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Amphetamine withdrawal differentially affects hippocampal and peripheral corticosterone levels in response to stress

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Abstract

Amphetamine withdrawal is associated with heightened anxiety-like behavior, which is directly driven by blunted stress-induced glucocorticoid receptor-dependent serotonin release in the ventral hippocampus. This suggests that glucocorticoid availability in the ventral hippocampus during stress may be reduced during amphetamine withdrawal. Therefore, we tested whether amphetamine withdrawal alters either peripheral or hippocampal corticosterone stress responses. Adult male rats received amphetamine (2.5 mg/kg, ip) or saline for 14 days followed by 2 weeks of withdrawal. Contrary to our prediction, microdialysis samples from freely-moving rats revealed that restraint stress-induced corticosterone levels in the ventral hippocampus are enhanced by amphetamine withdrawal relative to controls. In separate groups of rats, plasma corticosterone levels increased immediately after 20 min of restraint and decreased to below stress-naïve levels after 1 h, indicating negative feedback regulation of corticosterone following stress. However, plasma corticosterone responses were similar in amphetamine-withdrawn and control rats. Neither amphetamine nor stress exposure significantly altered protein expression or enzyme activity of the steroidogenic enzymes 11 β -hydroxysteroid dehydrogenase (11 β -HSD1) or hexose-6-phosphate dehydrogenase (H6PD) in the ventral hippocampus. Our findings demonstrate for the first time that amphetamine withdrawal potentiates stress-induced corticosterone in the ventral hippocampus, which may contribute to increased behavioral stress sensitivity previously observed during amphetamine withdrawal. However, this is not mediated by either changes in plasma corticosterone or hippocampal steroidogenic enzymes. Establishing enhanced ventral hippocampal corticosterone as a direct cause of greater stress sensitivity may identify the glucocorticoid system as a novel target for treating behavioral symptoms of amphetamine withdrawal.

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Keywords

Ventral hippocampus; Stress; Amphetamine withdrawal; Psychostimulant withdrawal; 11 β -hydroxysteroid dehydrogenase; Hexose-6-phosphate dehydrogenase

1. Introduction

Amphetamine dependence is a global health problem with a high incidence of relapse and few successful treatment options (Fone and Nutt, 2005; Heal et al., 2013; Pomerleau et al., 2012; Wilens et al., 2008). Amphetamine withdrawal is associated with anxiety and hypersensitivity to stressors in humans (Cleck and Blendy, 2008; Shoptaw et al., 2009) and rodents (Barr et al., 2010; Li et al., 2014; Russig et al., 2006; Tu et al., 2014; Vuong et al., 2010) that can induce relapse in humans (Gossop, 2009) and maintains the cycle of addiction (Koob et al., 2014; Shoptaw et al., 2009).

Stress induces serotonin release in the ventral hippocampus, which has been implicated in reducing anxiety and stress responsiveness (Graeff et al., 1996; Herman et al., 2003; Li et al., 2014; Tu et al., 2014). We have previously found that rats in the second week of withdrawal from repeated amphetamine exposure show heightened behavioral anxiety (Barr et al., 2010; Reinbold et al., 2014; Tu et al., 2014; Vuong et al., 2010), enhanced behavioral measures of stress-induced arousal (Li et al., 2014), and severely blunted stress-induced serotonin release in the ventral hippocampus (Li et al., 2014), which is known to cause increased behavioral anxiety (Tu et al., 2014). Therefore, it is important to understand the mechanism by which amphetamine withdrawal alters stress-induced serotonin levels in the ventral hippocampus.

One mechanism by which amphetamine withdrawal could alter stress-related serotonin function in the ventral hippocampus is via glucocorticoid actions in this region. Stress-induced serotonin release in the ventral hippocampus is mediated by corticosterone activation of local glucocorticoid receptors (Barr and Forster, 2011; Li et al., 2014). Amphetamine withdrawal causes a reduction in ventral hippocampus glucocorticoid receptor expression (Barr and Forster, 2011), which may partly explain the corresponding dampening of stress-evoked serotonin release observed in withdrawn rats (Li et al., 2014). However, glucocorticoid receptor expression is not totally abolished following amphetamine withdrawal, but only reduced by ~30% compared to controls, while mineralocorticoid receptor expression remains unaltered. This raises the possibility that other mechanisms regulating glucocorticoid availability during stress are affected by amphetamine withdrawal to account for blunted glucocorticoid receptor-dependent increases in serotonin release. For instance, stress-induced corticosterone levels in the dorsal hippocampus have been found to increase up to 200% from baseline in drug-naïve rats (Droste et al., 2008; Keeney et al., 2006). Therefore, it is conceivable that stress-induced levels of corticosterone in the ventral hippocampus are reduced or absent during amphetamine withdrawal, lowering corticosterone availability and contributing to the lack of glucocorticoid receptor-dependent stress-induced serotonin release in this region (Li et al., 2014) and to the resultant heightened anxiety states (Tu et al., 2014). This possibility was addressed by the current

study, using the same amphetamine treatment and withdrawal regime previously shown to produce heightened behavioral anxiety (Barr et al., 2010; Reinbold et al., 2014; Tu et al., 2014; Vuong et al., 2010), enhanced stress-induced behavioral arousal (Li et al., 2014), and blunted stress-induced serotonin release in the ventral hippocampus (Li et al., 2014).

Corticosterone is secreted primarily from the adrenal cortex in the periphery and readily crosses the blood brain barrier to act on target tissues, including the hippocampus (Pura and Kreze, 2005; Robel and Baulieu, 1994). Amphetamine withdrawal does not alter basal plasma corticosterone levels relative to saline controls at either 24 h or 4 weeks of withdrawal (Barr et al., 2010), but it is unknown whether stress-induced plasma corticosterone responses are affected. Therefore, we also tested whether amphetamine withdrawal is associated with reduced stress-induced corticosterone levels in the plasma to explain any alterations in ventral hippocampus concentrations.

Glucocorticoid activation of target tissues can also be regulated at the cellular level by extra-adrenal synthesis (Harris et al., 2001) by enzymes such as 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which reduces inert 11-dehydrocorticosterone (11-DHC) to active corticosterone (Harris et al., 2001; Taves et al., 2011). Of the intracellular enzymes that regulate steroidogenesis locally in central tissues including the hippocampus (Harris et al., 2001; Seckl, 1997; Taves et al., 2011), 11 β -HSD1 appears to play a major role in stress-induced alterations of hypothalamic-pituitary-adrenal output regulation (Atanasov et al., 2004; Edwards et al., 1988; Ergang et al., 2014; Harris et al., 2001; Muller et al., 2006; Odermatt and Kratschmar, 2012; Vodicka et al., 2014; Wyrwoll et al., 2011). For example, 11 β -HSD1 mRNA expression was increased in the ventral CA1 hippocampus following a variable 3-day stress protocol (Ergang et al., 2014) and following a resident intruder paradigm of repeated social stress (Vodicka et al., 2014), suggesting that stress may exert its effects on ventral hippocampus corticosterone availability by altering local 11 β -HSD1 activity. Another enzyme, hexose 6 phosphate de-hydrogenase (H6PD), directly interacts with 11 β -HSD1 in central tissue to stabilize 11 β -HSD1 reductase activity (White et al., 2007), regulating 11 β -HSD1-imposed glucocorticoid activation in peripheral and central tissues (Hewitt et al., 2005; White et al., 2007). Increased H6PD production contributes to 11 β -HSD1 up-regulation of glucocorticoids in liver tissues (Wang et al., 2011) but the role of H6PD expression in central glucocorticoid activation is largely unknown (Wang et al., 2011). Therefore, we also tested the hypothesis that amphetamine exposure alters expression and/or activity of either 11 β -HSD1 or H6PD in the ventral hippocampus to alter stress-induced levels of corticosterone during amphetamine withdrawal.

2. Results

2.1. Experiment 1 – amphetamine withdrawal enhances stress-induced corticosterone levels in the ventral hippocampus

2.1.1. Microdialysis probe placements and baseline corticosterone levels in the ventral hippocampus—Representative placements of probe membrane surfaces for the ventral hippocampus are drawn to scale and illustrated in Fig. 1A. Probe placements were similar between saline and amphetamine pretreated rats, and baseline levels of corticosterone also did not differ between saline (1.83 ± 0.07 ng/mL) and amphetamine

(1.65 ± 0.22 ng/mL) pretreatment ($t_{(11)} = 0.717$, $P = 0.488$). Data from rats where the probe missed the ventral hippocampus were excluded from the subsequent analyses.

2.1.2. Stress-induced corticosterone in the ventral hippocampus—

Amphetamine-pretreated rats undergoing withdrawal exhibited increased restraint-induced corticosterone in the ventral hippocampus (Fig. 1B) with a maximal post-stress average of 3.69 ± 0.74 ng/mL at 20 min post-stress. Two-way repeated measure ANOVA revealed significant effects of time ($F_{(9, 96)} = 4.175$, $P < 0.001$) and an interaction between treatment and time ($F_{(9, 96)} = 2.005$, $P = 0.047$) on corticosterone levels. There was no effect of stress over time in saline rats ($F_{(9, 43)} = 0.878$, $P = 0.552$) with maximal levels of corticosterone of 2.63 ± 0.39 ng/mL measured 20 min post-stress. However, an effect of stress over time was observed for amphetamine-pretreated rats ($F_{(9, 53)} = 4.267$, $P < 0.001$) that was apparent at 20 min post-stress as compared to pre-stress levels (Holm-Sidak $P = 0.002$). Ventral hippocampus corticosterone was significantly higher in amphetamine pretreated rats as compared to saline controls immediately following restraint stress (SNK, $P = 0.010$) and 20 min later (SNK, $P = 0.006$) (Fig. 1B).

2.2. Experiment 2 – mechanisms mediating enhanced corticosterone in the ventral hippocampus during amphetamine withdrawal

2.2.1. Amphetamine withdrawal does not alter stress-induced

corticosterone levels in the plasma—A significant effect of stress was observed on total ($F_{(2, 62)} = 88.427$, $P < 0.001$; Fig. 2A), free ($F_{(2, 61)} = 189.847$, $P < 0.001$; Fig. 2B), and bound ($F_{(2, 60)} = 11.702$, $P < 0.001$; Fig. 2C) plasma corticosterone levels. However, no significant effects of amphetamine or saline pretreatment were observed on any measure of plasma corticosterone (total: $F_{(1, 62)} = 0.030$, $P = 0.862$; free: $F_{(1, 61)} = 0.322$, $P = 0.572$; bound: $F_{(1, 60)} = 0.376$, $P = 0.542$). There was no significant interaction present between pretreatment and stress in any of the three measures (total: $F_{(2, 62)} = 0.218$, $P = 0.805$; free: $F_{(2, 61)} = 0.028$, $P = 0.972$; bound: $F_{(2, 60)} = 0.574$, $P = 0.566$) (Fig. 2).

Post-hoc analysis demonstrated that 20 min of restraint stress resulted in higher total (SNK, $P < 0.001$; Fig. 2A), free (SNK, $P < 0.001$; Fig. 2B), and bound (SNK, $P = 0.014$; Fig. 2C) plasma corticosterone levels immediately following restraint relative to stress-naïve levels, and relative to all measures of plasma corticosterone 1 h following restraint (total: SNK, $P < 0.001$; free: SNK, $P < 0.001$; bound: SNK, $P < 0.001$; Fig. 2). All measures of plasma corticosterone levels 1 h following restraint were also decreased relative to levels observed in stress-naïve controls (total: SNK, $P = 0.008$; free: SNK, $P = 0.012$; bound: SNK, $P = 0.016$).

2.2.2. Amphetamine withdrawal does not alter expression of 11 β -HSD1 or H6PD in the ventral hippocampus—

Neither amphetamine pretreatment nor stress exposure altered protein expression of 11 β -HSD1 or H6PD in the ventral hippocampus during withdrawal (Fig. 3). Specifically, protein expression of 11 β -HSD1 in the ventral hippocampus was not significantly affected by pretreatment ($F_{(1, 54)} = 0.001$, $P = 0.977$) or restraint stress ($F_{(2, 54)} = 0.301$, $P = 0.741$), and there was no significant interaction between the two factors ($F_{(2, 54)} = 0.309$, $P = 0.735$; Fig. 3A). Similarly, protein expression of H6PD in the ventral hippocampus was not significantly altered by pretreatment ($F_{(1, 51)} = 1.430$, P

= 0.237) or stress ($F_{(2, 51)} = 2.230$, $P = 0.118$), and there was no significant interaction between pretreatment and stress ($F_{(2, 51)} = 1.153$, $P = 0.324$; Fig. 3B).

2.2.3. Amphetamine withdrawal does not alter activity of 11 β -HSD1 in the ventral hippocampus—Neither amphetamine pretreatment nor stress exposure affected 11 β -HSD1 activity or corticosterone tissue concentration in the ventral hippocampus (Fig. 4). Specifically, corticosterone generated in vitro within ventral hippocampus tissue in the presence of its precursor 11-DHC (as a measure of 11 β -HSD1 activity) was at least 600% greater than corticosterone levels from samples in which 11-DHC was absent, but this increase was not significantly affected by pretreatment ($F_{(1, 46)} = 1.237$, $P = 0.272$) or stress ($F_{(2, 46)} = 2.038$, $P = 0.142$), and there was no significant interaction between the two factors ($F_{(2, 46)} = 0.0898$, $P = 0.914$; Fig. 4).

3. Discussion

3.1. Amphetamine withdrawal enhances stress-induced corticosterone levels in the ventral hippocampus

Given that corticosterone increases serotonin levels in the ventral hippocampus during stress via local glucocorticoid receptor activation (Barr and Forster, 2011), and stress-induced serotonin release in the ventral hippocampus is almost absent during amphetamine withdrawal (Li et al., 2014), we predicted that withdrawal from chronic amphetamine would result in decreased restraint-induced corticosterone in the ventral hippocampus. Instead, we observed an increase in stress-induced corticosterone levels in the ventral hippocampus during amphetamine withdrawal when compared to saline pretreated controls. The reduced stress-induced serotonergic response observed during amphetamine withdrawal, using the same stress paradigm as used here (Li et al., 2014), may instead be explained by a combination of reductions in ventral hippocampus glucocorticoid receptor levels (Barr and Forster, 2011) and increased extracellular serotonin reuptake (Barr et al., 2013), both of which have been observed in the ventral hippocampus following the same amphetamine treatment and withdrawal paradigm used here. It is also possible that surgical and microdialysis procedures may have altered glucocorticoid function in the ventral hippocampus. However, this possibility would be similar in saline and amphetamine-treated rats, since both groups in this and previous work (Li et al., 2014) underwent probe implantation and dialysis.

In the present study, 20 min of novel restraint stress exposure was found to increase free corticosterone levels in the ventral hippocampus by approximately 53% of baseline measures in saline pretreated controls, although this increase was not found to differ significantly from pre-stress levels. In contrast, forty minutes of exposure to a novel environment results in an immediate 3-fold increase in free corticosterone levels in the *dorsal* hippocampus (Droste et al., 2008), and 20 min of restraint stress increases corticosterone levels by 200% within the ventral hippocampus in male Wistar rats 6 months of age (Garrido et al., 2012). The discrepancy between the current and previous findings may result from variation in the type of stressor used or the age of the rats tested.

Overall, increased stress-induced corticosterone in the ventral hippocampus during amphetamine withdrawal may have consequences for the associated heightened anxiety and stress responsiveness (Li et al., 2014; Vuong et al., 2010). Amphetamine withdrawal is associated with a reduced glucocorticoid receptor to mineralocorticoid receptor ratio in the ventral hippocampus (Barr and Forster, 2011). Combined with the augmented ventral hippocampus corticosterone response seen in the current study, this suggests a more prominent effect of mineralocorticoid receptor occupancy in the ventral hippocampus during amphetamine withdrawal. Mineralocorticoid receptors in the hippocampus are thought to mediate the effects of corticosterone in increasing anxiety and fear states (Korte, 2001), thus pointing to a central role of enhanced hippocampal corticosterone and more prominent mineralocorticoid receptor activation in promoting anxiety states during amphetamine withdrawal and representing an important direction to explore in future studies.

3.2. Mechanisms mediating enhanced corticosterone in the ventral hippocampus during amphetamine withdrawal

In the present study, plasma corticosterone levels increased to above stress-naïve levels immediately following 20 min of restraint stress, then decreased to below stress-naïve levels 1 h after restraint. The latter observation is suggestive of negative feedback regulating plasma corticosterone levels post-stress (Ergang et al., 2014; Garrido et al., 2012; Groeneweg et al., 2011; Laryea et al., 2014; Russig et al., 2006; van Haarst et al., 1997). However, amphetamine withdrawal did not alter total, free, or bound levels of either basal (stress-naïve) or stress-induced plasma corticosterone levels relative to those observed in saline-treated controls, suggesting that the capacity for free corticosterone to reach the brain was similar between saline and amphetamine pretreated rats. This lack of difference in stress-induced corticosterone response following 2 weeks of withdrawal from repeated amphetamine is consistent with previous findings using different treatment regimes and/or withdrawal periods. Specifically, Russig et al. (2006) showed that escalating doses of amphetamine (1–10 mg/kg) given over 4 days did not alter corticosterone either basally or in response to 30 min of restraint applied after 2 weeks of withdrawal relative to control levels. Overall, this suggests that amphetamine withdrawal does not alter peripheral corticosterone responses to stress regardless of dose given or length of withdrawal. Further studies are required to determine if this is maintained in response to different types of stress, or is specific to restraint.

With regards to saline pretreated rats, the increase in free corticosterone levels in the plasma was not mirrored by a significant increase in stress-induced ventral hippocampal corticosterone. Similar findings have been observed by others (Droste et al., 2009a, 2009b; Garrido et al., 2012), supporting the suggestion that “a containment mechanism” may exist that prevents overexposure of the brain to glucocorticoid hormones (Droste et al., 2009a). Corticosterone binding globulin (CBG), 11 β -HSD1, and the multidrug resistance I (MDR1) type P glycoprotein in the blood-brain barrier have been proposed as three likely containment mechanisms (de Kloet et al., 2005; Droste et al., 2009a; Garrido et al., 2012) that could result in stress-induced increases of corticosterone in the plasma but not in the central nervous system.

Interestingly, the ratio of free to bound corticosterone was found to be approximately 1:3 in both control and withdrawal conditions in the current study whereas previous findings suggest this ratio is typically 1:10 for rodents (Moisan et al., 2014; Sivukhina and Jirikowski, 2014). Typically, approximately 95% of serum corticosteroids are bound by the binding proteins albumin or CBG, which bind 5–15% and 80–85% of circulating corticosteroids respectively (Baker, 2002; Breuner and Orchinik, 2002; Brien, 1981; Moisan et al., 2014; Pugeat et al., 1981; Sivukhina and Jirikowski, 2014; Sugio et al., 1999; Westphal, 1971). One explanation for the current discrepancy is 2 weeks of daily intraperitoneal injections may have altered the ratio of free to bound corticosterone. In support of this, a single intraperitoneal injection can elicit a corticosterone stress response in the plasma (Meijer et al., 2006) and stress exposure can reduce plasma CBG levels, contributing to greater prevalence of free unbound corticosterone (Fleshner et al., 1995; Spencer et al., 1996). Thus, it seems plausible that in the present study, 2 weeks of daily intraperitoneal injections may have reduce CBG expression or binding in the plasma during the second week of withdrawal, resulting in an altered ratio of bound to free corticosterone content. This possibility should be investigated in the future.

The current findings did not demonstrate an effect of either amphetamine withdrawal or restraint stress on the expression or activity of 11 β -HSD1, or on the expression of H6PD, in the ventral hippocampus. The lack of difference in stress-evoked 11 β -HSD1 expression or activity is in contrast to the finding that exposure to chronic or repeated stress results in increased 11 β -HSD1 mRNA expression in the CA1 region of the ventral hippocampus (Ergang et al., 2014; Vodicka et al., 2014). Combined, these results suggest that unlike repeated stress, acute stress does not increase 11 β -HSD1 and thus the capacity to synthesize corticosterone in the ventral hippocampus. However, the effects of a single stressor on these end measures may be expressed at time points beyond the 1 h post-stress time point used here. Overall, it appears that increased stress-induced corticosterone in the ventral hippocampus of rats undergoing amphetamine withdrawal cannot be explained by greater peripheral synthesis or release of corticosterone, or by enhanced free circulating corticosterone, nor by increased capacity for local synthesis of corticosterone in the ventral hippocampus.

One possible explanation for increased stress-induced corticosterone in the ventral hippocampus shown by amphetamine-withdrawn rats is that amphetamine exposure and withdrawal causes damage to the blood brain barrier that enables the passage of bound corticosterone into the central nervous system, ultimately resulting in greater free levels (as measured by microdialysis) upon dissociation from the binding protein. In support of this hypothesis, methamphetamine exposure and withdrawal has been reported to increase the permeability of the blood brain barrier to otherwise impermeable binding proteins (Kiyatkin and Sharma, 2012; Sharma and Ali, 2006). Furthermore, failure to show differences in bound steroid in the plasma, as in the current study, does not preclude increased binding protein in the cerebrospinal fluid (Schwarz and Pohl, 1994). It is therefore conceivable that chronic amphetamine exposure and withdrawal increases the permeability of the blood brain barrier to binding proteins such as CBG, which is known to play a critical role in maintaining a corticosterone pool accessible to central tissues following stress (Mattos et al., 2013). Greater amounts of bound corticosterone entering through a more permeable blood

brain barrier would then be available to the ventral hippocampus, where corticosterone could dissociate from CBG to become biologically active. This is in accordance with the higher levels of corticosterone in dialysates collected from amphetamine-withdrawn rats, which represent unbound (active) corticosterone levels.

Alternatively, it is also plausible that amphetamine exposure decreases expression or binding capacity of CBG in cerebrospinal fluid or central tissues such as the ventral hippocampus, resulting in greater free levels of stress-evoked corticosterone in this region. Expression of both CBG mRNA and protein have been identified that are localized to the hippocampus (Sivukhina et al., 2013a, 2013b, 2006), and it appears that CBG expression in hippocampal tissue is intrinsic rather than derived from cerebrospinal fluid or plasma (Jirikowski et al., 2007; Orchinik et al., 1997; Sivukhina and Jirikowski, 2014). Thus, it is possible that decreased extracellular CBG could prolong stress-induced elevations in free extracellular corticosterone levels in the ventral hippocampus that are not observed in the plasma.

Finally, although no effect of amphetamine withdrawal or restraint stress was observed on 11 β -HSD1 or H6PD expression or activity in ventral hippocampus tissue, it is possible that amphetamine exposure and withdrawal alters these measures in the cerebrospinal fluid-secreting cells of the choroid plexus epithelium to cause changes in regulation of corticosterone metabolism in the cerebrospinal fluid. In support of this, 11 β -HSD1 and H6PD mRNA expression have been identified in human and rabbit choroid plexus epithelial cells, suggesting the potential to regulate central glucocorticoid availability (Sinclair et al., 2007; Sinclair et al., 2010). Therefore, alterations in 11 β -HSD1 or H6PD expression or activity in the choroid plexus of amphetamine-withdrawn rats may represent a mechanism contributing to enhanced stress-induced corticosterone levels in the ventral hippocampus but not in the plasma.

3.3. Conclusions

Our findings demonstrate for the first time that withdrawal from chronic amphetamine exposure results in enhanced levels of stress-induced corticosterone in the ventral hippocampus, which may contribute to the related increases in anxiety and sensitivity to stress (Li et al., 2014; Tu et al., 2014; Vuong et al., 2010). We show further that enhancement in stress-induced hippocampal corticosterone is not mediated by either peripheral changes in plasma corticosterone levels or by functional expression of enzymes in the ventral hippocampus that regulate local corticosterone availability. Future work could explore the role of corticosteroid binding globulin in the delivery and regulation of hippocampal corticosterone during amphetamine withdrawal. Furthermore, identifying whether enhanced corticosterone in the ventral hippocampus contributes to enhanced sensitivity to stress during withdrawal may identify the glucocorticoid system as a target for treating amphetamine withdrawal.

4. Experimental procedures

4.1. Rodent model of amphetamine pretreatment and withdrawal

Procedures for all experiments in this study were approved by the Institutional Animal Care and Use Committee of the University of South Dakota, and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For all experiments, male Sprague-Dawley rats (Animal Resources Center, University of South Dakota) were weaned at 3 weeks of age and housed in pairs at a constant room temperature of 22° C (60% relative humidity) with a reverse 12-h light/dark cycle, (lights off at 10:00 a.m.) and freely accessible food and water. At 8 weeks of age, cage-paired rats were randomly assigned to receive either saline or d-amphetamine injections (2.5 mg/kg, ip.) for 14 consecutive days (Barr et al., 2010; Barr and Forster, 2011; Li et al., 2014; Tu et al., 2014; Vuong et al., 2010). Injections were administered during the dark phase of the reverse photoperiod (between 11:00 a.m. and 2:00 p.m.). Following this, rats underwent a 2-week withdrawal period, a protocol that reliably enhances anxiety-like behaviors (Reinbold et al., 2014; Tu et al., 2014; Vuong et al., 2010). This same amphetamine treatment and withdrawal regime has been previously shown to produce heightened anxiety-like behaviors (Reinbold et al., 2014; Tu et al., 2014; Vuong et al., 2010), stress-induced behavioral arousal (Li et al., 2014), and blunted stress-induced serotonin release in the ventral hippocampus (Li et al., 2014).

4.2. Experiment 1 – amphetamine withdrawal enhances stress-induced corticosterone levels in the ventral hippocampus

The purpose of this experiment was to determine the effects of amphetamine withdrawal on restraint stress-induced corticosterone in the ventral hippocampus.

4.2.1. Surgical procedures—During the first week of withdrawal from amphetamine, 13 rats underwent aseptic stereotaxic surgery under isoflurane anesthesia (3% isoflurane: 0.3 mL/min oxygen) (Li et al., 2014). Following anesthesia, rats were placed into a stereotaxic frame and implanted unilaterally with a guide cannula (21 gauge; Plastics One, Roanoke, VA, USA) 2 mm above the ventral hippocampus. Guide cannula coordinates relative to bregma were AP –5.2 mm, mL \pm 4.4 mm and DV –8.3 mm (Paxinos and Watson, 1998). The pedestal was anchored to the skull with a combination of support screws, glass ionomer cement (Fuji Plus dental acrylic; Patterson Dental, Minneapolis MN, USA) and cranioplastic acrylic (Plastics One). Following surgery, rats were treated with an analgesic (Ketoprofen, 5 mg/kg, im.; Med-Vet, Libertyville, IL, USA) and allowed to recover for 3 days (Li et al., 2014) before undergoing further experimental procedures.

4.2.2. Microdialysis—Approximately 18 h prior to experimentation, rats were anesthetized with isoflurane and implanted with a laboratory constructed microdialysis probe (Li et al., 2014) that projected 8.3 mm below the cortical surface and had a membrane length of 3.0 mm (MW cut-off 5–6 kDa; Farmer et al., 1996). Free corticosterone (0.35 kDa) can readily pass through the membrane, but bound corticosterone is excluded since the corticosterone binding globulin is 50–60 kDa.

The probes were attached to a liquid swivel (Instech Laboratories, Plymouth Meeting, PA, USA) that allowed for free movement of the rat within a 10-gallon aquarium. Artificial cerebrospinal fluid (4.295 g NaCl, 0.1005 g KCl, 0.062 g NaH₂PO₄, 0.0995 g Na₂HPO₄, 0.1015 g MgCl, and 0.088 g CaCl₂ in 500 mL distilled water; pH 7.2; Sigma-Aldrich, St Louis, MO, USA) was perfused through the probe overnight at a flow rate of 0.5 µL/min.

Flow rate was increased to 0.7 µL/min prior to sample collection, with collection of baseline samples initiated one hour after the start of the dark phase of the reverse photoperiod and continuing at 20 min intervals. Once 3 baseline samples had been collected, rats were subjected to restraint stress for 20 min. Restraint was carried out as previously reported (Li et al., 2014; Lukkes et al., 2009; Mo et al., 2008) by using polyvinyl chloride tubes (27.3 cm × 5.1 cm × 5.1 cm) (Stamper et al., 2015) that prevent movement while still enabling respiration, thus imposing a psychological but not physical stressor. Following the 20 min restraint, dialysate samples were collected for a further 120 min.

4.2.3. Histology—Following final collection of dialysates, rats were killed by an overdose of sodium pentobarbital (0.5 mL Fatal Plus, ip.; Vortech, Dearborn, MI, USA). Brains were removed and fixed in a 10% formalin solution (Fisher Scientific, Pittsburgh, PA, USA) for 3 days before sectioning (60 µm) on a sliding microtome. Coronal sections were then examined under a light microscope by two experimenters blind to outcome to confirm correct probe placement. Only rats with probes located to the ventral hippocampus were included in the subsequent analyses.

4.2.4. Hippocampal corticosterone measurement—Measurement of hippocampal corticosterone from dialysates was performed using an enzyme-linked immunoassay kit (Enzo Life Sciences, Farmingdale, NY). Briefly, dialysates (7.3 µL) were diluted with 102.7 µL of assay buffer for a 15-fold dilution. Duplicates of each sample were treated with 0.5 µL of SDR, and duplicates of each sample, standard, and control were then assayed. Corticosterone levels were detected using an automatic plate reader (Bio-Tek Instruments, Winooski, VT, USA) at an absorbance of 405 nm, with wavelength correction set at 595 nm. Samples were compared to known standards and absorbance values were used to calculate maximum binding percent (14.2–19% range) and percent of non-specific binding (1.9–2.9%). The detection limit sensitivity of the assay was 27.0 pg/mL. Dialysate levels were not corrected for differences in probe recovery. To account for this, dialysate levels were expressed as the % of the average of the baseline samples (Barr et al., 2013; Dorey et al., 2012; Droste et al., 2008, 2009a; Keeney et al., 2006; Li et al., 2014).

4.3. Experiment 2 – mechanisms mediating enhanced corticosterone in the ventral hippocampus during amphetamine withdrawal

The purpose of this experiment was to identify mechanisms underlying the enhanced stress-induced corticosterone levels observed in the ventral hippocampus during amphetamine withdrawal in Experiment 1. Specifically, we investigated whether this enhancement was a result of either augmented increases in stress-induced plasma corticosterone or elevated expression or activity of ventral hippocampus enzymes responsible for regulating local corticosterone metabolism. The steroidogenic enzymes 11β-hydroxysteroid dehydrogenase

type 1 (11 β -HSD1) and hexose 6 phosphate dehydrogenase (H6PD) were selected for testing based on literature suggesting these enzymes as the most likely candidates to affect corticosterone metabolism in the ventral hippocampus (Chapman et al., 2013; Ergang et al., 2014; Harris et al., 2001; Seckl, 1997; Taves et al., 2011; Vodicka et al., 2014). In contrast to earlier studies measuring mRNA expression of these enzymes (Ergang et al., 2014; Vodicka et al., 2014), we chose to use western immunoblotting to determine protein expression, since mRNA expression cannot account for either functional protein expression or post-translational modifications. The latter is important, since earlier studies suggest that 11 β -HSD1 can be glycosylated to alter enzyme activity, and glycosylation may stabilize 11 β -HSD1 reductase function (Seckl, 1997). Therefore, in addition to measuring protein levels of 11 β -HSD1 and H6PD, we also conducted an enzyme activity assay to test the hypothesis that amphetamine withdrawal is associated with increased activity of 11 β -HSD1 in the ventral hippocampus, which may contribute locally to enhanced stress-induced corticosterone in that region during amphetamine withdrawal.

4.3.1. Physical restraint—Restraint stress (as described for Experiment 1) was applied for 20 min to a separate group of amphetamine or saline pretreated rats (n = 70 total, 11–12 per treatment group) during the second week of withdrawal. Following restraint, rats from each pretreatment group were either decapitated immediately (n = 11–12/group), or were transferred back to the home cage for one hour where they remained until decapitation (n = 11–12/group). Stress-naïve controls (n = 12/group) were also sampled to provide baseline measures. All sampling (from 20 min restraint, 20 min restraint + 1 h recovery, and stress-naïve rats) occurred between 11:00 a.m. and 1:00 p.m., with collection from stress-naïve controls time-matched to the stress-groups.

4.3.2. Blood and tissue collection—To eliminate the confound of anesthesia, rats were rapidly decapitated following stress or control conditions. Trunk blood was collected in heparinized tubes and brains were rapidly removed and frozen on dry ice after collection. Blood samples were centrifuged at 5000 rpm, with plasma then drawn off. Both plasma and brain tissue were stored at –80 °C until processing.

4.3.3. Plasma corticosterone measurement—Corticosteroid binding globulin (CBG) has been implicated in regulating the fast actions of glucocorticoids on central tissues (Moisan et al., 2014) and in contributing to neuroendocrine stress response phenotypes in mice (Mattos et al., 2013; Richard et al., 2010), with chronic exposure to morphine increasing CBG in rats (Nock et al., 1997). To test whether amphetamine withdrawal alters plasma corticosterone levels or corticosterone binding to CBG, plasma levels of bound, unbound, and total corticosterone were quantified using an enzyme immunoassay kit (as described for ventral hippocampal samples in Experiment 1), with the following modifications: 10 μ L duplicate samples of plasma from each subject were diluted (1:100) with 990 μ L assay buffer, and all samples were assayed in duplicate, but one of each pair was treated with 0.5 μ L of SDR. This enabled measurement of total corticosterone levels from the non SDR-treated sample, while the SDR-treated sample provided levels of unbound corticosterone. Unbound levels were then subtracted from total levels to provide a measure of bound corticosterone. All corticosterone measurements were expressed as ng/mL of

plasma. The average percentages of maximum and non-specific binding were 18.3% and 2.5% respectively.

4.3.4. Western immunoblotting—Brain tissue was sliced into 300 5m coronal sections in a cryostat maintained at -10°C . The ventral hippocampus was located according to the rat brain atlas of Paxinos and Watson (1998) and microdissected on a freezing stage (Physitemp; North Central Instruments, Inc., Plymouth, MN, USA) using a 20 gauge cannula (Barr and Forster, 2011; Barr et al., 2013). Tissue samples were then expelled into 60 μL 4-(2-hydroxyethyl)-1-piper-azineethanesulfonic acid (HEPES) buffer containing protease inhibitor (28 $\mu\text{L}/\text{mL}$ HEPES) and homogenized by sonication. Protein concentrations of each sample were determined by Bradford immunoassay (Bradford, 1976) with remaining samples stored at -80°C until processed. Equal amounts of each sample (40 μg of protein per lane) were loaded onto 10% SDS-polyacrylamide gels for western immunoblotting. The blotted membranes were then blocked in Tris-buffered saline with 0.1% Tween-20 (TBST) and 5% non-fat dry milk (NFDM) for 1 h at room temperature prior to incubation for 18 h at 4°C in TBST with primary antibodies (rabbit host) against either 11 β -HSD1 (Cat #sc-20175, 1:200, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or H6PD (Cat #sc-67394, 1: 1000 Santa Cruz). Membranes were washed three times with TBST at room temperature, and then incubated for 2 h at room temperature with IRDye800-conjugated polyclonal goat anti-rabbit IgG secondary antibody (Cat #611-132-002, 1:1000, Rockland Inc., Gilbertsville, PA, USA). After this, membranes were washed three times with TBST before visualization. Primary antibodies for 11 β -HSD1 and H6PD were selected based on published literature (Kovacevic et al., 2014; Uschold-Schmidt et al., 2013; Vasiljevic et al., 2014; Xu et al., 2012). To control for protein loading, each membrane was also incubated in mouse anti-actin clone C4 primary antibody (Cat #MAB1501R, 1:2000, EMD Millipore Corp., Darmstadt, Germany) and IRDye 800-conjugated anti-mouse secondary antibody (Cat #610-132-003, 1:5000, Rockland Inc.). Membranes were scanned in a LI-COR scanning machine with an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA) using an 800 nm range filter to visualize proteins, as described previously (Barr and Forster, 2011). Optical densities of the protein bands for 11 β -HSD1 or H6PD for each individual sample were expressed as a mean percentage of the optical density for the β -actin control in each treatment condition. Solutions were made in our laboratory with reagents purchased from Sigma-Aldrich (St. Louis, MO, USA) with the exception of Complete Protease Inhibitor Cocktail Tablets (Roche Diagnostics Corp., Indianapolis, IN, USA).

4.3.5. Ex vivo enzyme activity assay—The 11 β -HSD1 activity assay employed here is based on previously published reports in frozen *ex vivo* hippocampal tissue (Jellinck et al., 1997; Low et al., 1994; Sooy et al., 2010). Ventral hippocampal tissue remaining from the western immunoblotting assay was microdissected from frozen sections and expelled into 60 μL of Krebs-Ringer buffer (Low et al., 1994) and sonicated (Fisher Scientific Model FB50 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA, USA). Protein concentrations of each sample were determined using Bradford immunoassay (Bradford, 1976), with remaining samples stored at 80°C until processing. Tissue samples were then diluted with Krebs-Ringer buffer to 0.5 mg/mL (Low et al., 1994; Sooy et al., 2010), and 70 μL aliquots of

diluted sample from each subject were incubated for 1 h at 37 °C (Sooy et al., 2010) with 70 µL of active or control buffer C: 1 mM EDTA, 300 mM NaCl, 100 mM potassium acetate, 10% glycerol, 10 mM D-glucose-6-phosphate dipotassium salt hydrate, pH 6.0, with “active” buffer C containing 10 nM 11-Dehydrocorticosterone (DHC) substrate and “control” buffer C containing no 11-DHC substrate (Brown et al., 1993; Sooy et al., 2010). Both reaction conditions were run in duplicate for each sample and all reactions were stopped with 140 µL ice-cold methanol and stored at 80 °C (Senesi et al., 2010). Samples were then centrifuged (Senesi et al., 2010) at 14,000 rpm at 4 °C for 11 min, and the supernatant drawn off and assessed for corticosterone levels (ng/mL) using a corticosterone enzyme immunoassay kit (Enzo Life Sciences) as described in Section 4.3.3, with the following modifications: supernatant was treated with 0.5 µL SDR, diluted (1:10) in corticosterone assay buffer and neutralized (pH 7.0). Samples for each subject were run in duplicate, and corticosterone levels in “active” buffer samples were expressed as percentage of “control” buffer samples to provide indirect measures of 11β-HSD1 activity. The average maximum binding was 17.8% and the average nonspecific binding was 2.8%. Levels of corticosterone in the samples were within the detection limit of the assay (27 pg/mL). All reagents were purchased from Sigma-Aldrich, except 11-dehydrocorticosterone (United States Biological, Swampscott, MA, USA).

4.4. Data analysis

Analyses were performed using SigmaStat v3.5 (SPSS Inc., Point Richmond, CA) with a p-value of $P < 0.05$ considered significant. A total of eight data points across all analyses were excluded using the Grubbs' outlier test (Grubbs, 1969). Baseline extracellular corticosterone concentrations (uncorrected for probe recovery) were compared between saline and amphetamine pretreated rats using a paired *t*-test. Hippocampal corticosterone (% of pre-stress levels) was then analyzed among treatment with two-way mixed design ANOVA with one repeated measure (time). One-way repeated ANOVA was used to detect a significant main effect of time within saline-pretreated or amphetamine-pretreated rats, with Holm-Sidak *post-hoc* tests for multiple comparisons against a baseline (−20 min) used to identify which time points differed. A significant interaction between treatment and time was followed up with Student-Newman-Keuls (SNK) *post-hoc* tests for multiple comparisons to identify which time points differed between treatments. Plasma corticosterone, hippocampal protein levels of 11β-HSD1 and H6PD, and corticosterone levels from the hippocampal 11β-HSD1 activity experiment were analyzed by 2-way ANOVA (amphetamine/saline pretreatment × stress condition, i.e., restraint only, restraint + 1 h recovery, stress-naive), with SNK *post-hoc* tests for multiple comparisons used to follow up main effects.

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Abbreviations

11-DHC 11-dehydrocorticosterone

11β-HSD1 11β-hydroxysteroid dehydrogenase

4	-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)
CBG	Corticosteroid binding globulin
H6PD	Hexose-6-phosphate dehydrogenase
SDR	Steroid displacement reagent
SNK	Student-Newman-Keuls

References

- Atanasov AG, Nashev LG, Schweizer RA, Frick C, Odermatt A. Hexose-6-phosphate dehydrogenase determines the reaction direction of 11 β -hydroxysteroid dehydrogenase type 1 as an oxoreductase. *FEBS Lett.* 2004; 571:129–133. [PubMed: 15280030]
- Baker ME. Albumin, steroid hormones and the origin of vertebrates. *J. Endocrinol.* 2002; 175:121–127. [PubMed: 12379496]
- Barr JL, Renner KJ, Forster GL. Withdrawal from chronic amphetamine produces persistent anxiety-like behavior but temporally-limited reductions in monoamines and neurogenesis in the adult rat dentate gyrus. *Neuropharmacology.* 2010; 59:395–405. [PubMed: 20638943]
- Barr JL, Forster GL. Serotonergic neurotransmission in the ventral hippocampus is enhanced by corticosterone and altered by chronic amphetamine treatment. *Neuroscience.* 2011; 182:105–114. [PubMed: 21420472]
- Barr JL, Scholl JL, Solanki RR, Watt MJ, Lowry CA, Renner KJ, Forster GL. Influence of chronic amphetamine treatment and acute withdrawal on serotonin synthesis and clearance mechanisms in the rat ventral hippocampus. *Eur. J. Neurosci.* 2013; 37:479–490. [PubMed: 23157166]
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; 72:248–254. [PubMed: 942051]
- Breuner CW, Orchinik M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol.* 2002; 175:99–112. [PubMed: 12379494]
- Brien TG. Human corticosteroid binding globulin. *Clin. Endocrinol.* 1981; 14:193–212.
- Brown RW, Chapman KE, Edwards CR, Seckl JR. Human placental 11 β -hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology.* 1993; 132:2614–2621. [PubMed: 8504762]
- Chapman K, Holmes M, Seckl J. 11 β -hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol. Rev.* 2013; 93:1139–1206. [PubMed: 23899562]
- Cleck JN, Blendy JA. Making a bad thing worse: adverse effects of stress on drug addiction. *J. Clin. Invest.* 2008; 118:454–461. [PubMed: 18246196]
- de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* 2005; 6:463–475. [PubMed: 15891777]
- Dorey R, Pierard C, Chauveau F, David V, Beracochea D. Stress-induced memory retrieval impairments: different time-course involvement of corticosterone and glucocorticoid receptors in dorsal and ventral hippocampus. *Neuropsychopharmacology.* 2012; 37:2870–2880. [PubMed: 22948976]
- Droste SK, de Groote L, Atkinson HC, Lightman SL, Reul JM, Linthorst AC. Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. *Endocrinology.* 2008; 149:3244–3253. [PubMed: 18356272]
- Droste SK, Collins A, Lightman SL, Linthorst AC, Reul JM. Distinct, time-dependent effects of voluntary exercise on circadian and ultradian rhythms and stress responses of free corticosterone in the rat hippocampus. *Endocrinology.* 2009a; 150:4170–4179. [PubMed: 19477935]
- Droste SK, de Groote L, Lightman SL, Reul JM, Linthorst AC. The ultradian and circadian rhythms of free corticosterone in the brain are not affected by gender: an in vivo microdialysis study in Wistar rats. *J. Neuroendocrinol.* 2009b; 21:132–140. [PubMed: 19076270]

- Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER, Monder C. Localisation of 11 beta-hydroxysteroid dehydrogenase—tissue specific protector of the mineralocorticoid receptor. *Lancet*. 1988; 2:986–989. [PubMed: 2902493]
- Ergang P, Kuzelova A, Sotak M, Klusonova P, Makal J, Pacha J. Distinct effect of stress on 11beta-hydroxysteroid dehydrogenase type 1 and corticosteroid receptors in dorsal and ventral hippocampus. *Physiol. Res*. 2014; 63:255–261. [PubMed: 24397806]
- Farmer CJ, Isakson TR, Coy DJ, Renner KJ. In vivo evidence for progesterone dependent decreases in serotonin release in the hypothalamus and midbrain central grey: relation to the induction of lordosis. *Brain Res*. 1996; 711:84–92. [PubMed: 8680878]
- Fleshner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF. A long-term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. *Endocrinology*. 1995; 136:5336–5342. [PubMed: 7588279]
- Fone KC, Nutt DJ. Stimulants: use and abuse in the treatment of attention deficit hyperactivity disorder. *Curr. Opin. Pharmacol*. 2005; 5:87–93. [PubMed: 15661631]
- Garrido P, de Blas M, Del Arco A, Segovia G, Mora F. Aging increases basal but not stress-induced levels of corticosterone in the brain of the awake rat. *Neurobiol. Aging*. 2012; 33:375–382. [PubMed: 20416975]
- Gossop M. Review: limited evidence to support pharmacological therapy for amphetamine withdrawal. *Evid. Based Ment. Health*. 2009; 12:122. [PubMed: 19854784]
- Graeff FG, Guimaraes FS, De Andrade TG, Deakin JF. Role of 5-HT in stress, anxiety, and depression. *Pharmacol. Biochem Behav*. 1996; 54:129–141. [PubMed: 8728550]
- Groeneweg FL, Karst H, de Kloet ER, Joels M. Rapid non-genomic effects of corticosteroids and their role in the central stress response. *J. Endocrinol*. 2011; 209:153–167. [PubMed: 21357682]
- Grubbs FE. Procedures for detecting outlying observations in samples. *Technometrics*. 1969; 11:1–21.
- Harris HJ, Kotelevtsev Y, Mullins JJ, Seckl JR, Holmes MC. Intracellular regeneration of glucocorticoids by 11beta-hydroxysteroid dehydrogenase (11beta-HSD)-1 plays a key role in regulation of the hypothalamic-pituitary-adrenal axis: analysis of 11beta-HSD-1-deficient mice. *Endocrinology*. 2001; 142:114–120. [PubMed: 11145573]
- Heal DJ, Smith SL, Gosden J, Nutt DJ. Amphetamine, past and present—a pharmacological and clinical perspective. *J. Psychopharmacol*. 2013; 27:479–496. [PubMed: 23539642]
- Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol*. 2003; 24:151–180. [PubMed: 14596810]
- Hewitt KN, Walker EA, Stewart PM. Minireview: hexose-6-phosphate dehydrogenase and redox control of 11{beta}-hydroxysteroid dehydrogenase type 1 activity. *Endocrinology*. 2005; 146:2539–2543. [PubMed: 15774558]
- Jellinck PH, Dhabhar FS, Sakai RR, McEwen BS. Long-term corticosteroid treatment but not chronic stress affects 11beta-hydroxysteroid dehydrogenase type I activity in rat brain and peripheral tissues. *J. Steroid Biochem Mol. Biol*. 1997; 60:319–323. [PubMed: 9219923]
- Jirikowski GF, Pusch L, Mopert B, Herbert Z, Caldwell JD. Expression of corticosteroid binding globulin in the rat central nervous system. *J. Chem. Neuroanat*. 2007; 34:22–28. [PubMed: 17467234]
- Keeney A, Jessop DS, Harbuz MS, Marsden CA, Hogg S, Blackburn-Munro RE. Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. *J. Neuroendocrinol*. 2006; 18:330–338. [PubMed: 16629831]
- Kiyatkin EA, Sharma HS. Environmental conditions modulate neurotoxic effects of psychomotor stimulant drugs of abuse. *Int. Rev. Neurobiol*. 2012; 102:147–171. [PubMed: 22748829]
- Koob GF, Buck CL, Cohen A, Edwards S, Park PE, Schlosburg JE, Schmeichel B, Vendruscolo LF, Wade CL, Whitfield TW Jr, George O. Addiction as a stress surfeit disorder. *Neuropharmacology*. 2014; 76(pt B):370–382. [PubMed: 23747571]
- Korte SM. Corticosteroids in relation to fear, anxiety and psychopathology. *Neurosci. Biobehav Rev*. 2001; 25:117–142. [PubMed: 11323078]

- Kovacevic S, Nestorov J, Matic G, Elakovic I. Dietary fructose-related adiposity and glucocorticoid receptor function in visceral adipose tissue of female rats. *Eur. J. Nutr.* 2014
- Laryea G, Muglia L, Arnet M, Muglia LJ. Dissection of glucocorticoid receptor-mediated inhibition of the hypothalamic-pituitary-adrenal axis by gene targeting in mice. *Front. Neuroendocrinol.* 2014
- Li H, Scholl JL, Tu W, Hassell JE, Watt MJ, Forster GL, Renner KJ. Serotonergic responses to stress are enhanced in the central amygdala and inhibited in the ventral hippocampus during amphetamine withdrawal. *Eur. J. Neurosci.* 2014; 40:3684–3692. [PubMed: 25234335]
- Low SC, Moisan MP, Noble JM, Edwards CR, Seckl JR. Glucocorticoids regulate hippocampal 11 beta-hydroxysteroid dehydrogenase activity and gene expression in vivo in the rat. *J. Neuroendocrinol.* 1994; 6:285–290. [PubMed: 7920594]
- Lukkes JL, Mokin MV, Scholl JL, Forster GL. Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Horm. Behav.* 2009; 55:248–256. [PubMed: 19027017]
- Mattos GE, Heinzmann JM, Norkowski S, Helbling JC, Minni AM, Moisan MP, Touma C. Corticosteroid-binding globulin contributes to the neuroendocrine phenotype of mice selected for extremes in stress reactivity. *J. Endocrinol.* 2013; 219:217–229. [PubMed: 24048966]
- Meijer MK, Spruijt BM, van Zutphen LF, Baumans V. Effect of restraint and injection methods on heart rate and body temperature in mice. *Lab. Anim.* 2006; 40:382–391. [PubMed: 17018209]
- Mo B, Feng N, Renner K, Forster G. Restraint stress increases serotonin release in the central nucleus of the amygdala via activation of corticotropin-releasing factor receptors. *Brain Res. Bull.* 2008; 76:493–498. [PubMed: 18534257]
- Moisan MP, Minni AM, Dominguez G, Helbling JC, Foury A, Henkous N, Dorey R, Beracochea D. Role of corticosteroid binding globulin in the fast actions of glucocorticoids on the brain. *Steroids.* 2014; 81:109–115. [PubMed: 24252379]
- Muller C, Hennebert O, Morfin R. The native anti-glucocorticoid paradigm. *J. Steroid Biochem Mol. Biol.* 2006; 100:95–105. [PubMed: 16713254]
- Nock B, Wich M, Cicero TJ. Chronic exposure to morphine increases corticosteroid-binding globulin. *J. Pharmacol. Exp. Ther.* 1997; 282:1262–1268. [PubMed: 9316834]
- Odermatt A, Kratschmar DV. Tissue-specific modulation of mineralocorticoid receptor function by 11beta-hydroxysteroid dehydrogenases: an overview. *Mol. Cell Endocrinol.* 2012; 350:168–186. [PubMed: 21820034]
- Orchinik M, Hastings N, Witt D, McEwen BS. High-affinity binding of corticosterone to mammalian neuronal membranes: possible role of corticosteroid binding globulin. *J. Steroid Biochem Mol. Biol.* 1997; 60:229–236. [PubMed: 9191981]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 4th. San Diego, CA: Academic Press; 1998. (Imprint of Elsevier)
- Pomerleau AC, Sutter ME, Owen KP, Loomis E, Albertson TE, Diercks DB. Amphetamine abuse in emergency department patients undergoing psychiatric evaluation. *J. Emerg. Med.* 2012; 43:798–802. [PubMed: 22538120]
- Pugeat MM, Dunn JF, Nisula BC. Transport of steroid hormones: interaction of 70 drugs with testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J. Clin. Endocrinol. Metab.* 1981; 53:69–75. [PubMed: 7195405]
- Pura M, Kreze A Jr. From the history of endocrinology: reminiscence of the discovery of adrenocortical hormones. *Cas. Lek. Cesk.* 2005; 144:648–650. (Discussion 650-1). [PubMed: 16193947]
- Reinbold ED, Scholl JL, Oliver KM, Watt MJ, Forster GL. Central CRF receptor antagonism reduces anxiety states during amphetamine withdrawal. *Neurosci. Res.* 2014; 89:37–43. [PubMed: 25205625]
- Richard EM, Helbling JC, Tridon C, Desmedt A, Minni AM, Cador M, Pourtau L, Konsman JP, Mormede P, Moisan MP. Plasma transcortin influences endocrine and behavioral stress responses in mice. *Endocrinology.* 2010; 151:649–659. [PubMed: 20022933]
- Robel P, Baulieu EE. Neurosteroids Biosynthesis and function. *Trends Endocrinol. Metab.* 1994; 5:1–8. [PubMed: 18407181]

- Russig H, Pryce CR, Feldon J. Amphetamine withdrawal leads to behavioral sensitization and reduced HPA axis response following amphetamine challenge. *Brain Res.* 2006; 1084:185–195. [PubMed: 16563358]
- Schwarz S, Pohl P. Steroids and opioid receptors. *J. Steroid Biochem Mol. Biol.* 1994; 48:391–402. [PubMed: 8142317]
- Seckl JR. 11beta-hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? *Front Neuroendocrinol.* 1997; 18:49–99. [PubMed: 9000459]
- Senesi S, Legeza B, Balazs Z, Csala M, Marcolongo P, Kereszturi E, Szelenyi P, Egger C, Fulceri R, Mandl J, Giunti R, Odermatt A, Banhegyi G, Benedetti A. Contribution of fructose-6-phosphate to glucocorticoid activation in the endoplasmic reticulum: possible implication in the metabolic syndrome. *Endocrinology.* 2010; 151:4830–4839. [PubMed: 20826560]
- Sharma HS, Ali SF. Alterations in blood-brain barrier function by morphine and methamphetamine. *Ann. N. Y. Acad. Sci.* 2006; 1074:198–224. [PubMed: 17105918]
- Shoptaw SJ, Kao U, Heinzerling K, Ling W. Treatment for amphetamine withdrawal. *Cochrane Database Syst. Rev.* 2009 Cd003021.
- Sinclair AJ, Onyimba CU, Khosla P, Vijapurapu N, Tomlinson JW, Burdon MA, Stewart PM, Murray PI, Walker EA, Rauz S. Corticosteroids, 11beta-hydroxysteroid dehydrogenase isozymes and the rabbit choroid plexus. *J. Neuroendocrinol.* 2007; 19:614–620. [PubMed: 17620103]
- Sinclair AJ, Walker EA, Burdon MA, van Beek AP, Kema IP, Hughes BA, Murray PI, Nightingale PG, Stewart PM, Rauz S, Tomlinson JW. Cerebrospinal fluid corticosteroid levels and cortisol metabolism in patients with idiopathic intracranial hypertension: a link between 11beta-HSD1 and intracranial pressure regulation? *J. Clin. Endocrinol. Metab.* 2010; 95:5348–5356. [PubMed: 20826586]
- Sivukhina E, Helbling JC, Minni AM, Schafer HH, Pallet V, Jirikowski GF, Moisan MP. Intrinsic expression of transcortin in neural cells of the mouse brain: a histochemical and molecular study. *J. Exp. Biol.* 2013a; 216:245–252. [PubMed: 22996440]
- Sivukhina E, Schafer HH, Jirikowski GF. Differences in colocalization of corticosteroid-binding globulin and glucocorticoid receptor immunoreactivity in the rat brain. *Ann. Anat.* 2013b; 195:219–224. [PubMed: 23279724]
- Sivukhina EV, Jirikowski GF, Bernstein HG, Lewis JG, Herbert Z. Expression of corticosteroid-binding protein in the human hypothalamus, co-localization with oxytocin and vasopressin. *Horm. Metab. Res.* 2006; 38:253–259. [PubMed: 16700007]
- Sivukhina EV, Jirikowski GF. Adrenal steroids in the brain: role of the intrinsic expression of corticosteroid-binding globulin (CBG) in the stress response. *Steroids.* 2014; 81:70–73. [PubMed: 24246737]
- Sooy K, Webster SP, Noble J, Binnie M, Walker BR, Seckl JR, Yau JL. Partial deficiency or short-term inhibition of 11beta-hydroxysteroid dehydrogenase type 1 improves cognitive function in aging mice. *J. Neurosci.* 2010; 30:13867–13872. [PubMed: 20943927]
- Spencer RL, Miller AH, Moday H, McEwen BS, Blanchard RJ, Blanchard DC, Sakai RR. Chronic social stress produces reductions in available splenic type II corticosteroid receptor binding and plasma corticosteroid binding globulin levels. *Psychoneuroendocrinology.* 1996; 21:95–109. [PubMed: 8778907]
- Stamper CE, Hennessey PA, Hale MW, Lukkes JL, Donner NC, Lowe KR, Paul ED, Spencer RL, Renner KJ, Orchinik M, Lowry CA. Role of the dorsomedial hypothalamus in glucocorticoid-mediated feedback inhibition of the hypothalamic-pituitary-adrenal axis. *Stress.* 2015; 18:76–87. [PubMed: 25556980]
- Sugio S, Kashima A, Mochizuki S, Noda M, Kobayashi K. Crystal structure of human serum albumin at 2.5 Å resolution. *Protein Eng.* 1999; 12:439–446. [PubMed: 10388840]
- Taves MD, Gomez-Sanchez CE, Soma KK. Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. *Am. J. Physiol. Endocrinol. Metab.* 2011; 301:E11–E24. [PubMed: 21540450]
- Tu W, Cook A, Scholl JL, Mears M, Watt MJ, Renner KJ, Forster GL. Serotonin in the ventral hippocampus modulates anxiety-like behavior during amphetamine withdrawal. *Neuroscience.* 2014; 281c:35–43. [PubMed: 25241066]

- Uschold-Schmidt N, Peterlik D, Fuchsl AM, Reber SO. HPA axis changes during the initial phase of psychosocial stressor exposure in male mice. *J. Endocrinol.* 2013; 218:193–203. [PubMed: 23720397]
- van Haarst AD, Oitzl MS, de Kloet ER. Facilitation of feedback inhibition through blockade of glucocorticoid receptors in the hippocampus. *Neurochem Res.* 1997; 22:1323–1328. [PubMed: 9355104]
- Vasiljevic A, Bursac B, Djordjevic A, Milutinovic DV, Nikolic M, Matic G, Velickovic N. Hepatic inflammation induced by high-fructose diet is associated with altered 11betaHSD1 expression in the liver of Wistar rats. *Eur. J. Nutr.* 2014
- Vodicka M, Ergang P, Mikulecka A, Rehakova L, Klusonova P, Makal J, Sotak M, Musilkova J, Zach P, Pacha J. Regulation of 11beta-hydroxysteroid dehydrogenase Type 1 and 7alpha-hydroxylase CYP7B1 during social stress. *PLoS One.* 2014; 9:e89421. [PubMed: 24586766]
- Vuong SM, Oliver HA, Scholl JL, Oliver KM, Forster GL. Increased anxiety-like behavior of rats during amphetamine withdrawal is reversed by CRF2 receptor antagonism. *Behav. Brain Res.* 2010; 208:278–281. [PubMed: 19958793]
- Wang Y, Nakagawa Y, Liu L, Wang W, Ren X, Anghel A, Lutfy K, Friedman TC, Liu Y. Tissue-specific dysregulation of hexose-6-phosphate dehydrogenase and glucose-6-phosphate transporter production in db/db mice as a model of type 2 diabetes. *Diabetologia.* 2011; 54:440–450. [PubMed: 21052977]
- Westphal U. Steroid-protein interactions. *Monogr. Endocrinol.* 1971; 4:1–567. [PubMed: 5162490]
- White PC, Rogoff D, McMillan DR, Lavery GG. Hexose 6-phosphate dehydrogenase (H6PD) and corticosteroid metabolism. *Mol. Cell Endocrinol.* 2007; 265–266:89–92.
- Wilens TE, Adler LA, Adams J, Sgambati S, Rotrosen J, Sawtelle R, Utzinger L, Fusillo S. Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. *J. Am. Acad. Child. Adolesc. Psychiatry.* 2008; 47:21–31. [PubMed: 18174822]
- Wyrwoll CS, Holmes MC, Seckl JR. 11beta-hydroxysteroid dehydrogenases and the brain: from zero to hero, a decade of progress. *Front Neuroendocrinol.* 2011; 32:265–286. [PubMed: 21144857]
- Xu D, Liang G, Yan YE, He WW, Liu YS, Chen LB, Magdalou J, Wang H. Nicotine-induced over-exposure to maternal glucocorticoid and activated glucocorticoid metabolism causes hypothalamic-pituitary-adrenal axis-associated neuroendocrine metabolic alterations in fetal rats. *Toxicol. Lett.* 2012; 209:282–290. [PubMed: 22265867]

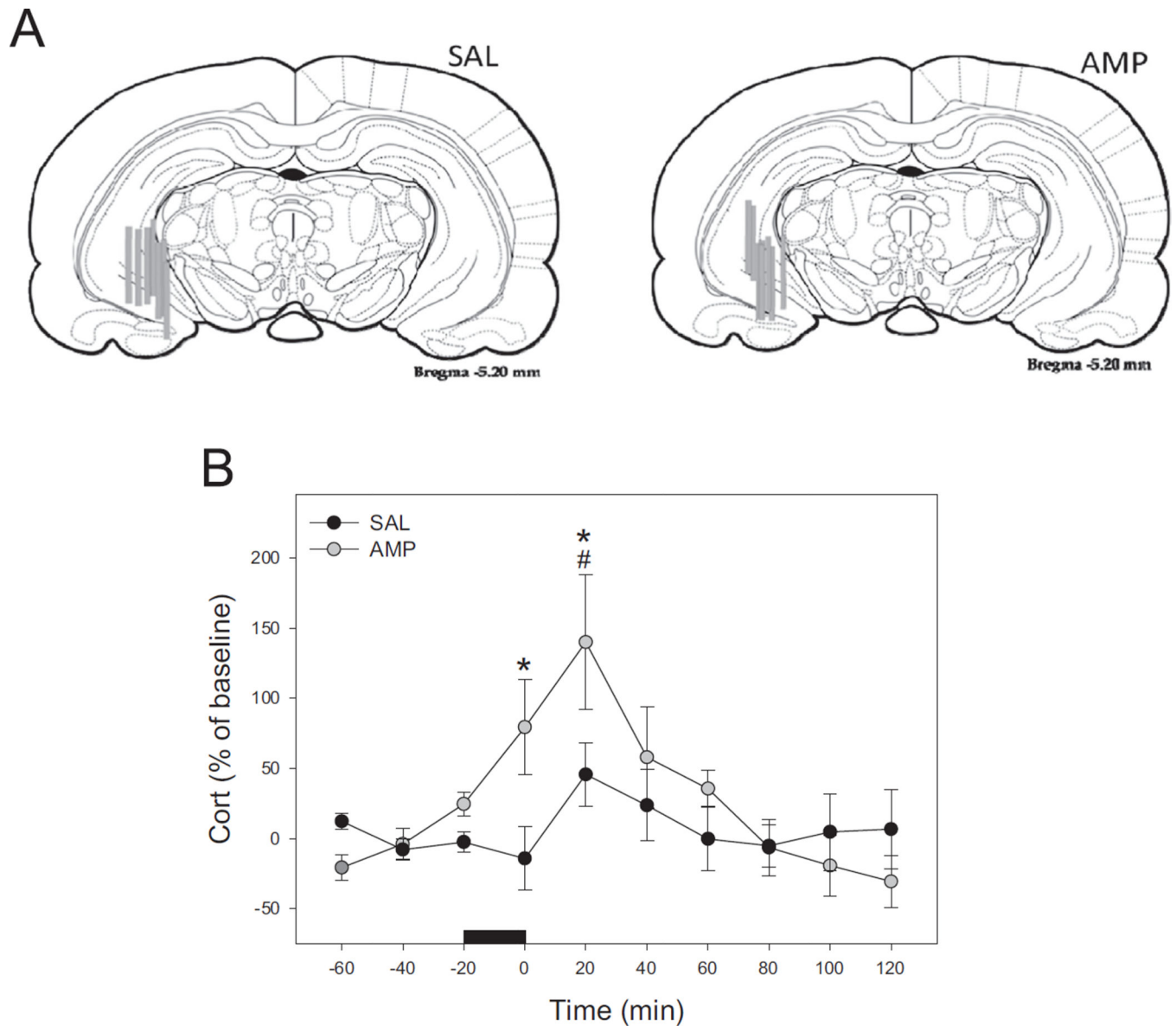
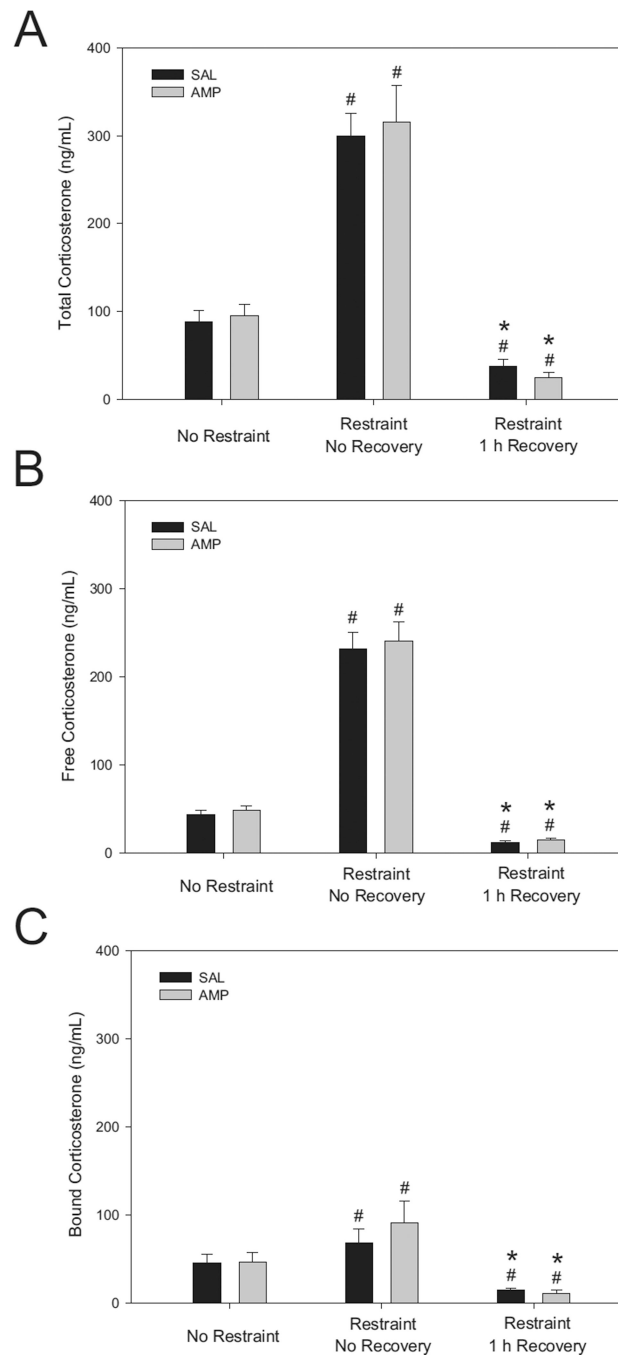
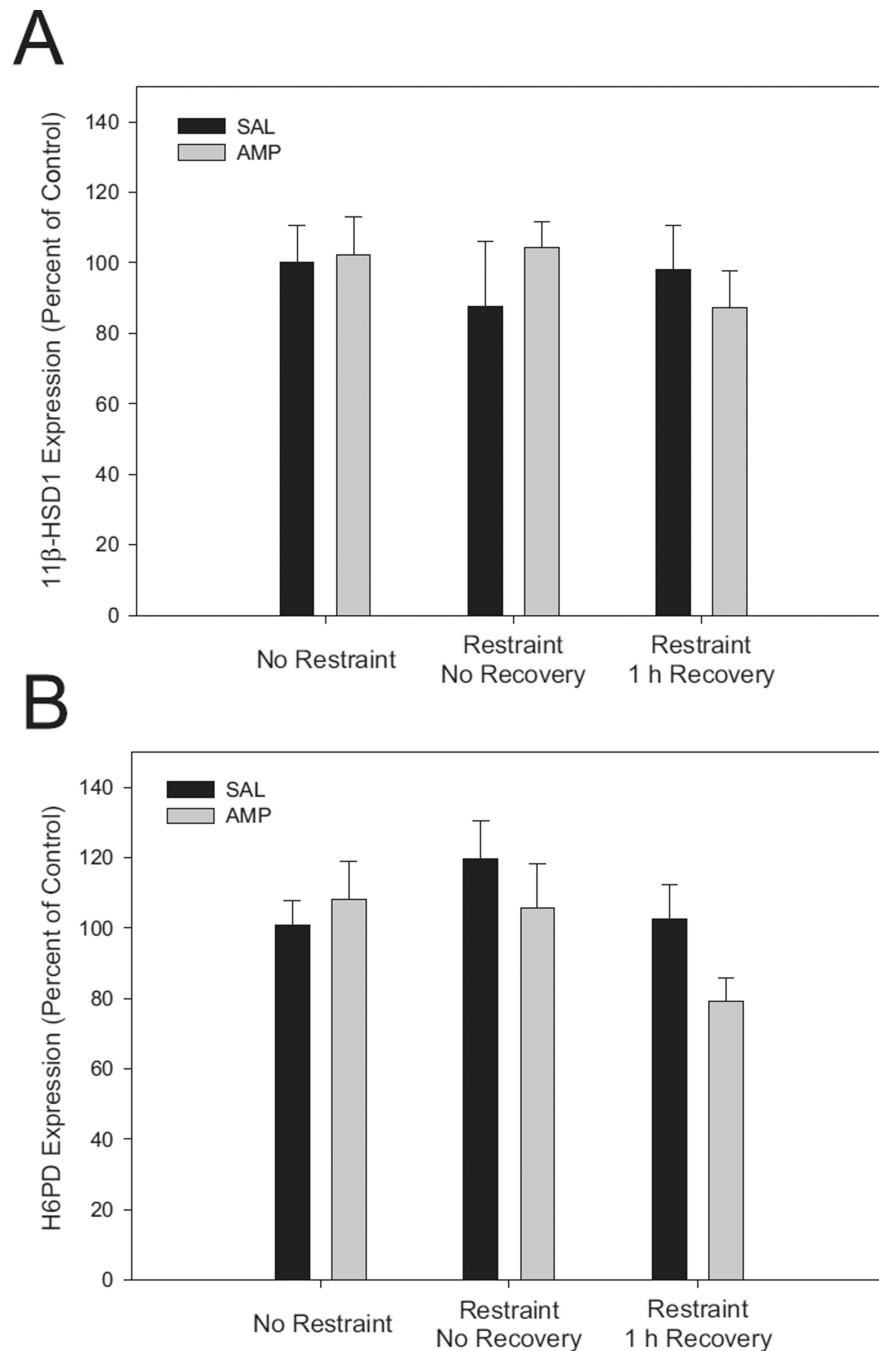


Fig. 1.

(A) Representative diagrams of microdialysis probe membrane placements in the ventral hippocampus of amphetamine and saline treatment groups. Figure adapted from Paxinos and Watson (1998) bregma -5.20 mm. (B) Restraint stress-induced corticosterone levels in the ventral hippocampus were increased in rats undergoing amphetamine withdrawal. Restraint stress was applied for 20 min during the sample period marked by the horizontal bar. N = 7–9 per group. *significant differences between amphetamine and saline treatment groups; #significantly different from baseline (-20 min) levels; P < 0.05.

**Fig. 2.**

(A) Total, (B) free, and (C) bound plasma corticosterone levels increase to above stress-naïve control levels immediately following 20 min of restraint stress, then decrease to below stress-naïve control levels after 1 h, with no significant difference observed between saline and amphetamine pretreated groups ($n = 9-12$ per treatment group per time point, mean \pm SEM). [#]significant difference compared to stress-naïve control group ($P < 0.05$). ^{*}significant difference compared to restraint (no recovery) group ($P < 0.001$).

**Fig. 3.**

Amphetamine withdrawal does not alter expression of (A) 11β-HSD1 or (B) H6PD in the ventral hippocampus before (No Restraint, $n = 11$), immediately (No Recovery, $n = 10$), or 1 h (1 h Recovery, $n = 10$) after restraint stress, compared to saline controls (11β-HSD1 $p = 0.977$, H6PD $p = 0.237$). Restraint stress did not significantly alter 11β-HSD1 or H6PD expression in the ventral hippocampus, compared to controls (11β-HSD1 $p = 0.741$, H6PD $p = 0.118$). Protein band density values are expressed as mean percent of β-Actin controls \pm SEM.

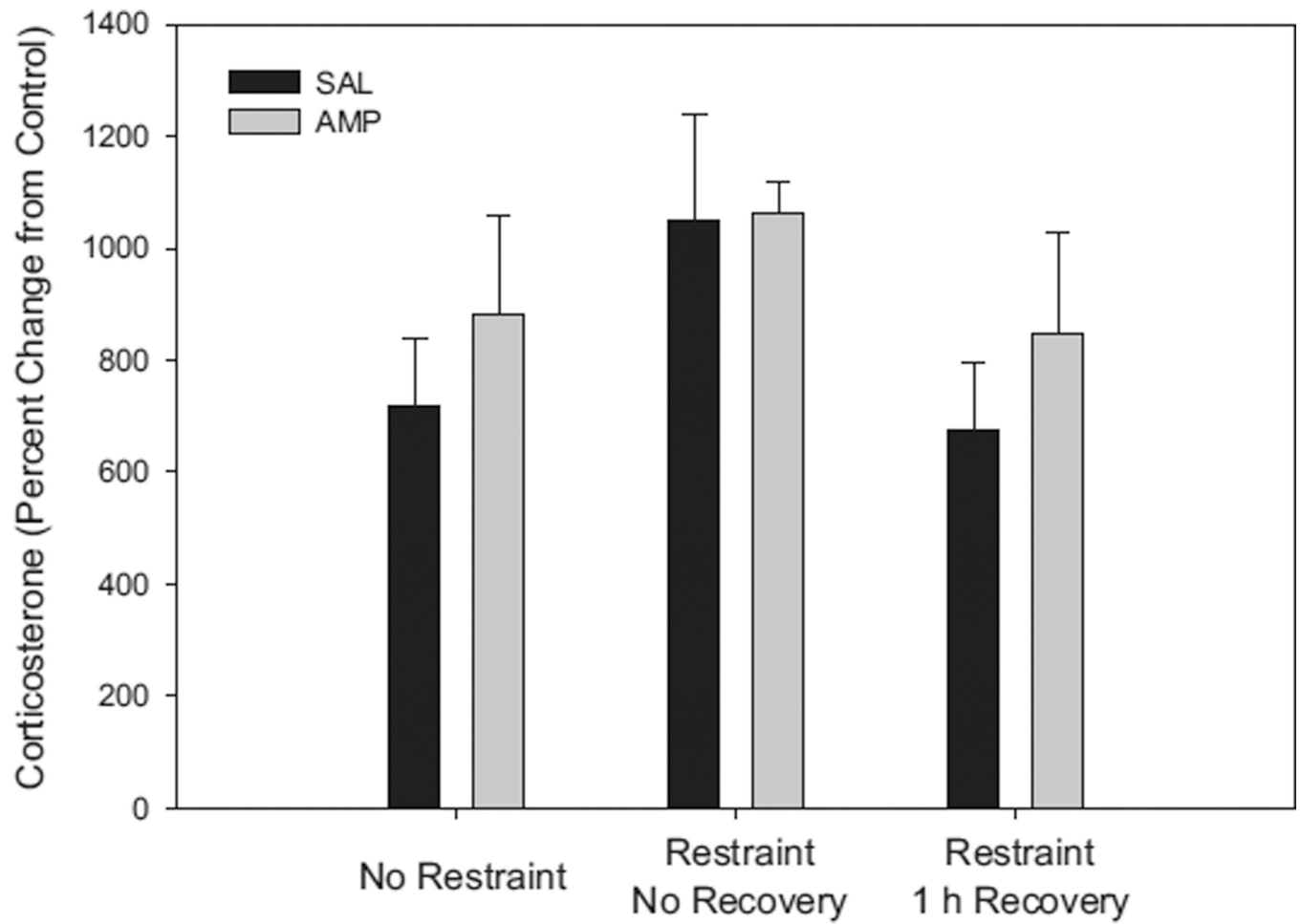


Fig. 4.

Amphetamine pretreatment (gray bars) does not alter 11 β -HSD1 activity in the ventral hippocampus immediately or 1 h after restraint stress (or in stress-naïve controls) relative to saline pretreatment (black bars; $n = 8-10$ per treatment group, mean \pm SEM).