. Hence, CREB activation may mediate a form of tolerance to cocaine's rewarding effects. Several studies observe CREB increases after acute cocaine exposure [133;210].

Jenab et al., (2005) recently demonstrated no change in pCREB protein levels after acute cocaine administration in male rats. In accordance with the previous study, we observed no changes in accumbal pCREB protein levels in either males or females compared to their saline controls [93]. However, contrary to these findings, Nazarian et al (2005) demonstrated that cocaine increased accumbal pCREB protein levels in both male and female rats. Conflicting data in observing elevated pCREB protein levels may be due to differences in experimental paradigms; Nazarian et al. (2005) observed a transient peak in pCREB protein levels in females 5 min after cocaine administration, and elevated pCREB protein levels in males occurred 30 min after cocaine administration, whereas, we measured pCREB protein levels 15 min and 1h after acute cocaine administration. In addition, Nazarian pretreated rats with saline for 5 days + treatment (30mg/kg cocaine,

i.p.), whereas our study administered one injection (20 mg/kg dose cocaine, i.p.). However, in accordance with Nazarian et al (2005), no sex differences in pCREB protein levels were observed in either the NAc or CPu [133]. Thus, activation of CREB in the NAc after acute cocaine, although it is a dogma, the underlying scientific verification remains controversial.

Our results extend the current literature by demonstrating, when analyzed according to estrous cycle, accumbal pCREB protein levels decreased in estrus females compared to their saline control. This effect was transient since only significant alterations were observed 15 min after cocaine administration. Indeed, CREB phosphorlyation has been shown to mediate cocaine reward and reinforcement behaviors; enhanced CREB phosphorylation can reduce CPP and self-administration [25;172]. Therefore, it is possible that decreases in pCREB protein levels observed in estrus females, may, in part, further contribute to the increased CPP and self-administration in estrus females [11;163;167].

In the NAc of OVX rats, we observed pCREB protein levels increased in vehicle-treated females, but decreased in estrogen + progesterone-treated females after acute cocaine administration. Previous studies demonstrated a direct correlation between PKA activity and increases in CREB phosphorylation within the NAc. Increases in PKA-CREB activation produced a decrease in the rewarding effects of cocaine, whereas PKA inhibition produced the opposite effect [172]. Additionally, direct elevations of CREB levels within the NAc by virus-mediated gene transfer also reduced the rewarding effects of cocaine [25]. Conversely, expression of mCREB produced the opposite pattern of results, causing increases in the rewarding effects of cocaine [172]. Similar effects on cocaine reward were observed in transgenic mice with mutations that increased or

decreased CREB function [120;209]. These findings suggest that CREB activity in this region may regulate the addictive qualities of cocaine. Interestingly, similar to our findings in accumbal PKA protein activation, when normalized to their respective controls, the vehicle-treated group had the highest pCREB protein levels when compared to estrogen-, progesterone, and estrogen + progesterone-treated groups, whereas the estrogen + progesterone-treated group had the lowest pCREB protein levels. In the current study, we demonstrated the presence of ovarian hormones attenuated pCREB protein levels in the NAc, and, may in part, be regulated by PKA-mediated phosphorylation of CREB. Thus, we provide evidence that ovarian hormones differentially modulate pCREB protein levels, further suggesting that PKA-mediated signal transduction pathways are the cellular mechanisms partially responsible for the known estrous cycle differences in the rewarding effects of cocaine.

Although the CREB-regulated target genes that contribute to the effects of cocaine on CREB are not well known, one particular target gene within the NAc is dynorphin. Cocaine-induced increases in preprodynorphin (PDYN) function as a negative feedback mechanism to reduce cocaine-induced DA increase [47;117;185;186;195]. Additionally, increases in dynorphin expression mediated by CREB appear to be associated with aversive effects, such as those that often accompany drug withdrawal. A previous report demonstrated increased PDYN mRNA levels in the CPu after a single injection of cocaine administration in OVX Fischer rats; PDYN mRNA levels increased in both vehicle-treated and estrogen + progesterone-treated group, but not in estrogen or progesterone-treated groups [94]. Our study demonstrated a similar pattern in accumbal PKA protein levels, however, no changes were observed in the CPu. It is possible that crosstalk may occur between the NAc and CPu to account for this observed phenomena.

We postulate that the similar pattern of activation of PDYN mRNA levels in vehicle-treated and estrogen + progesterone-treated groups provide more evidence that the PKA-CREB pathway have a significant role in mediating the known estrous cycle differences in the rewarding effects of cocaine in the striatum.

Estrogen and progesterone have a well established a direct effect on intracellular activation of PKA/CREB. Evidence suggests that CREB is important in the crosstalk between estrogen, estrogen receptors, and PKA-intracellular cascades [108]. A recent report demonstrated OVX decreased pCREB expression, while estrogen increased pCREB expression in the hippocampus after acute cocaine injection [175]. Moreover, estrogen rapidly phosphorylated CREB through non-genomic mechanisms in the hippocampus and several other brain regions [4;75;228]. Additionally, previous studies observed that progesterone reduced pCREB levels in hypothalamic nuclei and hippocampal cultures, and inhibited CREB phosphorylation, through the PKA pathway, in trophoblast cells [75;128;218]. Taken together, the current literature suggests endogenous ovarian hormones have complex interactions with PKA-mediated signaling cascades and that this interaction occurs at multiple levels. However, our data demonstrated a different pattern of estrogen and progesterone regulation compared to the hippocampus and hippcampal nuclei. This, in part, may be due to area-specific differences, and/or differences in hormone dosing. Further investigation of the relationship between ovarian hormones and PKA-mediated intracellular mechanisms in NAc and the striatum must be done in order to elucidate whether these interactions affect the known sex and estrous cycle differences in cocaine-induced behavioral activation.

Gene expression regulation is one mechanism by which drugs of abuse can induce relatively long-lasting changes in the brain and the formation of the addicted state [134].

Several cocaine administration paradigms consistently show increases in c-Fos mRNA and Fos protein levels in various CNS loci [72;84;194]. Previous observations by our group, report c-Fos mRNA levels increase in intact male, female and OVX female rats treated with vehicle, estrogen and/or progesterone after single cocaine injection in the CPu [94]. However, in contrast to previous findings, the present study observed no increase in c-Fos protein levels in male or female rats in the NAc or CPu. Inconsistencies in the resulting data may be due to differences in experimental paradigm, including differences in timing of observation and experimental assays may account for discrepancies in the results. Alternatively, Jenab et al., (2002) measured mRNA, whereas our study measured protein activation, therefore, it is possible that a longer time-course may demonstrate c-Fos changes in protein activation, by allowing additional time for transcription to take place. Similar to the report of Niyomchai et al., 2006, we found that estrogen and progesterone replacement did not differentially alter cocaine-induced c-Fos protein levels [141]. Therefore, we postulate that sex, estrous cycle, or hormone replacement, contribute to c-Fos protein activation, and, thus, c-Fos activation may play a limited role in the known sex differences to cocaine.

Current literature suggests that ΔFosB may mediate some of the long-term effects of drugs of abuse within brain pathways that underlie addiction [81;135]. Our study demonstrated that sex, estrous cycle, or hormone replacement did not differentially alter cocaine-induced ΔFosB protein levels. However, previous reports demotrate acute cocaine induces ΔFosB expression from 2-72h after acute cocaine administration at a dose of 25 mg/kg [35]. Because our study observed ΔFosB protein expression 15 min and 1h after cocaine administration, it is likely to be premature of ΔFosB induction in the striatum. Interestingly, ΔFosB protein levels decreased in vehicle females in the NAc

when compared to their saline controls. Therefore, we postulate that the absence of ovarian hormones may result in an attenuation of ΔFosB levels in OVX rats. Furthermore, in chronic paradigms, repeated exposure to psychostimulants induces ΔFosB in small, yet sustainable amounts [32]. Additionally, sensitized rats display increased ΔFosB expression in the NAc [22]. We postulate that chronic cocaine administration will result in a different pattern of activation in ΔFosB and of c-Fos protein levels during different stages of the estrous cycle and after hormone replacement. Taken together, further investigation the role of ΔFosB induction in a chronic cocaine paradigm is necessary to elucidate the signaling mechanisms involved in sex and estrous cycle differences observed after cocaine administration.

Overall, evidence from our study suggests that PKA and CREB are the most susceptible proteins to be regulated by ovarian hormones and acute cocaine administration. Therefore, we suggest the initial interaction between acute cocaine administration and ovarian hormones may further modulate PKA and CREB and their subsequent intracellular cascades, facilitating the development of sensitization and/or tolerance in females and during the estrous cycle to cocaine. Because sex, estrous cycle, and hormone replacement did not alter cocaine-induced protein activation in the CPu, we further postulate that sex and estrous cycle differences in the regulation of the reward circuitry, rather than only psychomotor, is crucial in underlying the known gender differences to cocaine. Taken together, we postulate that it is alterations within the PKA-CREB pathway that, in part, mediate increased sensitivity of females to the rewarding, motivational, and reinforcing effects of cocaine.

DARPP-32/PP-1/PP2B pathway was differentially affected by sex, estrous cycle, and hormone replacement after acute cocaine administration

Upon D1-like receptor activation, DARPP-32 is phosphorylated by PKA at Threonine 34 and activated [139;204]. In contrast, following activation of D2-like receptors, DARPP-32 is dephosphorylated through activation of PP2B and subsequent inhibition of PKA [101;140]. Hence, DARPP-32 protein levels can be bi-directionally regulated by dopaminergic signaling through either D1- or D2-like receptors. DARPP-32 inhibits PP-1 [143], which results in changes in the phosphorylation state of ion channels, receptors, and transcription factors, altering the responses to DA [61]. Previous studies shown alterations in the DARPP-32 pathway affect the response to cocaine.

The current study observed no effect of acute cocaine administration on Total DARPP-32 protein levels. Our findings are similar to a previous report demonstrating no sex differences or changes between groups in Total DARPP-32 protein levels after repeated exposure to cocaine in a self-administration paradigm [113]. Therefore, we postulate that Total DARPP-32 protein activation does not play a significant role influencing the known gender or estrous cycle differences in acute cocaine-induced behaviors.

Phosphorylation of DARPP-32 at Thr-34 site is required for cocaine's actions in the striatum [222]; ie acute cocaine administration increased phosphorylation of DARPP-32 at Thr-34 site [73;138]. Moreover, DARPP-32 KO mice and mice with site mutations of Thr-34, DA-typical cocaine-induced locomotor and rewarding effects are attenuated when compared to control mice [63;201;222]. A recent report observed increased P-Thr-34-DARPP-32 protein levels after a single injection of cocaine in the CPu, hippocampus, and prefrontal cortex in male rats [39]. However, we observed no change in P-Thr-34-

DARPP-32 protein levels in male rats after acute cocaine administration. Discrepancies in the data may be due to differences in experimental paradigm. Intrinsic strain differences may exist; enhanced P-Thr-34-DARPP-32 protein levels were demonstrated in Sprague-Dawley rats, whereas we demonstrated no change in Fischer rats. Additionally, different sacrifice time and/or drug treatments may also contribute to variations in resulting data; D'Addario et al., 2007, administered 10mg/kg cocaine dose, and sacrificed after 30 min post-injection, whereas we administered 20 mg/kg cocaine dose and sacrificed either 15 min or 1 h post-injection.

However, our results extend current literature in that sex and estrous cycle differences in P-Thr-34-DARPP-32 protein levels were observed in the NAc; females had higher P-Thr-34-DARPP-32 protein levels than males when normalized to their respective saline controls. Our results are in agreement with Lynch et al., 2006, who observed higher P-Thr-34-DARPP-32 protein levels in the NAc and striatum of females compared to males after repeated cocaine administration in a self-administration paradigm [113]. Furthermore, our study demonstrated females have prolonged activation of P-Thr-34-DARPP-32 protein levels. Because no differences were observed in Total DARPP-32 levels, we postulate that the sexual dimorphic behavioral response to cocaine correlates with underlying differences in the phosphorylation and/or dephosphorylation rate.

Additionally, the current study demonstrated differential activation of accumbal P-Thr-34-DARPP-32 protein levels during the estrous cycle after acute cocaine; P-Thr-34-DARPP-32 protein levels increased in estrus and diestrus females in the NAc, and diestrus females in the CPu when compared to their saline controls. However, we observed no change in P-Thr-34-DARPP-32 protein levels in proestrus females. Proestrus

is characterized by higher levels of estrogen and progesterone and this concentration of hormone may work to attenuate P-Thr-34-DARPP-32 protein activation. Furthermore, when normalized to their respective saline controls, accumbal P-Thr-34-DARPP-32 protein levels were higher in estrus females than proestrus females, whereas P-Thr-34-DARPP-32 protein levels were higher in diestrus than estrus females in the CPu. Therefore, we postulate that the prolonged motor responses after acute cocaine in females may, in part, be mediated by a longer time course of P-Thr-34-DARPP-32 activation, as demonstrated in estrus and diestrus females. A previous report demonstrated P-Thr-34-DARPP-32 mutant mice show increased self-administration at lower doses of cocaine, and take longer to meet criteria for acquisition of cocaine self-administration than wild-type [225]. Thus, prolonged elevation of P-Thr-34-DARPP-32 protein levels may be the underlying mechanism regulating differential reward sensitivity, locomotor, and/or other cocaine-induced behavioral differences observed in females during the estrous cycle.

Surprisingly, hormonal replacement did not alter the pattern of P-Thr-34-DARPP-32 protein activation after cocaine administration. It is feasible that DARPP-32 may need a more dynamic interaction between estrogen and progesterone replacement, and/or a different experimental dosing paradigm to alter protein activation. Interactions between gonadal hormones, both in terms of concentrations and temporal relationships have been postulated to be critical in the modulation of reproductive behaviors, as well as neuronal activity, and, thus, may also be crucial in cellular and molecular adaptations, including intracellular protein activation. Therefore, we postulate that the temporal pattern, time course, or concentration of hormone replacement may not have been optimal for observing significant alterations in DARPP-32 protein levels.

Since PP-2B has been found to dephosphorylate P-Thr-34-DARPP-32, elevated PP-2B protein levels may represent a positive feedback mechanism to counteract high levels of P-Thr-34-DARPP-32 activation [71;101]. Our results are novel in that they demonstrate PP-2B protein levels increase only in estrus females. The increase in PP2B activation may be a result of D2-receptor activation, and may act as a compensatory mechanism to counteract the increased and prolonged activation of P-Thr-34-DARPP-32 protein levels observed in estrus females. It has been previously demonstrated that repeated cocaine administration decreases PP-2B but not PP-1 protein levels in the NAc [89]. The modulatory effect of D1 receptor on NMDA-mediated Ca^{+2} influx is well established [30;65;182;211]. In addition, NMDA receptor activation is important for the behavioral and biochemical effects in response to acute cocaine [93;199]. Thus, acute cocaine administration may increase PP-2B protein levels via NMDA-receptor dependent manner and/or D1-receptor modulation, or both.

Increases in DARPP-32 phosphorylation at Thr34 amplifies the effects of D1 receptor/cAMP/PKA signaling cascade by inhibiting PP-1 and reducing dephosphorylation of downstream target proteins, such as ion channels and receptors, which may be necessary for generating the rapid increase in locomotor activity produced by acute administration of cocaine [62;222;222]. In the NAc, 15 min after cocaine administration, our study demonstrated that PP-1 protein levels decreased in males compared to their saline controls. The prolonged activation of P-Thr-34-DARPP-32 in females may suggest a different time course, and/or a delay in PP-1 inhibition, which may underlie the increases behavioral activation in females. Taken together, our study shows that the DARPP-32 pathway is differentially activated by sex, estrous cycle, and/or hormone replacement after acute cocaine.

Ovarian hormones have been shown to modulate the dopamine system at different levels and may be the mediating mechanism underlying the estrous cycle differences after cocaine administration [15;36;40;43;44;70;85;86;95]. Acute administration of estrogen acts directly on the striatum to increase dopamine release, turnover rates, and reuptake [10;86;87]. Estrogen potentiates stimulated DA levels in the striatum, and D2-like receptor levels vary across the estrous cycle [41]. Different temporal patterns of estrogen hormone replacement may produce conflicting behavioral outcomes. Previous studies by our lab and others, found estrogen administration, by subcutaneous injection, to have no effect on cocaine-induced behavioral responses, whereas estrogen replacement via Silastic capsules was shown to potentiate cocaine's behavioral effects [88;141;148;173;181]. Subcutaneous injections produce transient increases in levels of estrogen, while Silastic capsules maintain relatively steady levels of estrogen, thus, affecting the behavioral outcomes. However, in the present study, we found acute estrogen replacement via subcutaneous injection did not affect intracellular protein activation in either PKA-mediated pathway. Therefore, we suggest that acute estrogen replacement has no effect on intracellular protein activation.

Progesterone also increases mesocorticolimbic dopamine release and reuptake DA [42;45;49;50;192]. Previous studies by our lab and others, show discrepancies in progesterone's effects on behavioral responses depending on the behavioral activity being measured; acute progesterone attenuates or has no effect on cocaine-induced behavioral activity. Different doses and/or temporal patterns of progesterone replacement prior to exposure to cocaine, potentially affect these results on behavioral activation [142;148;154;173;181]. Progesterone replacement via Silastic capsules attenuated cocaine-induced CPP [166]. Acute progesterone replacement at a dose of 500 ug of

progesterone, 4 hrs before cocaine administration had no effect on cocaine-induced behaviors [154;181]. However, Niyomchai et al., 2006 demonstrated 500 ug of progesterone, 24 hrs before cocaine administration inhibited rearing responses. In the present study, 500 ug of progesterone, 4 hrs before cocaine administration had no effect on intracellular protein activation [142]. Overall, our results suggest that progesterone's attenuation of the subjective effects and locomotor response are not modulated by PKA-mediated mechanisms, however, we suggest that progesterone's effects may be due to its ability to modulate other cocaine-mediated pathways. For example, progesterone breaks down into its metabolite allopregnanolone, which may attenuate cocaine reward by modulating the $GABA_A$ and/or NMDA receptor [37;115]

Previous literature demonstrated that E+P co-administration has either no effect, inhibits, or stimulates cocaine-induced behavioral alterations and rewarding effects [92;126;148;166]. Co-administration of E+P produces a complex interaction, wherein increases or decreases in behavioral outcomes vary according to temporal interactions between the hormones [147]. The current study demonstrated that E+P co-administration can increase, decrease, and/or have no effect on intracellular mechanisms. Our results provide evidence that interactions between steroidal hormones result in differential protein activation within PKA-mediated pathways.

NAc vs. CPu effects

In the present study, alterations in PKA-mediated protein activation were observed, for the most part, in the NAc, a brain region implicated in mediation of the motivational and rewarding effects of drug addiction. However, conversely, relatively no changes were observed in PKA-mediated protein activation in the CPu, an area of the

brain thought to be primarily involved with control of voluntary movement. Thus, we postulate accumbal PKA and CREB protein activation mediate the known sex and estrous cycle differences initial rewarding and motivational aspects of cocaine addiction. Moreover, we postulate that interactions between ovarian hormones and PKA-mediated protein activation may be area-specific; the absence of cocaine-induced PKA-mediated protein activation in the CPu suggests that these two pathways may not be the mechanism involved in the known sex and estrous cycle differences in cocaine-induced behavioral activation. Furthermore, we observed higher protein levels in the NAc compared to the CPu, suggesting regional specific differences in protein levels may mediate the underlying differences in rewarding and motivating behavior.

Additionally, most of the alterations in protein levels observed in our study were transient, suggesting that different mechanisms may be are involved in mediating the intracellular response to acute and chronic cocaine administration paradigms. Considering the current and previous findings together, it appears that sex, estrous cycle, and hormone replacement differentially activate the homeostatic process in response to acute cocaine administration. These differences could, in turn, assist in better understanding of the mechanisms that underlie the known gender differences to cocaine.

Summary: Postulated Model

This dissertation provides evidence that fluctuating steroid hormones affect D1-PKA-mediated intracellular transduction pathways, contributing to the underlying mechanism responsible for known differences in reward and motivational behaviors in males and females. Additionally, we postulate that the estrous cycle differentially

modulates the rewarding and motivational effects of cocaine, by mediating PKA and CREB activation [Figure 97]. Overall, our studies suggest that estrogen and progesterone play a key role in the neuromodulation of responses to the initial and reinforcing effect of cocaine, further suggesting that hormone fluctuations underlie the cocaine-induced behavioral responses and contributing to the known sex and estrous cycle differences in cocaine addiction.

The clinical implications of our data suggest that the use of estrogen- and progesterone-based contraceptives, hormone replacement therapy, and/or the stage of the menstrual cycle could affect the way a woman experiences the subjective effects of cocaine and/or influence the quantity of cocaine that is self-administered. Further investigations into the influence of steroid hormones on cocaine-induced intracellular responses are needed to develop sex-specific treatments for cocaine addiction as well as to understand that biological and molecular basis of this disease.

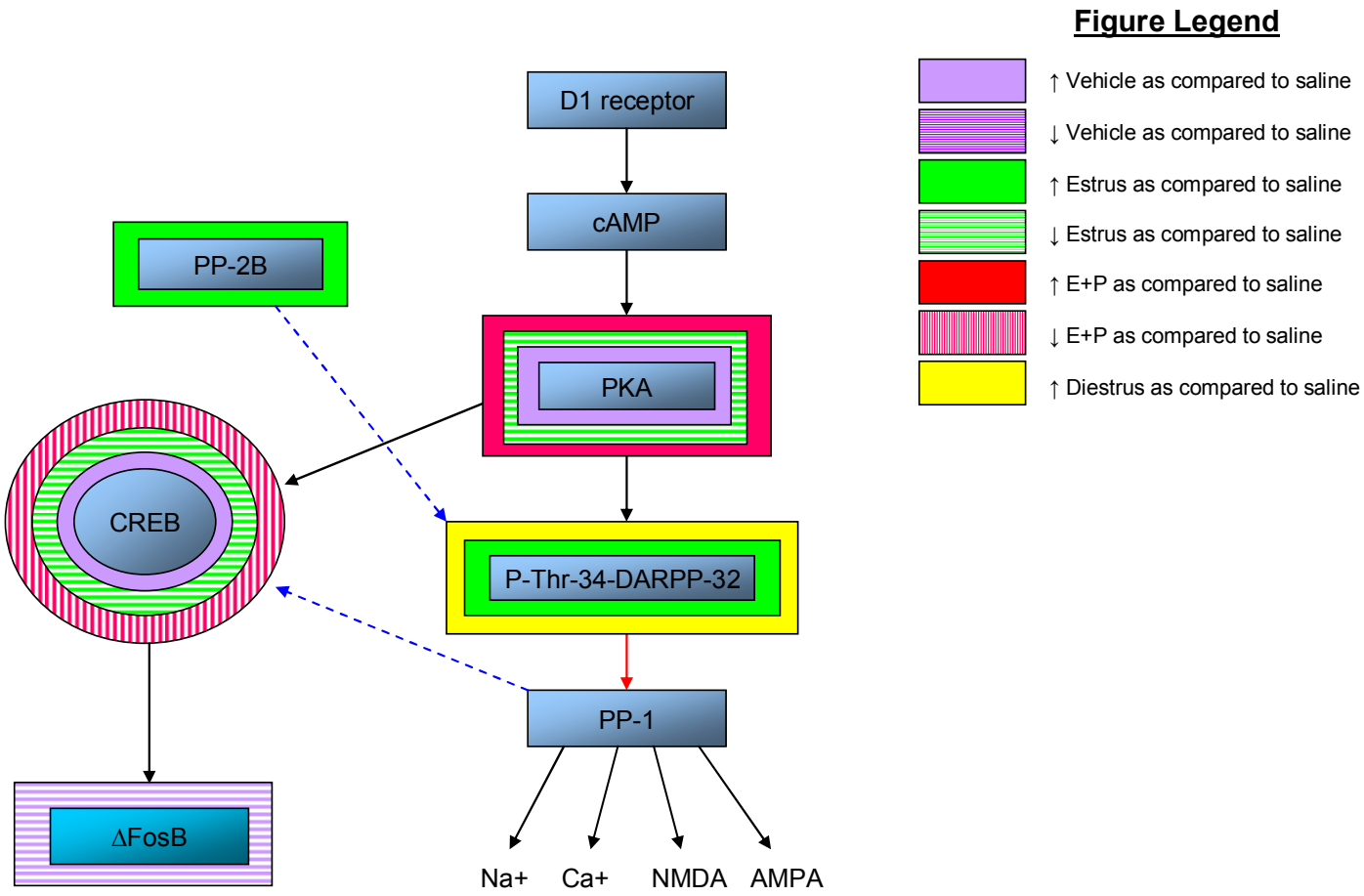


Figure 97: Proposed model of the influence of ovarian hormones on PKA-mediated intracellular mechanisms after acute cocaine administration.

Conclusion

The focus of this dissertation was to investigate and understand the underlying mechanisms of ovarian hormones effects on intracellular mechanisms in response to acute cocaine administration. However, the findings of present endeavors have produced more questions than answers. When considering all the findings as a whole, it becomes apparent that ovarian hormones play a crucial role in activating intracellular signal transduction proteins also modulated by cocaine. Thus, the existence of cocaine-induced sex and estrous cycle differences in signal transduction mechanisms may vitally contribute to the known sexually dimorphic response to cocaine.

Our study investigated whether sex, estrous cycle, and hormone replacement differences in protein activation occur in PKA-mediated pathways after acute cocaine. The current study demonstrated PKA and CREB were most susceptible to regulation by ovarian hormones, thus, PKA and CREB activation may play a crucial role in the mediating the known increased sensitivity and reward behaviors in females. However, several challenges remain in testing this hypothetical model. Due to the complexity of the systems studied in this dissertation, it is possible that alternative mechanisms not assessed in our experiments could have produced the obtained results.

Firstly, interactions between steroid hormone and cocaine are complex and occur at multiple levels. Cocaine-induced neuroendocrinological alterations (estrogen, progesterone) and their different receptor types (α, β) may overlap in their regulation of the genes and may have inhibitory or synergistic effects in influencing molecular, cellular, and transcription events.

The glutamate system has also been implicated in the behavioral and intracellular actions of cocaine. NMDA receptors have been studied for their role in behavioral sensitization and CREB phosphorylation. Previous studies demonstrated that NMDA receptor antagonists prevent the development of behavioral sensitization to cocaine in male rats [78;110;215]. NMDA can phosphorylate CREB by increasing intracellular Ca^{+2}, as result of the phosphorylation of the NR1 subunit of the NMDA receptor by either PKA or depolarization of the cell, or through phosphorylation of P-Thr-34-DARPP-32 [52]. The role of NMDA receptors cocaine-induced behaviors in female rats has yet to be elucidated.

However, we observed differential cocaine-induced P-Thr-34-DARPP-32 protein activation during in the estrous cycle; P-Thr-34-DARPP-32 protein levels increased estrus and diestrus females compared to their saline controls. More recently, it has been proposed that enhanced Thr34 phosphorylation also mediates the effects produced by prolonged administration of psychostimulants via regulation of the state of phosphorylation of two mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases 1 and 2 (ERK). The mitogen-activated protein kinase pathway (MAPK) has been implicated in mediating the rewarding effects in drugs of abuse [170]. Acute and chronic exposure to cocaine induced MAPK activation in the DA system [5]. Once activated, MAPKs translocate into the cell nucleus and activate transcription factors, such as c-Fos and CREB, and may contribute to the development of drug-induced long-term changes in the brain [200].

Recently our lab and others have demonstrated that cocaine-induced p-ERK is dependent on D1 receptor stimulation, which through the activation of adenylyl cyclase, increases PKA-mediated signaling, and, thus, may represent a distinct and/or redundant

intracellular cascade which may also underlie cocaine-induced long-term genomic alterations via regulation of ERK [91;93;161;200;203;223;224]. Additionally, MAPK cascade and the CREB protein signaling pathway also respond rapidly to estrogen and have been implicated in its neuroprotective effects [23;26;180]. Therefore, we postulate that the ability of MAPK and ERK pathways to modulate gene expression could be one of the contributing mechanisms to the increased sensitivity of female rats to cocaine than males. However, the interactions between steroid hormones and MAPK and ERK after acute and chronic cocaine remain to be elucidated.

Moreover, the hypothalamic pituitary adrenal (HPA) axis activation regulates stress responses. The hypothalamus secretes corticotropin-releasing hormone (CRH), activating the release of adrenocorticotropic hormone (ACTH) from the pituitary, which, in turn, triggers the secretion of corticosterone from the adrenal cortex. Cocaine causes activation of the HPA axis, increasing both ACTH and corticosterone secretion [104;125;165;169;187].

Sex differences have been observed in cocaine-induced activation of the HPA axis. Compared to males, females have greater ACTH and corticosterone plasma levels after acute cocaine exposure and a more prolonged response after cocaine administration than do males [33;105;206]. Interactions between the HPA axis and fluctuations of endogenous gonadal hormones also occur: corticosterone levels vary throughout the estrous cycle of the rat; specifically, levels are highest during proestrus and lowest during diestrus [206]. Estrous cycle also influences cocaine-induced ACTH and corticosterone release; rats in proestrus had greater cocaine-induced secretion of ACTH and corticosterone than rats in metestrus and diestrus [55]. Additionally, corticosterone levels are higher after OVX than in controls [34]. OVX decreased cocaine-induced ACTH and

corticosterone plasma levels in females, while castration had no effect on males [105]. Therefore, the interaction between cocaine's ability to modulate the HPA axis and ovarian hormones may affect underlying intracellular mechanisms and contribute to sex and estrous cycle differences in cocaine-induced behavioral and neurochemical responses.

Since previous literature demonstrated estrous cycle differences in cocaine-induced behavioral activation, grouping normal cycling female rats may abolish crucial effects of estrous cycle stage on protein activation within brain regions being observed. Thus, determining cycle stage is vitally important for interpreting results in experiments and may have contributed to discrepancies in the literature. However, one potential problem with our results was the technique used to determine estrous cycle stage. A previous report demonstrated that repeated lavage, attenuated estrous cycle effects and blocked the high cocaine-induced responding observed in proestrus and estrus [207]. Repeated lavage also facilitated DA release in the striatum and was able to establish CPP, suggesting that it serves as a reinforcing stimulus [207]. Moreover, vaginocervical stimulation increased phosphorylation of DARPP-32, likely evoked by increased extracellular dopamine [123]. These studies are relevant to the present findings because cocaine induces its behavioral and reinforcing effects by binding to the dopamine transporter and inhibiting the uptake of dopamine [79;159;214]. Taken together, repeated lavage may have affected protein activation in our study [207]. Therefore, further investigation of this complex interaction of ovarian hormones and DA-mediated intracellular mechanisms need to be examined using alternative techniques in order to collaborate with our data in the present study.

Although the hormone administration paradigms used in this study represent certain phases of the estrous cycle, levels of hormones are in constantly fluctuating; therefore, it is difficult to accurately simulate the circulating levels at a given phase. The changes that occur are the result of specific ratios of hormonal replacement and these ratios are not constant within a specific phase. Interestingly, the findings indicate that in some measure, similarities exist between OVX and the E+P groups that were not seen when estrogen and progesterone were administered alone. For example, in these groups, in the NAc, there was a cocaine-induced increase in PKA protein levels, while in pCREB protein levels, V increased, while E+P decreased in protein activation compared to their saline controls in the NAc. These observations were not seen in animals receiving only estrogen and progesterone, suggesting that the hormones interact in a synergistic manner in the mesolimbic pathway.

Further investigation, using different hormone replacement and cocaine administration paradigms would help elucidate how ovarian hormones modulate intracellular signal transduction pathways. A wide body of evidence demonstrates a sexually dimorphic response in behavioral sensitization, CPP, and other chronic paradigms. Observing how the estrous cycle and fluctuating ovarian hormones affect PKA-mediated pathways in a chronic paradigm will increase understanding of how PKA-mediated intracellular signal transduction pathways may affect sensitization, CPP, and/or the formation of the addicted state. Our study aimed to investigate the changes occurring acute administration, however, observation of protein levels occurring subsequent to chronic cocaine administration would further illuminate the underlying mechanism of the sexually dimorphic response.

Overall, our results demonstrated that PKA and pCREB protein levels decrease after acute cocaine administration during estrus, suggesting an increase in the initial rewarding effects of cocaine. However, previous literature demonstrated that human subjects experienced a decrease in the subjective effects of cocaine during the luteal stage and that rats in estrus demonstrate increased self-administration compared to other stages of the cycle [162;184]. Discrepancies in our results compared to others may be that women volunteers in human studies are regular cocaine users. Moreover, self-administration paradigms in rats require repeat cocaine exposure, whereas our results measured protein levels after the initial exposure to cocaine in naïve rats. Taken together, our results suggest that intrinsic differences in the initial intracellular protein response to acute cocaine may partially underlie estrous cycle and sex differences in the rewarding effects of cocaine.

Another limitation of our study was the western blot technique. Western blotting detects a specific protein in a given sample of tissue homogenate and determines its relative amount and relative size. Hence, it is a static measurement that is not useful in measuring the activity of the system. Conversely, protein level fluctuations in the dopamine system in response to cocaine administration can be transient and/or long-lasting. Moreover, the interactions between intracellular proteins in the dopamine system are dynamic and complex, however, western blotting technique can only measure protein levels at a fixed time point. Therefore, a time-course of each protein would help to elucidate the activity of each protein the dopamine system in response to cocaine administration.

Additionally, studies done in receptor knockout mice should also help to clarify the effects of ovarian hormones and estrous cycle. Since second messenger proteins

interact on several levels, KO mice would help to explain the interaction between hormones and second messenger pathways to help demonstrate at what levels interactions occur and the role they may play in cocaine addiction. Additionally, a time-course for each protein, done in normally cycling females, would be useful in determining when peak protein activation occurs in order to elucidate the changes occurring in the response to acute cocaine.

The findings presented in this dissertation provide further insight into gender differences in cocaine abuse. Although there is considerable evidence in clinical literature of these disparities, the mechanisms underlying the differences are not well understood. It is important to consider the gonadal hormones as a significant modulator of physiological effects in female rats. The dynamic fluctuations in estrogen and progesterone levels throughout the menstrual cycle plays a significant role modulating the effects of cocaine on several levels; the hormone concentrations, hormonally-mediated molecular and neurochemical mechanisms, and their impact on cocaine-induced protein activation are all crucial factors contributing to cycle-related fluctuations in cocaine effects.

The purpose of the current research was to better understand the mechanisms by which female rats respond to cocaine. Ultimately, the findings of these studies are aimed at advancing current knowledge in the way that women become addicted to cocaine. Understanding the dynamic interactions between steroid hormones and intracellular mechanisms are critical for the scientific community to develop improved approaches in gender-specific treatments for cocaine abuse.

Reference List

1. National Survey on Drug Use and Health: Monitoring the Future. National Survey Results on Drug Use, 1975-2003. College Students and Adults Ages 19-45, National Institute on Drug Abuse and University of Michigan 2 (2004).

2. Cocaine, National Institute on Drug Abuse (2005).

3. Drug Facts: Cocaine, Office of National Drug Control Policy (2005).

4. I.M.Abraham and A. E. Herbison, Major sex differences in non-genomic estrogen actions on intracellular signaling in mouse brain in vivo, Neuroscience 131 (2005), 945-951.

5. Adams JP and Sweatt JD, Molecular psychology: roles for the ERK MAP kinase cascade in memory, Annu Rev Pharmacol Toxicol 42 (2002), 135-163.

6. S.L.Andersen and M. H. Teicher, Sex differences in dopamine receptors and their relevance to ADHD, Neurosci Biobehav Rev 24 (2000), 137-141.

7. S.M.Anderson and R. C. Pierce, Cocaine-induced alterations in dopamine receptor signaling: Implications for reinforcement and reinstatement, Pharmacology & Therapeutics (2005).

8. D.A.Baker, R. A. Fuchs, S. E. Specio, T. V. Khroyan, and J. L. Neisewander, Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference, Synapse 30 (1998), 181-193.

9. Bazzett T.J. and J. B. Becker, Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding, Brain Res 637 (1994), 163-172.

10. J.B.Becker, Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis, Neurosci. Lett. 118 (1990), 169-171.

11. J.B.Becker, Gender differences in dopaminergic function in striatum and nucleus accumbens, Pharmacol Biochem Behav 64 (1999), 803-812.

12. J.B.Becker, M. E. Beer, and T. E. Robinson, Striatal dopamine release stimulated by amphetamine or potassium: influence of ovarian hormones and the light-dark cycle, Brain Res 311 (1984), 157-160.

13. J.B.Becker and V. D. Ramirez, Experimental studies on the development of sex difference in the release of dopamine from striatal tissue fragment in vitro, Neuroendocrinol. 32 (1981), 157-160.

14. J.B.Becker and V. D. Ramirez, Sex differences in the amphetamine stimulated release of catecholamines from rat striatal tissue in vitro, Brain Res 204 (1981), 361-372.

15. A.Beigon, C. T. Fischette, T. C. Rainbow, and B. S. McEwen, Serotonin receptor modulation by estrogen in discrete brain nuclei, Neuroendocrinol. 80 (1983), 5134-5137.

16. D.Benevides and J. Bibb, Role of Cdk5 in drug abuse and plasticity, Ann NY Acad Sci 1025 (2004), 335-344.

17. J.A.Bibb, J. Chen, J. R. Taylor, P. Svenningsson, A. Nishi, G. L. Snyder, Z. Yan, Z. K. Sagawa, C. C. Ouimet, A. C. Nairn, E. J. Nestler, and P. Greengard, Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5., Nature 410 (2001), 376-380.

18. J.A.Bibb, G. L. Snyder, A. Nishi, Z. Yan, L. Meijer, A. A. Fienberg, L. H. Tsai, Y. T. Kwon, J. A. Girault, A. J. Czernik, R. L. Huganir, H. C. Hemmings, A. C. Nairn, and P. Greegard, Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons., Nature 402 (1999), 669-671.

19. E.P.Bless, K. A. McGinnis, A. L. Mitchell, A. Hartwell, and J. B. Mitchell, The effects of gonadal steroids on brain stimulation reward in female rats, Behav Brain Res 82 (1997), 235-244.

20. R.Bosse, R. Rivest, and T. Di Paolo, Ovariectomy and estradiol treatment affect the dopamine transporter and its gene expression in the rat brain., Mol. Brain Res. 46 (1997), 343-346.

21. B.Bowman, S. R. Vaughan, D. Q. Walker, S. L. Davis, P. J. Little, N. M. Scheffler, B. F. Thomas, and C. M. Kuhn, Effects of sex and and gonadectomy on cocaine metabolism in the rat., J Pharmacol Exp Ther 290 (1999), 1316-1323.

22. H.C.Brenhouse and J. R. Stellar, c-Fos and ?FosB expression are differentially altered in distinct subregions of the nucleus accumbens shell in cocaine-sensitized rats, Neuroscience 137 (2006), 773-780.

23. D.N.Bryant, M. A. Bosch, O. K. Ronnekleiv, and D. M. Dorsa, 17-Betaestradiol rapidly enhances extracellular signal-regulated kinase 2 phosphorylation in the rat brain, Neuroscience 133 (2005), 343-352.

24. P.M.Callahan, R. De La Garza, and C. L. Cunningham, Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems, Pharmacol Biochem Behav 57 (1997), 601-607.

25. W.A.j.Carlezon, J. Thome, V. G. Olson, S. B. Lane-Ladd, E. S. Brodkin, N. Hiroi, R. S. Duman, R. L. Neve, and E. J. Nestler, Regulation of cocaine reward by CREB., Science 282 (1998), 2272-2275.

26. L.Carlstrom, Z. Ke, R. S. Cohen, and S. C. Pandey, Estrogen modulation of the cyclic AMP response element-binding protein pathway-effects of long-term and acute treatments, Neuroendocrinology 74 (2001), 227-243.

27. Carter,C.S., Neuroendocrinology of sexual behavior in the female. In: Becker,J.B., Breedlove,S.M., Crews,D. (Eds.), Behavioral Endocrinology, MIT Press, Cambridge, 1993, pp. 71-95.

28. W.A.Cass and G. A. Gerhardt, Direct in vivo evidencee that D2 dopamine receptors can modulate dopamine uptake, Neurosci. Lett. 176 (1994), 259-263.

29. S.A.Castner, L. Xiao, and J. B. Becker, Sex differences in striatal dopamine: *in vivo* microdialysis and behavioral studies, Brain Res. 610 (1993), 127-134.

30. C.Cepeda and M. S. Levine, Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum., Developmental Neuroscience 20 (1998), 1-18.

31. P.C.Chang, S. A. Aicher, and C. T. Drake, Kappa opioid receptors in rat spinal cord vary across the estrous cycle., Brain Res 861 (1999), 168-172.

32. J.Chen, M. B. Kelz, Hope BT, Y. Nakabeppu, and E. J. Nestler, Chronic Fos-related antigens: stable variants of delta FosB induced in brain by chronic treatments, J Neurosci 17 (1997), 4933-4941.

33. J.Chin, O. Sternin, and H. Fletcher, Sex differences in cocaine-induced locomotor and stereotypic behaviors in rats: an acute, chronic, and challenge study, Cell Mol Biol (2001).

34. J.Chin, O. Sternin, H. B. K. Wu, Burrell S., D. Lu, S. Jenab, L. I. Perrotti, and V. Quinones-Jenab, Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration., Brain Res 945 (2002), 123-130.

35. Chocyk, A., A. Czyrak, and K. Wedzony, Acute and repeated cocaine induces alterations in FosB/?FosB expression in the paraventricular nucleus of the hypothalamus, Brain Res. 1090 (2006), 58-68.

36. W.R.Crowley, Effects of ovarian hormones on norepinephrine and dopamine turnover in individual hypothalamic and extrahypothalamic nuclei, Neuroendocrinol. 34 (1982), 381-386.

37. M.Cyr, O. Ghribi, C. Thibault, M. Morissette, M. Landry, and T. Di Paolo, Ovarian steroids and selective estrogen receptor modulators activity on rat brain NMDA and AMPA receptors, Brain Res. Rev. 37 (2001), 153-161.

38. C.D'Addario, M. Di Benedetto, S. Candeletti, and P. Romualdi, The k-opioid receptor agonist U-69593 prevents cocaine-induced phosphorylaton of DARPP-32 at Thr34 in the rat brain, Brain Res. Bull. 73 (2007), 34-39.

39. C.D'Addario, M. Di Benedetto, S. Candeletti, and P. Roumaldi, The u-opioid receptor agonist U-69593 prevents cocaine-induced phosphorylation of DARPP-32 at Thr34 in the rat brain , Brain Res Bull 73 (2007), 34-39.

40. T.Di Paolo, R. Carmichael, F. Labrie, and J. P. Raymond, Effects of estrogen on the characteristics of {3-H}spiroperidol and [3-H]RU24213 binding in rat anterior pituitary gland and brain, Mol Cell Endo 16 (1979), 99-112.

41. T.Di Paolo, P. Falardeau, and M. Morissette, Striatal D-2 dopamine agonist binding sites fluctuate during the rat estrous cycle, Life Sci. 43 (1988), 665-672.

42. T.Di Paolo, D. Levesque, and M. Daigle, A physiological dose of progesterone affects rat striatum biogenic amine metabolism, Eur J Pharmacol 125 (1986), 11-16.

43. T.Di Paolo, P. Poyet, and F. Labrie, Effects of chronic estradiol and haloperidol treatment on striatal dopamine receptors, Euro. J. Pharm. 73 (1981), 105-106.

44. T.Di Paolo, P. Poyet, and F. Labrie, Effects of prolactin and estradiol on rat striatal dopamine receptors, Life Sci. 31 (1982), 2921-2929.

45. T.Di Paolo, G. Rouillard, and P. Bedard, 17β-estradiol at a physiological dose acutely increases dopamine turnover in rat brain, Euro. J. Pharm. 117 (1985), 197-203.

46. G.Diaz-Veliz, R. Baeza, F. Benavente, N. Dussaubat, and S. Mora, Influence of the estrous cycle and estradiol on the behavioral effects of amphetamine and apomorphine in rats, Pharmacol. Biochem. Behav. 49 (1994), 819-825.

47. G.DiChiara and A. Imperato, Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats, J Pharmacol Exp Ther 244 (1988), 1067-1080.

48. D.E.Dluzen and L. I. Anderson, Estrogen differentially modulates nicotine-evoked dopamine release from the striatum of male and female rats, Neurosci. Lett. 230 (1997), 140-142.

49. D.E.Dluzen and V. D. Ramirez, Bimodal effect of progesterone on in vitro dopamine function of the rat corpus striatum., Neuroendocrinology 39 (1984), 149-155.

50. D.E.Dluzen and V. D. Ramirez, Modulatory effects of progesterone upon dopamine release from the corpus striatum of ovariectomized estrogen-treated rats are stereo-specific., Brain Res. 538 (1991), 176-179.

51. A.W.Downs and N. B. Eddy, The effect of repeated doses of cocaine on the rat, J Pharmacol Exp Ther. 46 (1932), 199-202.

52. J.T.Dudman, M. E. Eaton, A. Rajadhyaksha, W. Macias, M. Taher, A. Barczak, K. Kameyama, R. Huganir, and C. Konradi, Dopamine D1 receptors mediate CREB phosphorylation via phosphorylating the NMDA receptor at Ser897-NR1, Journal of Neurochemistry 87 (2003), 922-934.

53. A.Dupont, T. Di Paolo, B. Gagne, and N. Barden, Effects of chronic estrogen treatment on dopamine levels and turnover in discrete brain nuclei of ovarianectomized rats, Neuroscience 22 (1981), 69-74.

54. A.M.Edelman, D. K. Blumenthal, and E. G. Krebs, Protein serine/threonine kinases, Ann. Rev. Biochem. 56 (1987), 567-613.

55. J.L.Falk, M. A. Fang, and C. E. Lau, Chronic oral cocaine self-administration: pharmacokinetics and effects on spontaneous and discriminative motor functions, J. Pharmacol. Exp. Ther. 257 (1991), 457-465.

56. Feldman,R.S., Meyer,J.S., and Quenzer,L.F., Principles of Neuropsychopharmacology, Sinauer Associates, Sunderland, 1997.

57. J.J.Fernández-Ruiz, J. C. Amor, and J. A. Ramos, Time-dependent effects of estradiol and progesterone on the number of striatal dopaminergic D2-receptors, Brain Res 476 (1989), 388-395.

58. E.D.Festa, S. Jenab, J. Chin, F. M. Gazi, H. B. K. Wu, S. J. Russo, and V. Quinones-Jenab, Frequency of cocaine administration affects behavioral and endocrine responses in male and female Fischer rats., Cell Mol Biol 49 (2003), 1275-1280.

59. E.D.Festa and Quinones-Jenab V, Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine, Horm. and Behav. 46 (2004), 509-519.

60. E.D.Festa, S. J. Russo, F. M. Gazi, T. Niyomchai, L. M. Kemen, S.-N. Lin, R. Foltz, S. Jenab, and V. Quinones-Jenab, Sex differences in cocaine-induced behavioral responses, pharmacokinetics, and monoamine levels., Neuropharmacology 46 (2004), 672-687.

61. A.A.Fienberg and P. Greegard, The DARPP-32 knockout mouse., Brain Res Rev. 31 (2000), 313-319.

62. A.A.Fienberg, N. Hiroi, P. G. Mermelstein, W. Song, G. L. Snyder, A. Nishi, A. Cheramy, J. P. O'Callaghan, D. B. Miller, D. G. Cole, D. Corbett, C. N. Haile, D. C. Cooper, S. P. Onn, A. A. Grace, C. C. Ouimet, F. J. White, S. E. Hyman, D. J. Surmeier, J. Girault, E. J. Nestler, and P. Greengard, DARPP-32: Regulator of the efficacy of dopaminergic neurotransmission, Science 281 (1998), 838-841.

63. A.A.Fienberg, A. Hiroi, P. G. Mermelstein, W. J. Song, G. L. Snyder, A. Nishi, A. Cheramy, J. P. O'Callaghan, D. B. Miller, D. G. Cole, R. Corbett, C. N. Haile, D. C. Cooper, S. P. Onn, A. A. Grace, C. C. Ouimet, F. J. White, S. E. Hyman, D. J. Surmeier, J. A. Girault, E. J. Nestler, and P. Greengard, DARPP-32: regulator of the efficacy of dopaminergic neurotransmission., Science 281 (2006), 838-842.

64. Fitzgerald,L.W. and Nestler,E.J., Cocaine regulation of signal transduction pathways. In: Hammer R (Ed.), Neurobiology of cocaine: molecular and cellular ascepts, CRC, Boca Raton, FL, 1995, pp. 219-240.

65. J.Flores-Hernandez, C. Cepeda, E. Hernandez-Echeagaray, C. R. Calvert, E. S. Jokel, A. Fienberg, P. Greengard, and M. S. Levine, Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32., J. Neurophysiol. 88 (2002), 3010-3020.

66. Freeman,M.E., The neuroendocrine control of the ovarian cycle of the rat. In: Knobil,E., Neill,J.D. (Eds.), The Physiology of Reproduction, Raven Press, Ltd., New York, 1994, pp. 613-658.

67. S.D.Glick and P. A. Hinds, Sex differences in sensitization to cocaine-induced rotation, Eur J Pharmacol 99 (1984), 119-121.

68. S.D.Glick, P. A. Hinds, and R. M. Shapiro, Cocaine-induced rotation: Sex-dependent differences between left- and right-sided rats., Science 221 (1983), 775-776.

69. S.B.Glickstein and C. Schmauss, Dopamine receptor functions: lessons from knockout mice, Pharmacol Ther 91 (2001), 63-83.

70. C.Goetz, F. Bourgoin, A. Cesselin, A. Brandi, D. Bression, M. Marinet, F. Peillo, and M. Hamon, Alterations in central neurotransmitter receptor binding sites following estradiol implantation in female rats, Neurochem. Int. 5 (1983), 375-383.

71. S.Goto, Y. Matsukado, Y. Mihara, N. Inoue, and E. Miyamoto, The distribution of calcineurin in rat brain by light and electron microscopic immunohistochemistry and enzyme-immunoassay., Brain Res 397 (1986), 161-172.

72. A.M.Graybiel, R. Moratalla, and H. A. Robertson, Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum., Proc Natl Acad Sci 87 (1990), 6912-6.

73. P.Greengard, The neurobiology of dopamine signaling., Biosci Rep 21 (2001), 247-269.

74. J.W.Grimm and R. E. See, Cocaine self-administration in ovariectomized rats is predicted by response to novelty, attenuated by 17-β estradiol, and associated with abnormal vaginal cytology, Physiol. Behav. 61 (1997), 755-761.

75. G.Gu, A. A. Rojo, M. C. Zee, J. Yu, and R. B. Simerly, Hormonal regulation of CREB phosphorylation in the anteroventral periventricular nucleus., J Neurosci. 16 (1996), 3035-3044.

76. G.Gupta and L. H. Tsai, A kinase to dampen the effects of cocaine, Science 292 (2001), 236-237.

77. M.Hagiwara, A. Alberts, P. Brindle, J. Meinkoth, Feramisco.J, T. Deng, M. Karin, S. Shenolikar, and M. Montminy, Transcriptional attenuation following cAMP induction requires PP-1-mediated dephosphorylation of CREB, Cell 70 (1991), 105-113.

78. J.L.Haracz, S. A. Belanger, J. S. MacDonall, and R. Sircar, Antagonist of N-methyl-D-aspartate receptors partially prevent the development of cocaine sensitization, Life Sci. 57 (1995), 2347-2357.

79. R.E.Heikkila, H. Orlansky, and G. Cohen, Studies on the distinction between uptake inhibition and release of [3H]dopamine in rat brain tissue slices, Biochem. Pharmacol. 24 (1975), 847-852.

80. A.Hiroi, G. J. Marek, Brown.J.R., H. Ye, F. Saudou, V. Vaidya, R. S. Duman, Greenberg ME, and E. J. Nestler, Essential role of the fosB gene in molecular, cellular, and behavioral actions of chronic electroconvulsive siezure, J Neurosci 18 (1997), 6952-6962.

81. N.Hiroi, J. Brown, C. Haile, H. Ye, M. Greenberg, and E. Nestler, Fos B mutant mice: loss of chronic cocaine induction of fos related proteins and heightened

sensitivity to cocaine's psychomotor and rewarding effects, Proc Natl Acad Sci 94 (1997), 10397-10402.

82. N.Hiroi, A. A. Fienberg, C. N. Haile, M. Alburges, G. R. Hanson, P. Greegard, and E. J. Nestler, Neuronal and behavioural abnormalities in striatal function in DARPP-32-mutant mice., European Journal of Neuroscience 11 (1999), 1114-1118.

83. B.Holmstedt, J. Lindgren, L. Rivier, and T. Plowman, Cocaine in the blood of coca chewers, European Journal of Ethnopharmacology 1 (1979), 69-78.

84. B.Hope, B. Kosofsky, S. Hyman, and E. Nestler, Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine, Proc Natl Acad Sci 89 (1992), 5764-5768.

85. R.E.Hruska, Elevation of Striatal Dopamine Receptors by Estrogen: Dose and Time Studies, J. Neurochem. 47 (1986), 1908-1915.

86. R.E.Hruska and K. T. Pitman, Distribution and localization of estrogen-sensitive dopamine receptors in the rat brain, J. Neurochem. 39 (1982), 1418-1423.

87. R.E.Hruska and E. K. Silbergeld, Increased dopamine receptor sensitivity after estrogen treatment using the rat rotation model, Science 208 (1980), 1466-1468.

88. M.Hu and J. B. Becker, Effects of sex and estrogen on behavioral sensitization to cocaine in rats., J Neurosci. 23 (2003), 693-699.

89. X.T.Hu, K. Ford, and F. J. White, Repeated cocaine administration decreases calcineurin (PP2B) but enhances DARPP-32 modulation of sodium currents in rat nucleus accumbens neurons., Neuropsychopharmacology 30 (2005), 916-926.

90. S.E.Hyman and R. C. Malenka, Addiction and the brain: the neurobiology of compulsion and its persistence, Nat. Rev. Neurosci. 2 (2001), 695-703.

91. S.Impey, K. Obrietan, S. T. Wong, S. Poser, S. Yano, G. Wayman, J. C. Deloume, G. Chan, and D. R. Storm, Cross talk between ERK and PKA is required for Ca 2 stimulation of CREB-dependent transcription and ERK nuclear translocation, Neuron 21 (1998), 869-883.

92. L.R.Jackson, T. E. Robinson , and J. B. Becker, Sex differences and hormonal influences on acquisition of cocaine self-administration in rats., Neuropsychopharmacology 1 (2006), 129-138.

93. S.Jenab, E. D. Festa, A. Nazarian, H. B. Wu, W. L. Sun, R. Hazim, S. J. Russo, and Quinones-Jenab V, Cocaine induction of ERK proteins in dorsal striatum of Fischer rats., Brain Res Mol Brain Res. 142 (2005), 134-138.

94. S.Jenab, T. Niyomchai, J. Chin, E. D. Festa, S. J. Russo, L. I. Perrotti, and V. Quinones-Jenab, Effects of cocaine on c-fos and preprodynorphin mRNA levels in intact and ovariectomized Fischer rats, Brain Res. Bull. 58 (2002), 295-299.

95. A.Jori and G. Cecchetti, Homovanillic acid levels in rat striatum during the oestrous cycle, J. Endo. 58 (1973), 341-342.

96. P.W.Kalivas, P. Duffy, L. A. DuMars, and C. Skinner, Neurochemical and behavioral effects of acute and daily cocaine, Journal of Pharmacological Experimental Therapeutics 245 (1988), 485-492.

97. K.M.Kantak, C. M. Goodwin, and V. Uribe, Influence of sex, estrous cycle, and drug-onset age in cocaine self-administration in rats (Rattus norvegicus)., Exp. Clin. Psychopharmacol. 15 (2007), 37-47.

98. S.Karch, Cocaine: history, use, and abuse, J R Soc Med 92 (1999), 393-397.

99. J.W.Kebabian and D. B. Calne, Multiple receptors for dopamine., Nature. 277 (1979), 93-96.

100. P.H.Kelly and S. D. Iversen, Selective 6OHDA-induced destruction of the mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats, Eur J Pharmacol 40 (1976), 45-56.

101. M.M.King, C. Y. Huang, P. B. Chock, A. C. Nairn, H. C. Hemmings, K. F. Chan, and P. Greengard, Mammalian brain phosphoproteins as substrates for calcineurin., J Biol Chem 259 (1984), 8080-8083.

102. G.F.Koob, Dopamine, addiction, and reward, Seminars in Neuroscience 4 (1992), 139-148.

103. T.R.Kosten, T. A. Kosten, C. J. McDougle, F. A. Hameedi, E. F. McCance, M. I. Rosen, A. H. Oliveto, and L. H. Price, Gender differences in response to intranasal cocaine administration to humans, Biol. Psychiatry 39 (1996), 147-148.

104. M.J.Kreek, Drug Addictions: Molecular and Cellular Endpoints, Ann. N. Y. Acad. Sci. 937 (2001), 27-49.

105. C.Kuhn and M. S. Francis, Gender differences in cocaine-induced HPA axis activation, Neuropsychopharmacology 16 (1997), 399-407.

106. K.Kumakura, M. Hoffman, D. Cocchi, M. Trabucci, P. F. Spano, and E. E. Muller, Long-term effect of ovariectomy on dopamine-stimulated adenylate cyclase in rat striatum and nucleus accumbens., Psychopharmacology 61 (1979), 13-16.

107. A.J.Lategan, M. R. Marien, and F. C. Colpaert, Effects of locus coeruleus lesions on the release of endogenous dopamine in the rat nucleus accumbens and caudate nucleus as determined by intracerebral microdialysis, Brain Res 523 (1990), 134-138.

108. G.Lazennec, J. A. Thomas, and B. S. Katzenellenbogen, Involvement of cyclic AMP response element binding protein (CREB) and estrogen receptor phosphorylation in the synergistic activation of the estrogen receptor by estradiol and protein kinase activators, The Journal of Steroid Biochemistry and Molecular Biology 77 (2001), 193-203.

109. D.Levesque, S. Gagnon, and T. Di Paolo, Striatal D_1 dopamine receptor density fluctuates during the rat estrous cycle, Neurosci. Lett. 98 (1989), 345-350.

110. Y.Li, X. T. Hu, T. G. Berney, A. J. Vartanian, C. D. Stine, M. E. Wolf, and F. J. White, Both glutamate receptor antagonists and prefrontal cortex lesions prevent

induction of cocaine sensitization and associated neuroadaptations, Synapse 34 (1999), 169-180.

111. S.E.Lukas, M. B. Sholar, L. H. Lundahl, X. Lamas, E. Kouri, J. D. Wines, L. Kragie, and J. H. Mendelson, Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers, Psychopharmacology 125 (1996), 346-356.

112. W.J.Lynch, M. N. Arizzi, and M. E. Carroll, Effects of sex and the estrous cycle on regulation of intravenously self-administered cocaine in rats, Psychopharmacology 152 (2000), 132-139.

113. W.J.Lynch, D. D. Kiraly, B. J. Calderone, Picciotto MR, and J. R. Taylor, Effect of cocaine self-administration on striatal PKA-regulated signaling in male and female rats, Psychopharmacology 191 (2006), 263-271.

114. W.J.Lynch, M. E. Roth, and M. E. Carroll, Biological basis of sex differences in drug abuse: preclinical and clinical studies, Psychopharmacology 164 (2002), 121-137.

115. M.D.Majewska, N. L. Harrison, R. D. Schwartz, J. L. Barker, and S. M. Paul, Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor, Science 232 (1986), 1007.

116. S.K.Mani, A. A. Fienberg, J. P. O'Callaghan, G. L. Snyder, P. B. Allen, P. K. Dash, A. N. Moore, A. J. Mitchell, J. Bibb, P. Greegard, and B. W. O'Malley,

Requirement for DARPP-32 in progesterone-facilitated sexual receptivity in female rats and mice., Science 287 (2000), 1053-1056.

117. J.Manzaneres, K. J. Lookingland, and K. E. Moore, Kappa opioid receptor mediated regulation of dopaminergic neurons in the rat brain, J Pharmacol Exp Ther 256 (1991), 505.

118. M.Maus, P. Bertrand, S. Drouva, R. Rasolonjanahary, C. Kordon, J. Glowinski, P. Premont, and A. and Enjalbert, Differential Modulation of D1 and D2 Dopamine-sensitive Adenylate Cyclases by 17Beta-Estradiol in cultured striatal neurons and anterior pituitary cells, J. Neurochem. 52 (1989), 410-418.

119. B.Mayr and M. Montminy, Transcriptional Regulation by the phosphorylation-dependent factor CREB, Nat Rev Mol Cell Biology 2 (2001), 588-609.

120. C.A.McClung and E. J. Nestler, Regulation of gene expression and cocaine reward by CREB and DeltaFosB., Nature Neuroscience 6 (2003), 1208-1215.

121. J.L.McDermott, Effects of estrogen upon dopamine release from the corpus striatum of young and aged female rats, Brain Res 060 (1993), 118-125.

122. B.S.McEwen and B. Parsons, Gonadal steroid action on the brain: neurochemistry and neuropharmacology, Annu Rev Pharmacol Toxicol 22 (1982), 598.

123. Meredith JM., Mating-related stimulation induces phosphorylation of dopamine and cyclic AMP-regulated phosphoprotein-32 in progestin receptor containing areas in the female rat brain, J Neurosci 18 (1998), 10189-10195.

124. M.J.Miserendino and E. J. Nestler, Behavioral sensitization to cocaine: modulation by the cyclic AMP system in the nucleus accumbens., Brain Res. 674 (1995), 299-306.

125. R.L.Moldow and A. J. Fischman, Cocaine induced secretion of ACTH, beta-endorphin , and corticosterone., Peptides 8 (1987), 819-822.

126. Morishima HO, Abe Y, Matsuo M, Akiba K, Masaoka T, and Cooper TB, Gender-related differences in cocaine toxicity in the rat., J Lab Clin Med 122 (2007), 157-163.

127. M.Morissette and T. Di Paolo, Effect of chronic estradiol and progesterone treatments of ovariectomized rats on brain dopamine uptake sites, J Neurochem 60 (1993), 1576-1583.

128. D.D.Murphy and M. Segal, Progesterone prevents estradiol-induced dendritic spine formation in cultured hippocampal neurons., Neuroendocrinology 72 (2000), 133-143.

129. Musto,D., America's first cocaine epidemic: what did we learn? In: Kosten,T.A., Kleber,H.D. (Eds.), Clinician's Guide to Cocaine Addiction Guilford, New York, 1992, pp. 3-15.

130. D.Musto, International traffic in coca through the early 20th century, Drug and Alcohol Dependence 49 (1998), 145-156.

131. Musto,D., The American Disease: Origines of Narcotic Control, Oxford Press, New York, 1999, 432 pp.

132. A.C.Nairn, P. Svenningsson, A. Nishi, G. Fisone, J. A. Girault, and P. Greengard, The role of DARPP-32 in the actions of drugs of abuse, Neuropharmacology 47 Suppl 1 (2004), 14-23.

133. A.Nazarian, Sex differences in behavioral and intracellular responses to cocaine, Dissertation: Hunter College of New York (2005).

134. E.J.Nestler, Molecular mechanisms of drug addiction, Neuropharmacology 47 (2004), 24-32.

135. E.J.Nestler, M. Barrot, and Self D.W., FosB: a molecular switch for addiction, Proc Natl Acad Sci 98 (2001), 11042-11046.

136. Nestler,E.J., Hyman,S.E., and Malenka,R.C., Molecular Basis of Neuropharmacology, McGraw-Hill, New York, 2001.

137. S.S.Newton, J. Thome, S. L. Wallas, Y. Shirayama, A. Dow, L. Schlesinger, C. Duman, N. Sakai, J. S. Chen, R. L. Neve, E. J. Nestler, and R. S. Duman, Inhibition of CREB or dynorphin in the nucleus accumbens produces an antipdepressant-like effect, J Neurosci 22 (2002), 10883-10890.

138. A.Nishi, J. A. Bibb, G. L. Snyder, H. Higashi, A. C. Nairn, and P. Greengard, Amplification of dopaminergic signaling by a positive feedback loop., Proceedings of the National Academy of Sciences USA 97 (2000), 12840-12845.

139. A.Nishi, G. L. Snyder, and P. Greengard, Bidirectional regulation of DARPP-32 phosphorylation by dopamine, Journal of Neuroscience 17 (1997), 8147-8155.

140. A.Nishi, G. L. Snyder, H. Higashi, A. C. Nairn, and P. Greengard, Requirement for DARPP-32 in mediating the effect of dopamine D2 activation, Eur. J. Neurosci. 11 (1999), 2589-2592.

141. T.Niyomachai, S. Jenab, E. D. Festa, A. Akhavan, and V. Quinones-Jenab, Effects of short- and long-term estrogen and progesterone replacement on behavioral responses and c-fos mRNA levels in female rats after acute cocain administration, Brain Res 1126 (2006), 193-199.

142. T.Niyomchai, S. J. Russo, E. D. Festa, A. Akhavan, S. Jenab, and V. Quinones-Jenab, Progesterone inhibits behavioral responses and estrogen increases corticosterone levels after acute cocaine administration, Pharmacol Biochem Behav 80 (2005), 603-610.

143. C.C.Ouimet, P. E. Miller, H. C. Hemmings, S. I. Walaas, and P. Greegard, DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine innervated brain regions III., Journal of Neuroscience 4 (1984), 111-124.

144. I.Perez-Otano, A. Mandelzys, and J. I. Morgan, MPTP-Parkinsonism is accompained by persistent expression of delta-FosB-like protein in dopaminergic pathways, Brain Res Mol Brain Res 53 (1998), 41-52.

145. J.Peris, N. Decambre, M. L. Coleman-Hardee, and J. W. Simpkins, Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [^{3}H]dopamine release, Brain Res. 566 (1991), 255-264.

146. L.I.Perrotti, D. Lu, T. Niyomachai, S. Cornejo, S. J. Russo, S. Jenab, and Quinones-Jenab V, Temporal effects of estrogen and progesterone on behavioral and endocrinological responses to acute cocaine administration, Cell Mol Biol 49 (2003), 1269-1274.

147. L.I.Perrotti, S. Russo, F. Lagos, O. Sternin, and V. Quiñones-Jenab, Temporal interactions between estrogen and progesterone affect cocaine-induced locomotor behaviors in ovariectomized Fischer rats., Soc. Neurosci. Abs. 26 (2000).

148. L.I.Perrotti, S. J. Russo, H. Fletcher, J. Chin, T. Webb, S. Jenab, and V. Quinones-Jenab, Ovarian hormones modulate cocaine induced locomotor and stereotypic activity, New York Academy of Science 937 (2001), 202-216.

149. R.C.Pierce and P. W. Kalivas, A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants, Brain Res 25 (1997), 192-216.

150. R.M.Post, A. Lockfeld, K. M. Squillace, and N. R. Contel, Drug-environment interaction: context dependency of cocaine-induced behavioral sensitization., Life Sci. 28 (1981), 755-760.

151. R.M.Post and H. Rose, Increasing effects of repetitive cocaine administration in the rat, Nature 260 (1976), 731-732.

152. V.Quinones-Jenab, L. I. Perrotti, Fabian.S.J., J. Chin, S. Russo, and S. Jenab, Endocrinological basis of sex differences in cocaine-induced behavioral responses, Ann NY Acad Sci 937 (2001), 140-171.

153. V.Quiñones-Jenab, L. I. Perrotti, A. Ho, S. Jenab, S. D. Schlussman, J. Franck, and M. J. Kreek, Cocaine affects progesterone plasma levels in female rats, Pharmacol. Biochem. Behav. 66 (2000), 449-453.

154. V.Quinones-Jenab, L. I. Perrotti, J. Mc Monagle, A. Ho, and M. J. Kreek, Ovarian hormone replacement affects cocaine-induced behaviors in ovariectomized female rats., Pharmacol. Biochem. Behav. 67 (2000), 417-422.

155. V.Quiñones-Jenab, L. I. Perrotti, J. Mc Monagle, A. Ho, and M. J. Kreek, Steroid replacement affects cocaine-induced behaviors in ovariectomized Fischer rats, Pharmacol. Biochem. Behav. 67 (2000), 417-422.

156. V.Quiñones-Jenab, A. Ho, S. D. Schlussman, J. Franck, and M. J. Kreek, Estrous cycle differences in cocaine-induced stereotypic and locomotor behaviors in Fischer rats., Behav Brain Res 101 (1999), 15-20.

157. G.Rakajumar, P. Chiu, B. Johnson, and R. K. Mishra, 17B estradiol induced increase in brain dopamine D2 receptor: antagonism by MIF-1, Peptides 8 (1987), 997-1002.

158. U.Rapp, P. Kourounakis, and H. Seyle, Effects of steroids on diethylstilbestrol on cocaine toxicity, plasma concentrations, and urinary excretion, Drug Research **29** (1979), 48-50.

159. M.C.Ritz, M. Landry, S. R. Goldberg, and M. J. Kuhar, Cocaine receptors on dopamine transporters are related to self-administration of cocaine, Science 237 (1987), 1219-1223.

160. S.Robbins, R. N. Ehrman, A. R. Chidress, and C. P. O'Brian, Comparing levels of cocaine cue reactivity in male and female outpatients, Drug Alcohol Depend 53 (1999), 223-230.

161. Roberson ED, English JD, Adams JP, Selcher JC, Kondratick C, and Sweatt JD, The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus., J Neurosci 19 (1999), 4337-4348.

162. D.C.Roberts, S. A. L. Bennett, and G. J. Vikers, The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats, Psychopharmacology 98 (1989), 408-411.

163. D.C.S.Roberts, S. A. L. Bennett, and G. J. Vickers, The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats, Psychopharmacology 98 (1989), 408-411.

164. T.E.Robinson and K. C. Berridge, Mechanisms of action of addictive stimuli: incentive-sensitization and addiction., Addiction 96 (2001), 103-114.

165. P.Romualdi, A. Donatini, S. Izenwasser, B. M. Cox, and S. Ferri, Chronic intracerebroventicular cocaine differentially affects prodynorphin gene expression in rat hypothalmus and caudate-putamen., Mol. Brain Res. 40 (1996), 153-6.

166. S.J.Russo, E. D. Festa, S. J. Fabian, F. M. Gazi, M. Kraisch, S. Jenab, and V. Quinones-Jenab, Gonadal hormones differentially modulate cocaine-induced place preference in male and female rats., Neurosci. 120 (2003), 523-533.

167. S.J.Russo, S. Jenab, S. J. Fabian, E. D. Festa, L. M. Kemen, and V. Quinones-Jenab, Sex differences in the conditioned rewarding effects of cocaine., Brain Res 970 (2003), 214-220.

168. N.Sakai, J. Thome, J. S. Chen, M. B. Kelz, C. Steffen, E. J. Nestler, and C. Duman, Inducible and brain region specific CREB transgenic mice, Mol Pharmacol. 61 (2002), 1453-1464.

169. Z.Sarnyai, F. S. Dhabhar, B. S. McEwen, and M. J. Kreek, Neuroendocrine-related effects of long-term "binge" cocaine administration: Diminished individual differenes in stress-induced corticosterone response., Neuroendocrinology 68 (1998), 334-344.

170. H.J.Schaefers and Weber M.J., Mitogen-activated protein kinases: specific messages from ubiquitous messengers, Mol Cell Biol 19 (1999), 2435-2444.

171. J.A.Schroeder, M. Hummel, and E. M. Unterwald, Repeated intracerebroventricular forskolin administration enhances behavioral sensitization to cocaine., Behav Brain Res 153 (2004), 255-260.

172. Self D.W., L. M. Genova, B. T. Hope, W. J. Barnhart, J. J. Spencer, and E. J. Nestler, Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior., J Neurosci. 18 (1998), 1848-1859.

173. S.L.Sell, J. M. Scalzitti, M. L. Thomas, and K. A. Cunningham, Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats., J. Pharmacol. Exp. Ther. 293 (2000), 879-886.

174. H.Seyle, Protection by estradiol against cocaine, coniine, ethylmorphine, LSD, and strychinine., Horm Behav 2 (1971), 337-341.

175. Sharma K, R. D. Mehra, and U. Vij, Chronic exposure to estrogen and tamoxifen regulates synaptophysin and phosphorylated cAMP response element-binding (CREB) protein expression in CA1 of ovariectomized rat hippocampus, Brain Res. 1132 (2007), 10-19.

176. A.J.Shaywitz and M. E. Greenberg, CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals, Ann. Rev. Biochem. 68 (1999), 821-861.

177. H.Shimizu and G. A. Bray, Effects of castration, estrogen replacement and estrus cycle on monoamine metabolism in the nucleus accumbens, measured by microdialysis, Brain Res 621 (1993), 200-206.

178. C.W.Shindler and G. N. Carmona, Effects of dopamine agonists and antagonist on locomotor activity in the male and female rats, Pharmacol Biochem Behav. 72 (2002), 857-863.

179. D.R.Sibley, F. J. Monsma, and Y. Shen, Molecular neurobiology of dopaminergic receptors, In Rev Neurobiol 35 (1993), 391-415.

180. C.A.Singer, X. A. Figueroa-Masot, R. H. Batchelor, and D. M. Dorsa, The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons., J Neurosci 19 (1999), 2463.

181. R.Sircar and D. Kim, Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats., J Pharmacol Exp Ther 289 (1999), 54-65.

182. G.L.Snyder, A. A. Fienberg, R. L. Huganir, and P. Greengard, A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/ protein phosphatase-1 pathway regulated dephosphorylation of the NMDA receptor., Journal of Neuroscience 18 (1998), 10297-10303.

183. M.Sofuoglu, D. Babb, and D. K. Hatsukami, Effects of progesterone treatment on smoked cocaine response in women., Pharmacol Biochem Behav 72 (2002), 431-435.

184. M.Sofuoglu, S. Duidish-Poulsen, D. Nelson, P. R. Pentel, and D. K. Hatsukami, Sex and menstrual cycle differences in the subjective effects from smoked cociane in humans, Exp. Clin. Psychopharmacol. 7 (1999), 274-283.

185. R.Spanagel, A. Herz, and T. S. Shippenberg, The effects of opioid peptides on dopamine relase in the nucleus accumbens: an in vivo microdialysis study, J. Neurochem. 55 (1990), 1734-1740.

186. R.Spangler, L. Zhou, C. Maggos, S. D. Schlussman, A. Ho, and M. J. Kreek, Prodynorphin, proenkephalin, and kappa opioid receptor mRNA responses to acute "binge" cocaine, Mol. Brain Res. 44 (1997), 139-142.

187. R.Spangler, Y. Zhou, S. D. Schlussman, A. Ho, and M. J. Kreek, Behavioral stereotypies induced by 'binge' cocaine administration are independent of drug-induced increased in corticosterone levels, Behav Brain Res 86 (1997), 201-204.

188. P.Svenningsson, A. Fienberg, P. B. Allen, C. LeMoine, M. Lindskog, G. Fisone, Greengard P, and B. B. Fredholm, Dopamine D1 receptor-induced gene transcription is modulated by DARPP-32, J Neurochem 75 (2000), 248-257.

189. P.Svenningsson, M. Lindskog, C. Ledent, M. Parmentier, Greengard P, B. B. Fredholm, and G. Fisone, Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32kDA in vivo by dopamine D1, and dopamine D2 and adenosine A2A receptors, Proceedings of the National Academy of Sciences USA 97 (2000), 1856-1860.

190. R.Z.Terwilliger, D. Beitner-Johnson, K. A. Sevarino, S. M. Crain, and E. J. Nestler, A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function., Brain Res. 548 (1991), 100-110.

191. T.L.Thompson and R. L. Moss, Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects., J Neurochem. 62 (1994), 1750-1756.

192. T.L.Thompson and R. L. Moss, Modulation of mesolimbic dopaminergic activity over the rat estrous cycle., Neurosci Lett 229 (1997), 145-148.

193. Timmons,C.R. and Hamilton,L.W., Drugs, Brains, and Behavior, Prentice Hall, 1990.

194. G.Torres and C. Rivier, Induction of c-fos in rat brain by acute cocaine and fenfluranmine exposure: a comparison study., Brain Res 647 (1994), 1-9.

195. E.M.Unterwald, Regulation of Opioid Receptors by Cocaine, Ann N. Y. Acad Sci 937 (2001), 74-92.

196. E.M.Unterwald, J. Fillmore, and M. J. Kreek, Chronic repeated cocaine administration increases dopamine D1 receptor-mediated signal transduction, European Journal of Pharmacology 318 (1996), 31-35.

197. I.Ushijima, A. Carino, and A. Horita, Involvement of D1 and D2 dopamine systems in the behavioral effects of cocaine in rats., Pharmacol Biochem Behav 52 (1995), 737-741.

198. H.Usui, R. Inoue, C. Tanabe, Y. Nishito, M. Shimizu, H. Hayashi, H. Kagamiyama, and M. Takeda, Activation of protein phosphatase 2A by cAMP-dependent protein kinase-catalyzed phosphorylation of the 74kDA B (delta) regulatory subunit in vitro and identification of the phosphorylation sites., FEBS Letters 430 (1998), 312-216.

199. I.T.Uzbay, C. J. Wallis, H. Lal, and M. J. Forster, Effects of NMDA receptor blockers on cocaine-stimulated locomotor activity in mice, Behav Brain Res 108 (2000), 57-61.

200. E.Valjent, J. C. Corvol, C. Pages, J. M. Besson, R. Maldanado, and J. Caboche, Involvement of the extracellular signal regulated kinase cascade for cocaine rewarding properties, J Neuroscience 20 (2000), 8701-8709.

201. E.Valjent, V. Pascoli, P. Svenningsson, S. Paul, E. Herve, J. C. Corvol, A. Stipanovich, J. Caboche, P. J. Lombroso, A. C. Nairn, P. Greengard, D. Herve, and J. A. Girault, Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum., Proc Natl Acad Sci U. S. A 102 (2005), 491-496.

202. F.Van Haaren and M. E. Meyer, Sex differences in locomotor activity after acute and chronic cocaine administration., Pharmacol. Biochem. Behav. 39 (1991), 923-927.

203. M.R.Vossler, H. Yao, M. G. Pan, C. S. Rim, and P. J. Stork, cAMP activates MAP kinase and Elk-1 through a B-raf- and Rap1-dependent pathway, Cell 89 (1997), 73-82.

204. S.I.Walaas, D. W. Aswad, and P. Greengard, A dopamine- and cyclic AMP-regulated phosphoprotein enriched in dopamine innervated brain region., Nature 301 (1983), 69-71.

205. Q.D.Walker, J. Cabassa, A. S. Hilmar, and C. M. uhn, Sex differences in cocaine-stimulated motor behavior: Disparate effects of gonadectomy., Neuropsychopharmacology 25 (2001), 118-130.

206. Q.D.Walker, R. Frances, J. Cabbassa, and C. M. Kuhn, Effect of ovarian hormones and estrous cycle on stimulation of the hypothalamo-pituitary-adrenal axis by cocaine., J Pharmacol Exp Ther 297 (2001), 291-298.

207. Q.D.Walker, C. J. Nelson, D. Smith, and C. M. Kuhn, Vaginal lavage attenuates cocaine-stimulated activity and establishes place preference in rats., Pharmacol Biochem Behav. 73 (2002), 743-752.

208. Q.D.Walker, M. B. Rooney, Wightman R.M., and C. M. Kuhn, Dopamine release and uptake in are greater in female than male rat striatum as measured by fast cyclic voltammetry., Neuroscience 95 (2000), 1061-1070.

209. C.L.Walters and J. A. Blendy, Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse., J Neurosci. 21 (2001), 9438-9444.

210. C.L.Walters, Y. C. Kuo, and J. A. Blendy, Differential distribution of CREB in the mesolimbic dopamine reward pathway., Journal of Neurochemistry 87 (2003), 1237-1244.

211. R.S.Westphal, S. J. Tavalin, J. W. Lin, N. M. Alto, I. D. Fraser, L. K. Langeberg, M. Sheng, and J. D. Scott, Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex., Science 289 (1999), 93-96.

212. F.J.White and D. C. Cooper, The vicious cycle of addiction, Nat Med 7 (2001), 416-417.

213. S.M.White and C. H. T. Lambe, The pathophysiology of cocaine abuse, Journal of Clinical Forensic Medicine 10 (2003), 27-39.

214. R.A.Wise and P. P. Rompre, Brain dopamine and reward, Annu. Rev. Psychol. 40 (1989), 191-225.

215. M.E.Wolf and M. Jeziorski, Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization., Brain Res 613 (1993), 291-294.

216. Wu Q.et al., Preferential increases in nucleus accumbens dopamine following systemic cocaine administration are due to unique characteristics for dopamine neurotransmission, Journal of Neuroscience 21 (2001), 6338-6347.

217. L.Xiao and J. B. Becker, Hormonal activation of the striatum and the nucleus accumbens modulates paced mating behavior in the female rat, Horm Behav 32 (1997), 114-124.

218. T.Yamamoto, K. Matsumoto, H. Kurachi, Y. Okamoto, Y. Nishio, M. Sakata, K. Tasaka, and Y. Murata, Progesterone inhibits transcriptional activation of human chorionic gonadotropin-a gene through protein kinase A pathway in trophoblast cells, Mol Cell Endocrinol. 182 (2001), 215-224.

219. Z.Yan, J. Feng, A. A. Fienberg, and P. Greengard, D(2) dopamine receptors induce mitogen-activated protein kinase and cAMP response element-binding

protein phosphorylation in neurons., Proceedings of the National Academy of Sciences USA 96 (1999), 11607-11612.

220. S.T.Young, L. J. Porrino, and M. J. Iadarola, Cocaine induces striatal c-fos immunoreactive proteins via dopaminergic D1 receptors, Proc Natl Acad Sci 88 (1991), 1291-1295.

221. L.Zaborsky, G. F. Alheid, M. C. Benfield, L. E. Eiden, L. Heimer, and M. Palkovitz, Cholecystoinin innervation of the ventral striatum: a morphological and radioimmunological study, Neuroscience 14 (1985), 427-453.

222. V.Zachariou, V. Sgambato-Faure, T. Sasaki, P. Svenningsson, O. Berton, A. A. Fienberg, A. C. Nairn, P. Greengard, and E. J. Nestler, Phosphorylation of DARPP-32 at Threonine-34 is required for cocaine action., Neuropsychopharmacology 31 (2006), 555-562.

223. Zhang J and Xu M, Opposite regulation of cocaine-induced intracellular signaling and gene expression by dopamine D1 and D3 receptors., Ann. N. Y. Acad. Sci. 1074 (2006), 1-12.

224. L.Zhang, D. Lou, H. Jiao, D. Zhang, X. Wang, Y. Xia, J. Zhang, and M. Xu, Cocaine induced intracellular signaling and gene expression are oppositly regulated by dopamine D1 and D3 receptors, J Neuroscience 24 (2004), 3344-3354.

225. Y.Zhang, P. Svenningsson, R. Picetti, S. D. Schlussman, A. C. Nairn, A. Ho, P. Greengard, and M. J. Kreek, Cocaine self-administration in mice is inversely

related to phosphorylation at Thr34 (protein kinase A site) and Ser130 (kinase CK1 site) of DARPP-32., J Neurosci 26 (2006), 2645-2651.

226. W.Zhou, K. A. Cunningham, and M. L. Thomas, Estrogen regulation of gene expression in the brain: a possible mechanism altering the response to psychostimulants in female rats., Brain Res Mol Brain Res. 100 (2002), 75-83.

227. W.Zhou, N. Koldzic-Zivanovic, C. J. Clarke, R. de Beun, K. Wasserman, B. S. Bury, K. A. Cunningham, and M. Thomas, Selective estrogen receptor modulator effects in rat brain, Neuroendocrinol. 75 (2002), 24-33.

228. Y.Zhou, J. J. Watters, and D. M. Dorsa, Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain., Endocrinology 137 (1996), 2163-2166.

229. X.Zhuang, L. Belluscio, and R. Hen, Golfalpha mediates dopamine D1 receptor signaling., J Neuroscience 20 (2000).