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Chronic administration of glucocorticoid receptor ligands increases anxiety-like behavior and selectively increase serotonin transporters in the ventral hippocampus

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Abstract

Organic cation transporter-3 (OCT3) is widely distributed in the brain with high expression in portions of the stress axis. These high capacity, polyspecific transporters function in monoamine clearance and are sensitive to the stress hormone corticosterone. In rats, withdrawal from chronic amphetamine increases OCT3 expression in specific limbic brain regions involved anxiety and stress responses, including the ventral hippocampus, central nucleus of amygdala (CeA) and dorsomedial hypothalamus. (DMH). Previous studies show that glucocorticoid receptor (GR) agonists increase OCT1 mRNA and OCT2 mRNA expression in non-neural tissues. Thus, we hypothesized that corticosterone increases OCT3 expression in the brain by activating GRs. Male Sprague-Dawley rats were pre-treated daily with the GR antagonist mifepristone (20 mg/kg; sc.) or vehicle followed 45 min later by injections of corticosterone or vehicle for 2 weeks. Corticosterone treatment significantly increased OCT3 expression in the ventral hippocampus and increased anxiety-like behavior. However, these effects were not blocked by mifepristone. Interestingly, treatment with mifepristone alone reduced plasma corticosterone levels and increased serotonin transporter and GR expression in the ventral hippocampus but did not significantly affect OCT3 expression or behavior. No treatment effects on OCT3, serotonin transporter or GR expression were observed in the DMH, CeA or dorsal hippocampus. Our findings suggest that corticosterone increases OCT3 expression in the ventral hippocampus

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Declarations of Interest

All authors report no declarations of interest.

by a mechanism independent of GRs, and that mifepristone and corticosterone can act in an independent manner to affect HPA axis-related physiological and behavioral parameters.

Keywords

Organic cation transporter; corticosterone; mifepristone; stress

1. Introduction

Organic cation transporter 3 (OCT3s), a member of the solute carrier family-22, function as high capacity, low affinity transporters for a variety of cations. The presence of these transporters was characterized in brain tissue in 1998 (Grundermann et al., 1998, Wu et al., 1998). Subsequent studies found that OCT3 is widely distributed in the CNS with high expression levels in a number of brain regions that comprise components of the stress axis (Vialou et al., 2004, Amphoux et al., 2006, Gasser et al., 2009). In the brain, OCTs have been proposed to modify neurotransmission by altering neurotransmitter clearance from extracellular fluid, including the monoamine neurotransmitters, histamine, and acetylcholine (Busch et al., 1996, Grundemann et al., 1998, Koepsell et al., 2007), particularly under conditions during which neurotransmission is enhanced and low capacity, high affinity transporters are saturated (for review, see Daws, 2008, Daws et al., 2013). This function might be especially important in modulating rapid changes in neurotransmission as a response to stress since corticosterone blocks OCT3-mediated transport (Grundermann et al., 1998, Wu et al., 1998, Gasser et al., 2006, Feng et al., 2009, Banganz et al., 2010).

Much of the work implicating OCT3-mediated transport as a modulator of stress has focused on changes in monoaminergic neurotransmission and/or behavior. For example, treatment with corticosterone or decynium-22, both of which can block OCT3 in the rat dorsomedial hypothalamus, which is involved in the integration of the stress response (reviewed in DiMicco et al., 2002) increases extracellular serotonin (Feng et al., 2005; Feng et al., 2009) and alters postsynaptic clearance rates of organic cations, such as serotonin (Gasser et al., 2006). In mice with deficits in serotonin transporter (SERT), OCT3 expression is upregulated in the hippocampus (Schmitt et al., 2002, Baganz et al., 2008). These mice exhibit decreased 5-HT clearance and reduced immobility time when treated with decynium-22, which the authors interpreted as antidepressant-like behavior (Baganz et al., 2008) and the response appears to be corticosterone-dependent (Bagnanz et al., 2010). In the amygdala, OCT3s are expressed in the intercalated cell groups (Hill and Gasser, 2013) and corticosterone-mediated inhibition of OCT3s in this region may play a role in regulating extracellular dopamine. In addition, OCT3 deficient mice exhibit decreased immobility time in forced swim (Kitaichi et al., 2005) and increased open arm/open center time in elevated plus maze (EPM) and open field tests (Wultsch et al., 2009; but see Vialou et al., 2008). Combined, these studies suggest that the behavioral stress response can be modulated by corticosterone blockade of OCT3-mediated monoamine clearance.

In experimental animals, withdrawal from amphetamine is characterized by increases in behavioral measures of anxiety and stress sensitivity (Voung et al., 2010; Barr et al., 2010;

Li et al., 2014, Tu et al., 2014). Previously, we reported that acute (24 h) or chronic (2 weeks) withdrawal from chronic amphetamine increases OCT3 expression in the ventral hippocampus of rats (Barr et al., 2013; Solanki et al., 2016), possibly contributing to the reduction in corticosterone or stress-induced increases in extracellular serotonin in rats chronically pre-treated with amphetamine by increasing serotonin clearance (Barr and Forster, 2011; Barr et al., 2013; Li et al., 2014). The decrease in ventral hippocampal serotonin, which normally functions to decrease anxiety (Joca et al., 2003, Joels et al., 2008), is likely to contribute to the presence of heightened anxiety-like behaviors during amphetamine withdrawal (Tu et al., 2014). In addition, we found that OCT3 and serotonin transporter (SERT) expression in the central nucleus of amygdala (CeA) increased following both acute and chronic amphetamine withdrawal and that OCT3 expression increased in the dorsomedial hypothalamus (DMH) following acute (24 h) drug withdrawal (Solanki et al., 2016). Thus, changes in monoamine transport mechanisms may contribute to dysregulation of limbic regions during amphetamine withdrawal that heighten stress susceptibility (Scholl et al., 2010; Li et al., 2014a).

A potential mechanism by which OCT3 and/or SERT expression in limbic brain regions may be altered by stress or drugs of abuse is through glucocorticoid activation of glucocorticoid receptors (GRs). There is evidence that GR activation can alter OCT expression in peripheral tissues. For example, the GR agonist, dexamethasone, upregulates organic cation transporter 1 (OCT1) in human primary hepatocytes (Rulcova et al., 2013). Similarly, glucocorticoid agonists hydrocortisone and dexamethasone upregulate organic cation transporter 2 (OCT2) mRNA and increased OCT2 mediated 14C tetraethylammonium uptake in Madin-Derby canine kidney cells (Shu et al., 2001). However, mechanisms by which stress or amphetamines can alter OCT3 expression in the brain are unknown. Several studies show that stress and psychostimulants, including amphetamine, increase corticosterone (Knych and Eisenberg., 1979; Swerdlow et al., 1993; Parrott et al., 2014; Gomez-Roman et al., 2015; Sial et al., 2015; Qulu et al., 2015). Combined, previous studies suggest that increased corticosterone following amphetamine administration may increase OCT3 expression in limbic brain regions by acting on GRs. In this study, we hypothesized that corticosterone induces increases in OCT3 expression in the brain through the activation of GRs.

2. Material and Methods

2.1. Animals and drug treatment:

Forty-eight male Sprague-Dawley rats were obtained from the Animal Resource Center of the University of the South Dakota. Rats were housed in pairs at 22°C room temperature and 60% relative humidity in a 12 h light: 12 h dark reversed light/dark cycle with lights off at 1000. Food and water were available *ad libitum*. Procedures were approved by the Institutional Animal Care and Use Committee of the University of South Dakota, and studies were performed during the dark phase of the light cycle.

2.1.1. Mifepristone-Corticosterone treatment: At 8 weeks of age, rats were divided in four groups and were treated with the respective drugs for 14 consecutive days. All

injections were administered during the dark phase, between 1100–1500. Animals were pre-treated with either GR antagonist mifepristone (20 mg/kg; sc.) or propylene glycol vehicle. This concentration of mifepristone blocks GR-mediated effects on amphetamine self-administration (Stairs et al., 2011). Forty-five minutes following pre-treatment, rats were treated with either corticosterone (40 mg/kg, ip.) or vehicle (2-Hydroxypropyl)-β-cyclodextrin (HBC). This concentration of corticosterone administered chronically significantly reduces the time spent in open arms of EPM, which is indicative of elevated anxiety states (Lim et al., 2012).

2.2. Elevated plus maze testing and tissue collection:

Rats were tested for anxiety-like behavior 24 h after the last injection. Anxiety testing was performed using an EPM apparatus (Noldus Information Technology, Wageningen, The Netherlands). The EPM is comprised of four (12 cm wide × 100 cm long) arms, two of which are open and two are enclosed with 40 cm high walls, connected to one another by a central area. To test anxiety-like behavior, a rat was placed in the center space and allowed to freely access all the four arms of EPM for five minutes. Movement of animals was recorded by an automated software (Ethovision XT v5.1, Noldus Technologies) to record the time spent in open and closed arms, and within the center. All behavioral testing was done in the dark phase, between 1100 and 1400.

Four hours following anxiety testing (1500–1800), animals were decapitated; trunk blood and brains were collected and stored on ice at 4°C and on dry ice at −80°C respectively until further use. Blood was centrifuged at 5,000 rpm, supernatant layer of plasma was collected and stored at −80°C.

2.3. Measurement of plasma corticosterone levels:

Plasma corticosterone levels were measured using corticosterone Enzyme Linked Immunosorbent Assay (ELISA) kit (R&D systems, Minneapolis, MN, USA) following manufacturer's instructions (Forster et al., 2008). Briefly, 10 μL of plasma was mixed with 0.5 μL of steroid displacement reagent, the mixture was then diluted with 990 μL of assay buffer and vortexed. This procedure yielded 100-fold dilution of the original plasma samples. Samples, standards (range 32–20,000 pg/mL), and corticosterone controls were assayed in duplicate. At the end of the assay, corticosterone concentration was determined using colorimetric absorbance scanner (Bio-Tek instruments, Winooski, VA, USA) at the scanning wavelength of 405 nm with wavelength correction set at 595 nm. The sensitivity of this assay was 22 pg/mL and non-specific binding was 5.03%.

2.4. Measurement of OCT3, SERT and GR Expression:

Given that SERT expression often changes concurrently with OCT3 expression in the brain (Solanki et al., 2016), we assessed SERT along with OCT3 expression in the brain regions of interest. These brain regions included the ventral hippocampus, the CeA and the DMH, based on previous findings showing amphetamine treatment increased OCT3 expression and function in these regions (Barr et al., 2013; Solanki et al., 2016). The dorsal hippocampus was included as control region as OCT3 expression is not altered in this region by stimulant

treatment (Solanki et al., 2016). Furthermore, we determined whether the glucocorticoid treatment altered the expression of GRs in these same brain regions.

2.4.1. Cryostat brain sectioning and tissue microdissection: Brains were sectioned in a cryostat (−10°C) at the thickness of 300 μM in the coronal plane. Sections were thaw mounted on glass-slides and stored at −80°C until microdissection. The ventral and dorsal hippocampus, DMH and CeA were identified using the Paxinos & Watson brain atlas (1997). Brain regions were bilaterally microdissected on a freezing stage (Physiotemp, North Central Instruments) and tissue collected in 40 μL of HEPES buffer (1.19%, pH 7.5) containing 0.1 μM of protease inhibitor. Microdissected tissue was sonicated to disrupt the cells (Fisher scientific, PA, ISA) and protein concentration was determined using the Bradford method (Bradford et al., 1976) (BioRad Laboratories, Hercules, CA, USA) and read on microplate reader (Bio-Tek Instruments, Winooski, VT, USA) (Solanki et al., 2016).

2.4.2. Western blot: Procedures were followed as described in Solanki et al., 2016. Briefly, homogenized samples were mixed with 1.5 M loading buffer containing β-mercaptoethanol followed by 3 min of boiling. Once the samples cooled, they were loaded (50 μg for dorsal and ventral hippocampus; 40 μg for CeA; 35 μg for DMH) on 10% sodium dodecyl sulfate polyacrylamide gel for electrophoresis (BioRad Laboratories) at 90 V for 1.5–2 hrs. Following electrophoresis, proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (0.2 μm, BioRad Laboratories) by a semi dry blotting apparatus (BioRad Laboratories) for 90 min. Then, the PVDF membrane was blocked in 5% skim milk and 1% bovine serum albumin overnight at 4°C. The PVDF membrane was incubated with rabbit polyclonal OCT3 primary antibody (1:1000 dilution; OCT31-A. Alpha Diagnostic International, TX, USA; Solanki et al., 2016; Barr et al., 2013; Gasser et al., 2009) for 24 h at 4°C and washed in tris buffered saline with tween-20 (TBST) 3 times (10 min each). The membranes were then incubated with IRDye800-conjugated goat anti-rabbit IgG secondary antibody (1:5000 dilution; 611-132-122, Rockland, PA) and washed three times in TBST. Membranes were scanned and subsequently washed in TBST and reprobed with Actin (1:2000 dilution; Anti-Actin, clone 4; MAB1501R, Millipore, CA, USA), SERT (1:200 dilution; goat polyclonal against SERT; sc-1458. Santa-Cruz Biotechnology Inc, CA, USA; Solanki et al., 2016; Barr et al., 2013) and GR (1:200 dilution; rabbit polyclonal IgG; sc-1004, Santa-Cruz Biotechnology, USA; Barr & Forster, 2011). The secondary antibodies used to tag above proteins were Cy2 AffiniPure goat anti-mouse secondary IgG antibody (1:5000 dilution; 115-225-003, Jackson Immunoresearch laboratories Inc., PA, USA), AMCA-AffiniPure rabbit anti-goat IgG secondary antibody (1:5000 dilution; 305-155-003, Jackson Immunoresearch, PA, USA) and IRDye800-conjugated goat anti-rabbit IgG secondary antibody (1:5000 dilution; 611-132-122, Rockland, PA). Membranes were scanned using an infrared LiCor imaging system with the 800-channel filter (Odyssey CLx, LiCor Biosciences, NE, USA) to visualize protein brands. Optical densities from each individual sample were corrected against their actin density, and expressed as a percentage of the loading control.

2.5. Statistics:

Separate two-way ANOVA were used to determine the effects of treatment on EPM measures, plasma corticosterone, and OCT3, GR and SERT expression. Significant interactions were followed by Student-Newman-Keul's (SNK) *post hoc* test for multiple comparisons. Linear regression analysis were used to assess a relationship between distance moved and time spent in open arms of the EPM. All analyses were performed using SigmaStat v.3.5, with significance determined with $P < 0.05$.

3. Results

3.1. Effect of corticosterone and mifepristone on anxiety-like behavior:

A significant main effect of corticosterone treatment was observed on anxiety-like behavior within the EPM (Fig. 1A). Specifically, rats treated with corticosterone showed significantly decreased time spent in open arms of the maze ($F_{(1, 30)} = 4.771$, $P = 0.037$). There was no main effect of mifepristone pre-treatment ($F_{(1, 30)} = 1.432$, $P = 0.241$), nor a significant interaction between mifepristone pre-treatment and corticosterone treatment ($F_{(1, 30)} = 0.139$, $P = 0.712$). There was a significant main effect of corticosterone treatment on total distance moved in the maze ($F_{(1,29)} = 9.771$, $P = 0.004$; Fig. 1B). Specifically, there was reduced mobility in corticosterone treatment groups relative to the controls. Mifepristone pre-treatment had no effect on total distance moved in the maze ($F_{(1,29)} = 0.831$, $P = 0.370$). There was also no significant interaction between mifepristone pre-treatment and corticosterone treatment ($F_{(1,29)} = 0.085$, $P = 0.773$). Thus, the decrease in time spent in open arms in corticosterone treated group may partly be due to corticosterone-induced decreased mobility in these groups. Somewhat supportive of this, a weak but non-significant relationship was observed between these two behavioral variables in corticosterone treated groups ($r^2 = 0.165$; $F_{(1,16)} = 4.36$, $P = 0.053$; Fig. 1C).

3.2. Effect of corticosterone and mifepristone on circulating plasma corticosterone levels:

There was a significant main effect of mifepristone pre-treatment on circulating plasma corticosterone levels ($F_{(1,43)} = 4.637$, $P = 0.037$; Fig. 2). Specifically, mifepristone treatment decreased plasma corticosterone compared to vehicle-treated rats. Corticosterone treatment had no effect on plasma corticosterone levels ($F_{(1,43)} = 0.000$, $P = 0.997$) and the interaction between pre-treatment with mifepristone and treatment with corticosterone on plasma corticosterone levels was not significant ($F_{(1,43)} = 2.371$, $P = 0.131$).

3.3. Effect of corticosterone and mifepristone treatment on OCT3, SERT and GR protein expression:

There was a significant main effect of corticosterone-treatment on OCT3 expression in the ventral hippocampus ($F_{(1,42)} = 4.570$, $P = 0.038$; Fig. 3A), with corticosterone increasing OCT3 immunoreactivity in this region. Mifepristone pre-treatment did not significantly affect OCT3 expression in the ventral hippocampus ($F_{(1,42)} = 0.134$, $P = 0.716$; Fig. 3A), and there was no significant interaction between mifepristone and corticosterone on OCT3

expression in the ventral hippocampus ($F_{(1,42)}$ = 0.538, P= 0.467). All OCT3 bands (45kDa) expressed as percent of Actin (40kDa) as the loading control.

In contrast, there were significant main effects of mifepristone pre-treatment on SERT and GR expression in ventral hippocampus. Mifepristone pre-treatment significantly increased SERT ($F_{(1,41)}$= 5.309, P= 0.026; Fig. 3B) and GR ($F_{(1,42)}$= 0.035, P =0.035; Fig. 3C) expression compared to propylene glycol vehicle treatment. Corticosterone treatment did not significantly affect SERT ($F_{(1,41)}$= 0.135, P =0.715; Fig. 3B) or GR ($F_{(1,42)}$= 1.731, P =0.195; Fig. 3C) expression in the ventral hippocampus. Furthermore, there was no significant interaction between mifepristone and corticosterone on SERT ($F_{(1,41)}$= 1.325, P =0.256; Fig. 3B) and GR ($F_{(1,42)}$= 0.222, P = 0.640; Fig. 3C) expression in the ventral hippocampus. All SERT bands (70kDa) and GR bands (80kDa) expressed as percent of Actin (40kDa) as the loading control.

Corticosterone or mifepristone did not significantly affect OCT3, SERT or GR expression in any of the other brain regions studied (P > 0.05 for all comparisons; Tables 1–3).

4. Discussion

Fourteen days of corticosterone treatment increased anxiety-like behavior, and decreased overall activity in the EPM compared to HBC vehicle-treated controls, and pre-treatment with the GR antagonist mifepristone did not block these behavioral effects of corticosterone. Moreover, mifepristone, by itself, did not have any effect on anxiety-like behavior in the EPM, similar to chronic intracerebroventricular administration of mifepristone (Oitzl et al., 1998). Our finding that chronic corticosterone treatment elevates anxiety states adds to other studies suggesting that chronic corticosterone administration is associated with elevated anxiety- and depression-like conditions in rats (Murray et al., 2008; Diniz et al., 2011; Lim et al., 2012). However, we show that corticosterone treatment also significantly reduced total distance moved in open arms. Linear regression analysis showed a weak non-significant relationship (accounting for 16.5% of the variance) between total distance moved and time spent in the open arms. Thus, increases in anxiety measures in corticosterone-treated groups may be in part, due to a decrease in overall movement, which should be examined in more detail in future work.

Acute concentration of circulating plasma corticosterone levels remained unchanged when tested following chronic corticosterone treatment, similar to a previous report in which plasma corticosterone levels were measured 24 h following corticosterone injection (Quadrilatero and Hoffman-Goetz, 2005). Hence, it is likely that the elevated anxiety state observed following chronic corticosterone treatment is not related to this peripheral marker of hypothalamus-pituitary-adrenal (HPA) axis action. However, mifepristone treatment decreased plasma corticosterone levels compared to the both the vehicle-treated control and the corticosterone-treated groups, suggesting enhancement of negative feed-back of the HPA axis. Theoretically, GR activation is thought to negatively regulate HPA axis leading to reduction in plasma corticosterone levels (De Kloet et al., 1998; Smith et al., 2006). Hence, GR antagonism would be predicted to increase plasma corticosterone levels, as shown by some studies (Ghosal et al., 2014; Flores et al., 2006; Lenze et al., 2014) which is opposite

to our finding. This difference may be explained by mifepristone acting as a partial agonist at GRs, and the observation that partial agonistic activity may overcome antagonistic effect (Zhang et al., 2007; Gruol et al., 1993; Havel et al., 1996). Thus, it is possible that chronic mifepristone treatment at the dose and period used here results in a net agonistic instead of antagonistic action.

The effects of chronic mifepristone on plasma corticosterone may also be related to mifepristone-induced changes in neural GR expression. Zhang et al. (2007) demonstrated that mifepristone exerted partial agonistic activity on GRs and increased GR expression. Similarly, we observed increased GR expression in the ventral hippocampus following mifepristone treatment, which is likely to increase GR-mediated negative feed-back of the HPA axis to reduce plasma corticosterone levels (Smith et al., 2006; De Kloet et al., 1998).

We also observed differential effects of mifepristone and corticosterone on OCT3 protein expression. Chronic corticosterone treatment increased OCT3 expression in the ventral hippocampus, an effect that was not blocked by GR antagonism by mifepristone. In contrast, neither CORT nor mifepristone alone significantly affected OCT3 expression in the DMH, CeA or dorsal hippocampus. Mifepristone treatment alone increased both SERT and GR expression in the ventral hippocampus and this effect was not observed in any of the other brain regions studied. Expression of SERT and GR were not significantly affected by chronic corticosterone treatment in any brain region studied. Overall, these results indicate that the chronic administration of glucocorticoid ligands has regionally specific effects on the expression of OCT3, SERT and GRs and suggests that the increase in OCT3 expression in the ventral hippocampus is independent of GR activation by corticosterone.

The current findings in the ventral hippocampus are in line with increased OCT3 expression in the ventral hippocampus of rats that exhibit heightened anxiety in response to acute withdrawal from amphetamine (Barr et al., 2013). In contrast, SERT knockout mice exposed to chronic stress showed decreased expression of OCT3 in the hippocampus (Baganz et al., 2010). This finding was explained by suggesting that repeated blockade of OCT3 by stress-induced corticosterone may act similarly to repeated antagonism of other transporters, such as SERT, in down regulating transporter expression (Baganz et al., 2010). It is possible that these disparate results may be related to the SERT knockout mice, which already had a compensatory increased expression of hippocampal OCT3 (Baganz et al., 2010).

Serotonin clearance represents a likely mechanism for OCT3 modulation of stress effects in the hippocampus. The hippocampus is believed to function in adaptive responses to stress (reviewed in McEwen, 2002, Radley et al., 2015). In SERT deficient mice, corticosterone and decynium-22 blockade of OCTs in the CA3 of the dorsal hippocampus decrease serotonin clearance and behaviorally, these animals exhibit antidepressant-like effects (Baganz et al., 2008, 2010). In studies examining the underlying mechanisms associated with amphetamine withdrawal, we found that OCT3 expression was increased in amphetamine-treated rats (Barr et al., 2013, Solanki et al., 2016). In these rats, corticosterone- and decynium-22-induced increases in serotonin the ventral hippocampus were attenuated relative to controls, possibly because higher concentrations of the drugs may be required to counteract serotonin clearance augmented through higher expression

of OCT3 relative to controls (Barr and Forster, 2011, Barr et al., 2013). Consistent with this possibility, rats undergoing amphetamine withdrawal exhibit pronounced deficits in stress-induced increases in hippocampal serotonin (Li et al., 2014). The changes in hippocampal serotonin appear to be directly related to heightened anxiety since local hippocampal serotonergic lesions increase anxiety and the increased anxiety-like behavior exhibited during amphetamine withdrawal is reversed by intra-hippocampal infusions blocking serotonin reuptake. Combined, these results suggest that decreases in serotonin in the ventral hippocampus contribute to the increase in anxiety-like behavior exhibited during amphetamine withdrawal (Vuong et al., 2010).

Alterations in protein expression of OCT3, GR and SERT in response to corticosterone or mifepristone treatment was only observed in the ventral hippocampus and not in the CeA, DMH or dorsal hippocampus. The ventral hippocampus contains the highest concentration of GRs in the brain (Sapolsky et al., 1984; Smith et al., 2006) which may make it more vulnerable to chronic corticosterone and mifepristone treatment as compared to other brain regions. In the DMH, OCT3s are highly expressed in the ependymal layer lining the ventricles and are believed to contribute to the corticosterone inhibition of serotonin clearance corticosterone-mediated inhibition of serotonin clearance under conditions of increased serotonergic activity (Feng et al., 2009). In the DMH, OCT3 expression increased at 24 h post-amphetamine withdrawal but not at 2 weeks. In the CeA, OCT3s are present in modest levels (Gasser et al., 2006) including expression in the intercalated cell groups (Hill et al., 2013). In response to both acute and chronic amphetamine withdrawal, expression of both OCT3 and SERT increase in the CeA (Solanki et al., 2016). Our results suggest that these changes in OCT3 and/or SERT following amphetamine withdrawal appear to be independent of GR activation.

Our findings support a role for corticosterone in upregulating OCT3 protein expression in the ventral hippocampus, similar to the glucocorticoid-induced upregulation of OCT1 and OCT2 found in non-neural tissues (Rulcova et al., 2013; Shu et al., 2001). However, it was surprising that the corticosterone-induced increase in OCT3 expression in the ventral hippocampus was not blocked by the GR antagonist mifepristone, suggesting a GR-independent mechanism, or alternatively, a shift from antagonistic effects to agonistic effects (Zhang, 2007) coinciding with the increased GR expression in the vHipp. Our findings also suggest that chronic corticosterone treatment does not regulate SERT expression in any of the regions studied. However, administration of chronic mifepristone upregulated SERT protein expression in the ventral hippocampus. Mifepristone has been shown to block SERT (Li et al., 2014) which may have contributed to the increase in SERT expression. Increases in SERT expression have also been found following chronic social defeat stress, which were prevented by combined treatment of mifepristone and spironolactone (Zhang et al., 2012) suggesting a role for mineralocorticoid receptors rather than GR alone. Combined, the results suggest that chronic mifepristone treatment has the potential to alter serotonergic transmission in the ventral hippocampus.

Conclusions:

Our results suggest that OCT3 expression in the ventral hippocampus is upregulated by chronic corticosterone treatment, an effect that is independent of GR activation. This effect is specific to the ventral hippocampus, and may contribute the increased anxiety states induced by either chronic corticosterone or repeated exposure to stressors. The molecular mechanism by which corticosterone increased OCT3 expression in the ventral hippocampus; and whether blockage of ventral hippocampal OCT3s alleviates anxiety-like behavior is yet to be determined. Furthermore, chronic mifepristone treatment increases SERT and GR expression, again specific to the ventral hippocampus, and the latter finding may underlie reduced plasma corticosterone observed after chronic mifepristone treatment. Mechanisms of mifepristone-mediated reductions in plasma corticosterone and increases in GRs and SERT should be further elucidated as mifepristone is clinically used to treat psychotic major depression in patients with elevated HPA axis activity. Overall, our results contribute to the understanding how central regulation of OCT3 expression is altered by stress exposure, as well as provide new insights into the potential effects of chronic mifepristone treatment.

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Abbreviations

CeA	Central nucleus of amygdala
DMH	Dorsomedial hypothalamu
EPM	Elevated plus maze
ELISA	Enzyme-linked immunosorbent assay
GR	Glucocorticoid receptor
HPA	Hypothalamus-pituitary-adrenal
OCT1	Organic cation transporter 1
OCT2	Organic cation transporter 2
OCT3	Organic cation transporter 3
PVDF	Polyvinylidene difluoride
SERT	Serotonin transporter
SNK	Student-Newman-Keul’s
TBST	Tris buttered saline with tween-20

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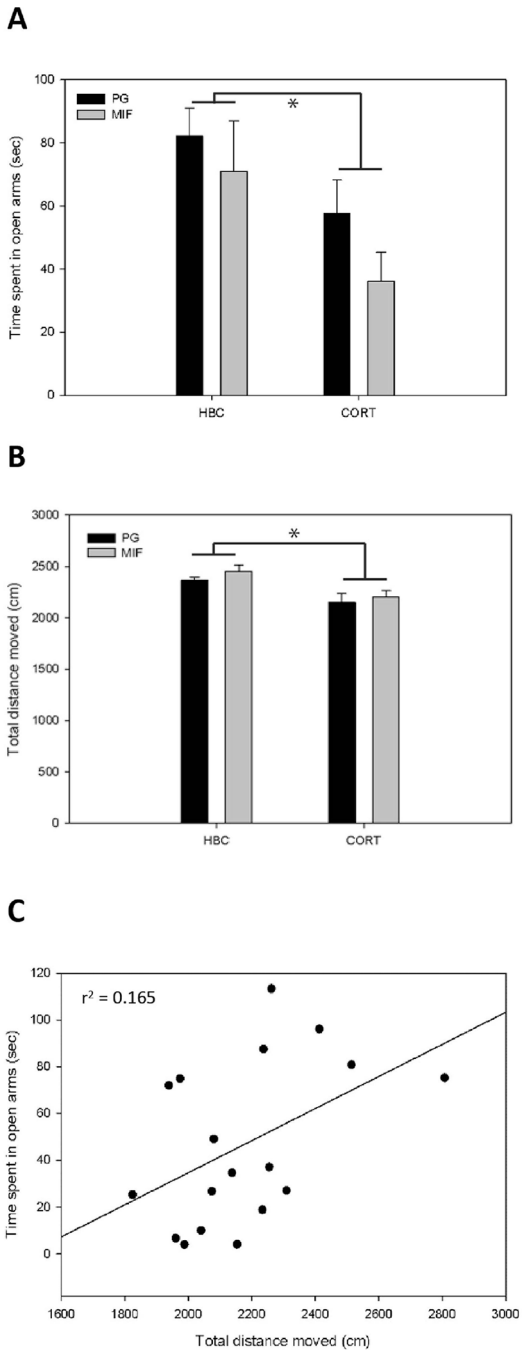

Figure. 1:

Effect of mifepristone pre-treatment and corticosterone treatment on behavior in the EPM. (**A**) Corticosterone, but not mifepristone treatment significantly reduced the time spent in the open arms of the maze compared to the HBC-PG vehicle treatment. (**B**) Corticosterone, but not mifepristone treatment significantly reduced the total distance moved in the maze compared to the HBC-PG vehicle treated group during the 5 min EPM anxiety test. Means expressed as percentage of control, data in the graphs represent Mean ± SEM; *indicates significant difference between groups, P< 0.05. **(C)** There was a weak non-significant

relationship between total distance moved and time spent in open arms within the two corticosterone treated groups, PG/CORT and MIF/CORT. (P> 0.05).

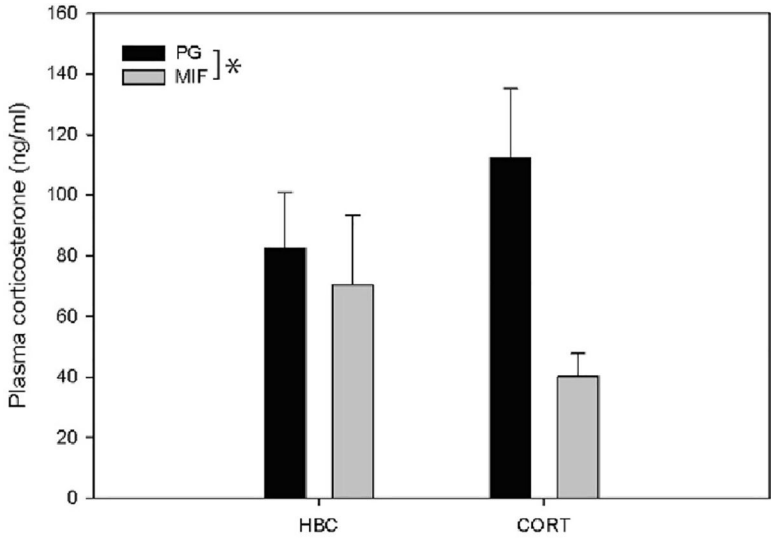

Figure. 2:

Effect of mifepristone pre-treatment and corticosterone treatment on plasma corticosterone levels as measured by ELISA: Mifepristone but not corticosterone treatment decreased plasma corticosterone levels 28 hours post-treatment (plasma collected 1500–1800; data expressed as Mean ± SEM). *indicates significant difference between groups, $P < 0.05$.

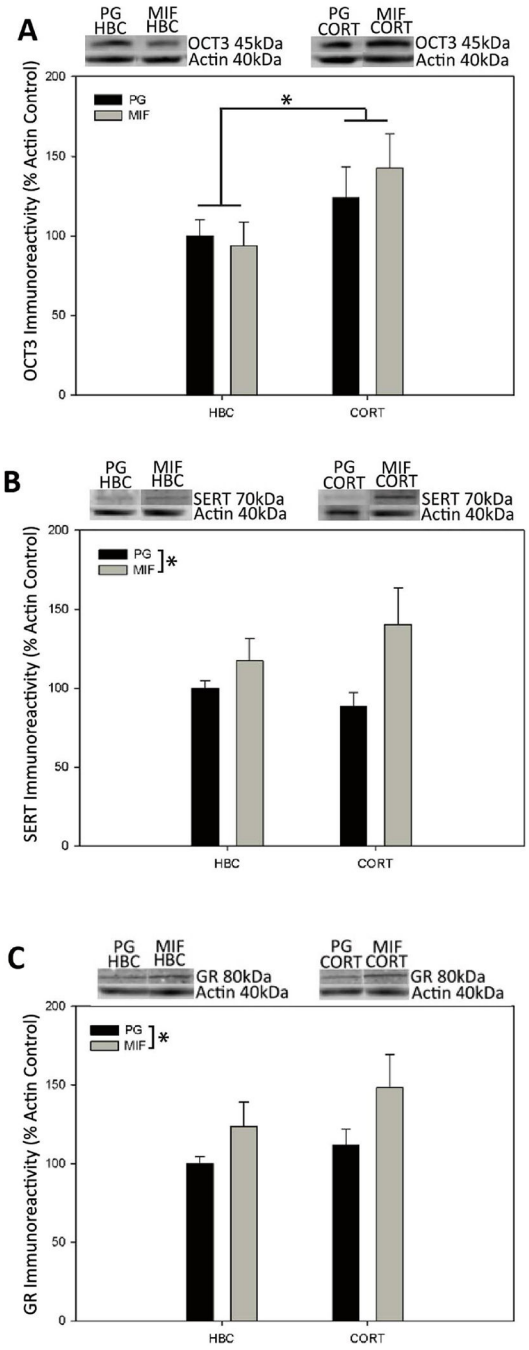

Figure 3:

Effect of mifepristone pre-treatment and corticosterone treatment on protein expression in ventral hippocampus as measured by western immunoblot (**A**) Corticosterone but not mifepristone treatment increased OCT3 expression. Mifepristone but not corticosterone treatment increased SERT (**B**) and GR expression (**C**). Means expressed as percentage of control, data in the graphs represent Mean ± SEM; *indicates significant difference between groups, $P < 0.05$.

OCT3 expression is upregulated by corticosterone in the ventral hippocampus

Mifepristone treatment increases SERT and GR expression in the ventral hippocampus
Regulation of OCT3 expression in the ventral hippocampus is altered by corticosterone

Table 1:

OCT3 expression was unchanged in the CeA, DMH and dorsal hippocampus following mifepristone and corticosterone treatment. Data expressed as % of control, P> 0.05.

Brain region	PG + HBC Mean ± SEM	PG + CORT Mean ± SEM	MIF + HBC Mean ± SEM	MIF + CORT Mean ± SEM
CeA	100.0 ± 7.49	108.01 ± 16.71	104.64 ± 7.02	92.39 ± 8.22
DMH	100.00 ± 3.63	102.16 ± 8.33	88.48 + 4.48	104.85 ± 11.72
dHipp	100.00 ± 10.72	130.28 ± 22.86	99.07 ± 12.00	108.25 ± 22.20

Table 2:

SERT expression was unchanged in the CeA, DMH and dorsal hippocampus following mifepristone and corticosterone treatment. Data expressed as % of control, P> 0.05.

Brain region	PG + HBC Mean ± SEM	PG + CORT Mean ± SEM	MIF + HBC Mean ± SEM	MIF + CORT Mean ± SEM
CeA	100.00 ± 7.9	104.94 ± 13.81	99.92 ± 15.70	76.48 ± 6.25
DMH	100.00 ± 14.22	93.23 ± 18.09	69.53 ± 11.72	100.53 ± 31.89
dHipp	100.00 ± 5.10	96.99 ± 11.37	98.79 ± 13.66	82.47 ± 6.82

Table 3:

GR expression was unchanged in the CeA, DMH and dorsal hippocampus following mifepristone and corticosterone treatment. Data expressed as % of control, P> 0.05.

Brain region	PG + HBC Mean ± SEM	PG + CORT Mean ± SEM	MIF + HBC Mean ± SEM	MIF + CORT Mean ± SEM
CeA	100.00 ± 10.04	105.27 ± 13.88	85.89 ± 12.46	84.02 ± 6.84
DMH	100.00 ± 6.60	127.09 ± 19.93	89.95 ± 12.25	104.92 ± 18.74
dHipp	100.00 ± 5.03	100.51 ± 15.09	109.75 ± 12.35	80.11 ± 9.28