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Corticosterone enhances N-methyl-D-aspartate receptor signaling to promote isolated ventral tegmental area activity in a reconstituted mesolimbic dopamine pathway

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Abstract

Elevations in circulating corticosteroids during periods of stress may influence activity of the mesolimbic dopamine reward pathway by increasing glutamatergic *N*-methyl-D-aspartate (NMDA) receptor expression and/or function in a glucocorticoid receptor-dependent manner. The current study employed organotypic co-cultures of the ventral tegmental area (VTA) and nucleus accumbens (NAcc) to examine the effects of corticosterone exposure on NMDA receptor-mediated neuronal viability. Co-cultures were pre-exposed to vehicle or corticosterone (CORT; 1 μ M) for 5 days prior to a 24 hour co-exposure to NMDA (200 μ M). Co-cultures pre-exposed to a non-toxic concentration of corticosterone and subsequently NMDA showed significant neurotoxicity in the VTA only. This was evidenced by increases in propidium iodide uptake as well as decreases in immunoreactivity of the neuronal nuclear protein (NeuN). Co-exposure to the NMDA receptor antagonist 2-amino-7-phosphonovaleric acid (APV; 50 μ M) or the glucocorticoid receptor (GR) antagonist mifepristone (10 μ M) attenuated neurotoxicity. In contrast, the combination of corticosterone and NMDA did not produce any significant effects on either measure within the NAcc. Cultures of the VTA and NAcc maintained without synaptic contact showed no response to CORT or NMDA. These results demonstrate the ability to functionally reconstitute key regions of

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the mesolimbic reward pathway ex vivo and to reveal a GR-dependent enhancement of NMDA receptor-dependent signaling in the VTA.

Keywords

stress; dopamine; reward; organotypic; corticosterone

1. Introduction

Psychological stress often contributes to substance abuse and the development of drug dependence (Jacobsen et al., 2001). The glucocorticoid cortisol is a neuroactive hormone product of hypothalamic-pituitary-adrenal (HPA) axis stimulation that may influence synaptic physiology in the mesocorticolimbic dopamine pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) and other regions. Tidey and Miczek (1996) showed that animals exposed to a tail pinch had increased mesolimbic dopamine levels. Similarly, extracellular levels of dopamine in the NAcc shell were increased in rats immediately following a mild footshock stressor (Kalivas and Duffy, 1995). Removal of endogenous glucocorticoids, via adrenalectomy, resulted in decreased extracellular dopamine concentrations in the NAcc shell at baseline and following a stress or cocaine challenge (Barrot et al., 2000). Administration of a glucocorticoid receptor (GR) antagonist resulted in decreased basal dopamine levels in a dose-dependent manner in the same area (Marinelli et al., 1998). Piazza and colleagues (1993) showed that animals self-administered corticosterone (CORT) at concentrations achieved in serum after stress. Thus, while the importance of CORT elevations in this pathway is not clearly understood, it may well reflect regulatory modulation of reward pathways at rest and/or following exposure to stress-inducing stimuli that is mediated by GRs. However, the underlying signaling mechanisms involved have not been elucidated.

Glucocorticoid modulation of glutamate receptor activity may contribute to GR-mediated effects in specific subregions of the mesolimbic reward pathway. Chronic CORT treatment has been shown to increase mRNA levels of GluN2A and GluN2B subunits of N-methyl-D-aspartate (NMDA) receptors in several models systems (Cohen et al., 2011; Costa-Nunes et al., 2014; Prendergast and Mulholland, 2012; Weiland et al., 1997). These ionotropic receptors are highly permeable to Ca^{2+} and promote Ca^{2+} -dependent excitotoxicity in models of ischemia/hypoxia, traumatic brain injury, and ethanol withdrawal (for review, see Lau and Tymianski, 2010). Functionally, these effects of CORT are associated with prolonged NMDA receptor mediated Ca^{2+} signaling (Takahashi et al., 2002; Xiao et al., 2010) and increased sensitivity of NMDA receptor populations to agonists in hippocampal cultures, resulting in pyramidal cell loss in a GR-dependent manner (Mulholland et al., 2004a; Mulholland et al., 2004b; Mulholland et al., 2006). Previous studies have indicated that chronic exposure to elevated, yet physiologically-relevant, concentrations of CORT may result in enhanced vulnerability to glutamatergic insults in the hippocampus, implicating these systems in stress-associated cognitive decline (Mulholland et al., 2004a; Takahashi et al., 2002; Wiegert et al., 2005). The current study sought to determine if CORT pre-exposure

also enhances NMDA-induced signaling and neurotoxicity in of the mesolimbic reward pathway using an *in vitro* reconstitution model.

2. Materials and Methods

2.1 Organotypic co-culture preparation

In vitro work is well suited to examine CORT-induced differences, as *in vivo* studies with rodents are confounded by diurnal fluctuations in CORT concentrations and *in vitro* models allow for precise control over CORT concentrations. In addition, though the effects of *in vitro* aging on receptor density have not yet been examined in co-cultures of the NAcc and VTA, Martens and Wree (2001) noted that NMDA receptor distribution was comparable in hippocampal slices aged *in vitro* and those taken from aged-matched rats and previous studies have found that a number of synaptic components remain at a steady level following a brief initial depression (Bahr et al., 1995). Additionally, numerous studies have investigated the use of long-term organotypic co-cultures of the VTA/NAcc and reported in tact morphology, re-innervation of dopaminergic tracts into the NAcc, and survival up to 3 months (Jaumotte and Zigmond, 2005; Lyng et al., 2007; Ostergaard et al., 1990). Thus, organotypic cell culture studies allow for long-term and detailed manipulations investigating mechanisms of brain activity while minimizing environmental influences and may provide a better understanding of CORT-NMDA interactions *in vivo*. Eight day old male and female Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were humanely sacrificed and the brains aseptically removed. Following removal, brains were transferred to ice-cold dissecting media (4°C), composed of Minimum Essential Medium (MEM; Invitrogen, Carlsbad, CA), 25 mM HEPES (Sigma, St. Louis, MO), and 50 µM streptomycin/penicillin (Invitrogen). Following aseptical removal, brains were sliced midsagittally and sectioned coronally at 400 µm thickness using a McIlwain Tissue Chopper (Mickle Laboratory Engineering Co. Ltd., Gomshall, UK). After sectioning, slices were placed into culture medium, containing dissecting medium along with distilled water, 36 mM glucose (Fisher, Pittsburg, PA), 25% (v/v) Hanks'balanced Salt Solution (HBSS; Invitrogen) and 25% heat-inactivated horse serum (HIHS; Sigma) and 0.05% streptomycin/penicillin (Invitrogen). Using a dissecting microscope, intact slices containing the NAcc and VTA were identified, yielding approximately 4-6 slices of each region per animal. Two pair of co-cultures (each pair containing one NAcc and one VTA in slight contact) was plated onto Millicell-CM 0.4 µm biopore membrane inserts (Fisher) with 1 mL of pre-incubated culture media added to the bottom of each well of a six well plate. Using the dissecting microscope, slices were oriented such that the NAcc and VTA were in direct contact with each other. Additional cultures were obtained from each region and plated with one slice in each well (i.e. slices not co-cultured) to test the hypothesis that synaptic reconnection was required to produce functional responses to experimental manipulations. Plates were then incubated at 37°C with a gas composition of 5%CO₂/95% air for 21 days to allow the co-cultures to grow together and attach to inserts. During this time, the culture media was refreshed every 3 days. Care of all animals was carried out in agreement with the University of Kentucky's Institutional Animal Care and Use Committee as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

2.2 Characterization of co-culture model

Several measures were taken in order to better characterize the NAcc/VTA co-cultures. Autoradiography was conducted for quantification of dopamine transporter binding sites in control-treated cultures, after Maragos et al. (2002) without modification. Briefly, binding was carried out after 21 days of culturing to match the *in vitro* aging cultures employed in drug exposure studies (below). High pressure liquid chromatography (HPLC) analysis of extracellular dopamine in co-cultures was assessed after Meyer et al. (2013) without modification, with the exception that 1 mL of artificial cerebrospinal fluid was placed on top of each insert for collected 45 minutes prior stabilization for HPLC analysis. In addition, immunohistochemistry for tyrosine hydroxylase (the rate limiting enzyme in the synthesis of dopamine) and myelin basic protein (to stain myelinated axon fibers) was also conducted on control-treated co-cultures. To assess immunoreactivity, co-cultures were fixed by transferring the insert containing the co-culture to a plate containing 1 mL of 10% formalin solution. One mL of formalin was also placed on top of the insert and the plates were allowed to sit for 30 minutes. The slices were then washed with 1 × phosphate buffered saline (PBS) twice and stored with 1 mL of 1 × PBS overnight at 4°C. Following overnight storage, the inserts were transferred to a plate containing 1 mL of permeabilization buffer (200 mL 1 × PBS (Invitrogen), 200 µL Triton X-100 (Sigma), 0.010 mg Bovine Serum (Sigma)) in each well and 1 mL of permeabilization buffer was also placed on top of each insert containing the slices for 45 minutes. Tissue was then washed twice with 1 × PBS. Inserts were then transferred to a plate containing 1 mL of 1 × PBS on the bottom of each well and were treated with 1 mL of permeabilization buffer containing mouse anti-tyrosine hydroxylase (1:200; Sigma) and rabbit anti-myelin basic protein (1:100; Sigma) on top of each well. Plates were then stored at 4°C for 24 hours. The slices were washed gently with 1 × PBS twice and were transferred to a plate containing 1 mL of 1 × PBS on bottom. At this point, slices were treated with 1 mL of permeabilization buffer containing the goat anti-mouse secondary antibody conjugated to fluorescein isothiocyanate (FITC; 1:100; Sigma) for tyrosine hydroxylase-labeled cultures and the goat anti-rabbit secondary antibody conjugated to tetramethylrhodamine isothiocyanate (TRITC; 1:200; Sigma) for myelin basic protein-labeled cultures on top of the insert and were stored at 4°C for 24 hours. Slices were then washed twice with 1 × PBS and placed into a plate containing 1 mL of 1 × PBS on the bottom of each well. The slices were imaged immediately with PBS under each insert.

2.3 CORT pre-exposure

Male and female co-cultures (pairs of VTA-NAcc co-cultures/well, representing different animals) or slices that were not co-cultured were continuously exposed to corticosterone (CORT; 1 µM; Sigma) for 5 days beginning at 21 days *in vitro* (DIV), followed by a 24 hour co-exposure to NMDA. At 21 DIV, all co-cultures were then transferred to plates containing 1 mL of culture media with 0.2% dimethyl sulfoxide (DMSO: vehicle control; Fisher) or 1 mL of culture media containing 1 µM of CORT. For studies investigating the potential role of the GR, a subset of these cultures was also exposed to the GR antagonist mifepristone (10 µM; Sigma) for 5 days beginning at 21 DIV. Mifepristone and CORT stock solutions were dissolved DMSO and diluted with culture media to reach a final concentration of 0.2% DMSO. Concentrations of CORT and mifepristone were chosen based on physiological relevance to exposure to a variety of stressors as well as previous organotypic experiments

(Bielajew et al., 2002; Little et al., 2008; Livezey et al., 1985; Mulholland et al., 2005; Mulholland et al., 2004b; Mulholland et al., 2006; Sharrett-Field et al., 2013). All six-well plates were then returned to an incubator maintained at 37°C with a gas composition of 5%CO₂/95% air. This exact treatment regimen was repeated at 24 DIV.

2.4 NMDA co-exposure

At 26 DIV all co-cultures were transferred to plates containing 1 mL of culture media with CORT, vehicle, or 1 mL of culture media with CORT/vehicle and NMDA (200 µM; Sigma). This concentration of NMDA was chosen based on previous reports suggesting that higher concentrations (>100 µM) are necessary for NMDA-induced cytotoxicity in co-cultures containing the VTA-NAcc pair (Maeda et al., 1998). To test the involvement of the NMDA receptor, a subset of cultures was also co-exposed to the competitive NMDA receptor antagonist 2-amino-7-phosphonovaleric acid (APV; 50 µM; Sigma), or NMDA+APV. For studies investigating the potential role of the GR, a second subset of co-cultures was co-exposed to NMDA and mifepristone, as described above. All culture media contained propidium iodide (PI; 3.74 µM; Molecular Probes, Eugene, OR) in order to assess cytotoxicity.

2.5 PI Uptake and NeuN Immunoreactivity

Cytotoxicity (PI staining of neurons and glia with damaged membranes) was evaluated in all regions of the co-cultures using fluorescent microscopy. Previous literature has demonstrated that PI reliably correlates with other measures of cell death, including fluoro-jade staining for histology and the release of lactate dehydrogenase within the culture media (for a review, see Zimmer et al., 2000). Following imaging for PI uptake, co-cultures were subsequently fixed to assess immunoreactivity of the neuronal nuclear protein (NeuN) according to methods detailed above except that slices were incubated overnight with 1 mL of permeabilization buffer containing mouse anti-NeuN (1:200; Millipore, Billerica, MA) on top of each well.

2.6 Imaging of TH, MBP, NeuN Immunoreactivity and PI uptake

All imaging was conducted using SPOT advanced version 4.0.2 software for Windows (W. Nuhsbaum Inc.; McHenry, IL, USA) using a 2.5× objective with a Leica DMIRB microscope (W. Nuhsbaum Inc.; McHenry, IL, USA) fitted for fluorescence detection and connected to a personal computer through a SPOT 7.2 color mosaic camera (W. Nuhsbaum), as previously described (Mulholland et al., 2004a; 2004b). Densitometry using Image J software (National Institutes of Health, Bethesda, MD) was used to measure the intensity of the immunoreactivity and PI fluorescence. A background measurement was also taken from the visual field surrounding each co-culture and was subsequently subtracted from the region measurements of each co-culture. The intensity was measured in each of the two regions of interest: the NAcc and the VTA. Within each region, measurements were converted to percent control for comparison across repetitions.

2.7 Statistical analysis

Each well contained two co-cultures which were analyzed individually. Treatment effects were examined using a two-way analysis of variance (ANOVA; treatment $\times$ sex). If sex differences were not observed, data derived from male and female cultures were combined and a one-way ANOVA for treatment was examined. Experiments were conducted a minimum of 2 times with a total of 8 different rat litters (8-12 pups/litter). Each experimental condition contained 2-4 co-cultures each litter, yielding a total of 4-16 slices per sex per treatment group. Data from each replication was converted into percent control values and was subsequently combined. Post-hoc tests were conducted using Fisher's LSD to examine further effects. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Characterization of co-culture model

Co-cultures of the NAcc and VTA were plated such that the two regions were touching with juncture points at the mid-ventral surface of each culture and allowed to culture for 21 days *in vitro*. Initial studies were completed to characterize the NAcc/VTA co-culture model and likely re-innervation of dopaminergic phenotypes. Figure 1A illustrates a bright field picture indicating the orientation of both the NAcc and VTA, as well as, the junction of the co-cultures. Densely labeled autoradiography of the dopamine transporter using ^{125}I -RTI-55 binding (Figure 1B) was observed in the region bridging the co-cultures. Dopamine levels observed in extracellular fluid was found to be nearly 3 nM. Immunoreactivity of tyrosine hydroxylase (Figure 1C) and myelin basic protein (Figure 1C) was concentrated in the region bridging the junction of the co-cultures and in accumbens terminal field, suggesting innervation within the co-culture model that is dopaminergic in nature.

3.2 Effects of CORT on NMDA-induced on propidium iodide uptake

This set of experiments was designed to assess the neurotoxic effects of 5 days of previous CORT pre-exposure followed by 24 hours of co-exposure to CORT and NMDA, and to investigate the potential roles of the NMDA receptor and the GR in the observed cytotoxicity. No differences in uptake were observed between sexes within each treatment group, and male and female data were combined. A one-way ANOVA for treatment was conducted within each region. In the VTA, a significant main effect of treatment was observed (Figure 2A; $F(11, 198)=4.277$, $p < 0.001$). Regardless of acute treatment (NMDA/APV/mifepristone), vehicle-pretreated co-cultures showed no significant toxicity in the VTA (data not shown). Further, exposure to CORT (for a total of 6 days) in NMDA-naïve tissue produced no significant changes in toxicity compared to vehicle treated co-cultures. However, significant toxicity was observed in the VTA after 5 days of pre-exposure to CORT followed by 24 hour exposure to CORT+NMDA. The NMDA-induced toxicity in CORT pre-treated co-cultures was significantly attenuated by co-exposure to APV ($p < 0.001$) or pre-treatment with mifepristone ($p < 0.01$; Figure 2). Thus, chronic CORT pre-exposure sensitized the VTA to subsequent NMDA receptor-dependent toxicity, and this effect was also dependent on activation of the GR during the CORT pre-exposure. No significant effects of any treatment on PI uptake were found when the VTA and NAcc were cultured separately.

Within the NAcc, a significant main effect of treatment was observed. Five days of pre-exposure to either vehicle or CORT in the presence of mifepristone significantly enhanced subsequent NMDA-induced toxicity produced by the 24 hour co-exposure to 200 μ M NMDA. Exposure to CORT (for a total of 6 days) in NMDA-naïve tissue did not alter PI uptake and no toxicity was observed in co-cultures pre-treated with CORT and later co-exposed to NMDA (~97% vehicle control). Representative images of these effects are presented in Figure 2B. No effects were observed in NAcc cultures maintained without VTA co-cultures.

3.3 Effects of CORT and NMDA on NeuN Immunoreactivity

This set of experiments also sought to determine the effects of 5 days of previous CORT pre-exposure followed by 24 hours of co-exposure to CORT and NMDA and/or the competitive NMDA receptor antagonist APV on NeuN immunoreactivity. There were no sex differences in NeuN immunoreactivity, thus, male and female data were combined. A one-way ANOVA for treatment was conducted within each region. In the VTA, a significant main effect of treatment was observed (Figure 3A; $F(11, 90)=3.172$, $p<0.001$). Regardless of acute treatment (NMDA/APV/mifepristone), no loss of NeuN immunoreactivity within the VTA of control cultures was seen (data not shown). Exposure to CORT for a total of 6 days in NMDA-naïve tissue produced no significant changes in the density of NeuN compared to vehicle treated co-cultures. In agreement with PI data described above, a significant loss of NeuN immunoreactivity was observed in the VTA after 5 days of pre-exposure to CORT (but not vehicle) followed by 24 hour exposure to CORT+NMDA ($p<0.01$). The CORT +NMDA-induced loss of NeuN was attenuated by co-exposure to the NMDA receptor antagonist APV ($p<0.05$) and pre-treatment with mifepristone during the 5 day exposure to CORT ($p<0.01$). Thus, within the VTA, chronic CORT pre-exposure followed by acute NMDA exposure produced significant losses in NeuN immunoreactivity that was dependent on both NMDA receptor- and GR-activation. No significant effects of treatment or interactions were found in the NAcc region. Representative images of effects in the VTA are presented in Figure 3B. In VTA and NAcc cultures maintained in isolation (i.e. not co-cultured), no NeuN immunoreactivity was detected.

4. Discussion

The present studies demonstrate that prolonged elevations in CORT likely alter the sensitivity of glutamatergic VTA neurons to excitatory effects of endogenous, and possibly exogenous, signaling molecules. Additional studies also demonstrated that this effect of CORT was dependent upon GR activation and demonstrate the importance of intracellular GR binding in mediating the effects of CORT in this reward pathway, though mifepristone does have effects at progesterone receptors. It is important to note that neither CORT nor NMDA produced any changes in PI uptake in either region in cultures that were maintained alone (i.e. not co-cultured), likely because of the lack of viability of the slices as reflected in the absence of NeuN immunoreactivity. When considered in kind with findings of what appears to be a reconstitution or possible chemoattractant-mediated recapitulation of synaptic contacts between the midbrain and striatal cultures, as reflected in TH and MBP co-labeling; nM concentrations of dopamine in extracellular fluid; and dense DA transporter

labeling across the surgical gap, these findings suggest that the functional effects of CORT and NMDA in these brain regions require the integrity of intrinsic synaptic contact.

It is interesting to note that CORT-induced potentiation of NMDA receptor-mediated neurotoxicity only occurred in the VTA and not in the NAcc. This does not likely reflect differences in synaptic NMDA receptor density or differences in receptor stoichiometry (Albin et al., 1992; Wang et al., 1995), although the polyamine sensitive GluN2B subunit appears to be the most prevalent subunit in mesencephalon cultures (Allgaier et al., 1999). The GluN2B subunit is highly sensitive to polyamines and CORT has been shown to increase polyamine expression in some regions (Ientile et al., 1988) or to produce increased expression of GluN2B subunits (Karst and Joels, 2003; Weiland et al., 1997), providing a possible mechanism for the regional specificity of CORT. The results of the current study are in agreement with literature implicating the importance of the GR in the CORT-induced potentiation of excitatory signaling (Alexander et al., 2009; Mulholland et al., 2004a; Mulholland et al., 2004b). In cortical co-cultures of neurons and astrocytes, the synthetic glucocorticoid dexamethasone and to a lesser extent corticosterone acutely reduced cytosolic Ca^{2+} concentration and was found to be neuroprotective against glutamate toxicity (Suwanjang et al., 2013); however, many methodological differences exist between the aforementioned study and the current study, including cell culture preparation and length of treatment times. In addition, other studies have found that high concentrations of CORT increased intracellular levels of Ca^{2+} (Karst et al., 1994; Kerr et al., 1992; Xiao et al., 2010) as well as induced the release of glutamate and other excitatory amino acids (Moghaddam et al., 1994; Stein-Behrens et al., 1992; Stein-Behrens et al., 1994).

Alternatively, there may be a differential distribution of GRs between the NAcc and VTA. Although the distribution of GRs is generally thought to be ubiquitous throughout much of the rodent brain, results from previous studies have been contradictory regarding the relative density within the NAcc and VTA. For example, Aronsson et al. (1988) found moderate levels of GR mRNA in the VTA compared to weak GR mRNA in the NAcc. Most studies, however, have found approximately equal distribution of GRs via immunohistochemistry and/or in situ hybridization within the striatum and midbrain regions (Ahima and Harlan, 1990; Morimoto et al., 1996). Additionally, several studies have noted strong immunoreactivity for the GR among dopaminergic neurons, especially those originating in the VTA (Barik et al., 2010; Diaz et al., 1997; Harfstrand et al., 1986).

The results of the current study suggest that chronic CORT may lead to increased neuronal vulnerability to future insults via increased NMDA receptor expression and/or function. The ability of CORT to potentiate subsequent NMDA receptor-mediated excitotoxic insults within areas of the brain key to drug reward and reinforcement may be of crucial importance as several drugs of abuse, such as methamphetamine, cocaine, and even ethanol, are known to produce increases in extracellular glutamate release within the striatum resulting in neurotoxicity at times (Abekawa et al., 1994; Ding et al., 2013; Griffin et al., 2014; Rossetti and Carboni, 1995; Rossetti et al., 1999; Suto et al., 2010; Wakabayashi and Kiyatkin, 2012). Numerous *in vivo* studies have demonstrated enhanced striatal dopamine and glutamate efflux in animals exposed to stressors and methamphetamine (Matuszewich and Yamamoto, 2004) and research has indicated that exposure to chronic CORT significantly

enhanced methamphetamine-induced dopaminergic nerve terminal damage (Kelly et al., 2012). Others have demonstrated the importance of the GR in the development of ethanol dependence and subsequent ethanol withdrawal (Jacquot et al., 2008; Reynolds et al., 2015; Rotter et al., 2012; Sharrett-Field et al., 2013). Taken together, these findings demonstrate the ability of stress and the resulting rise in CORT levels to sensitize the reward pathway, and in particular the VTA, to future excitatory amino acid signaling such as that seen with psychostimulant and ethanol use.

In conclusion, the present studies demonstrated that long-term exposure to a physiologically relevant concentration of CORT can potentiate NMDA receptor-mediated signaling within the VTA, but not the NAcc, in a GR-dependent manner. In agreement with previous studies examining CORT effects in the hippocampus, these results suggest that chronic CORT may lead to an increase in the expression and/or function of NMDA receptor systems in an area of the brain that is critical for drug reward and reinforcement. Future studies should investigate the possibility of increased expression of the polyamine sensitive GluN2B subunit among other NMDA receptor subunits within the VTA following chronic CORT treatment and the potential role of changes in phosphorylation state of the NMDA receptor in mediating the effects of chronic CORT. In addition, perturbation of glutamatergic signaling within the mesolimbic pathway is often achieved via various substances of abuse, thus these findings further our understanding of the means by which stress and substance abuse intersect to impair brain function.

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Abbreviations

ACTH	adrenocorticotrophic hormone
ANOVA	analysis of variance
APV	DL-2-amino-7-phosphonovaleric acid
Ca²⁺	calcium
CORT	corticosterone
CRH	corticotrophin releasing hormone
DMSO	dimethyl sulfoxide
FITC	fluorescein isothiocyanate
GR	glucocorticoid receptor
HPA	hypothalamus-pituitary-adrenal
MEM	minimum essential medium
MR	mineralocorticoid receptor
NAcc	nucleus accumbens
NeuN	neuronal nuclear protein
NMDA	N-methyl-D-aspartate
PBS	phosphate-buffered saline
PI	propidium iodide
VTa	ventral tegmental area

Highlights

- Midbrain and striatal cultures functionally reintegrate synaptic contacts ex vivo.
- Corticosterone causes glutamate receptor-dependent cell loss in midbrain neurons.
- Ventral striatum is insensitive to effects of either corticosterone or an excitotoxin.

Figure 1. Characterization of co-cultures (2.5× magnification)
(A) bright-field image of co-cultures; (B) Autoradiographic localization of ¹²⁵I-RTI-55 binding (dopamine transporter) and point of junction; (C) merged image of tyrosine hydroxylase (FITC; green) and myelin basic protein (TRITC; red) immunoreactivity at point of junction.

Figure 2. Effects of CORT and NMDA on propidium iodide uptake

(A) Pre-treatment with CORT prior to NMDA exposure resulted in significant neurotoxicity, an effect which was not seen in vehicle pre-treated cultures. Co-exposure to the competitive NMDA receptor antagonist APV or pre-treatment with the glucocorticoid receptor antagonist mifepristone significantly attenuated the ability of CORT to potentiate NMDA-induced toxicity. Dashed line represents DMSO-treated value. Data represented as percentage of DMSO-treated cultures (mean $\pm$ SEM). * $p < 0.05$ vs. DMSO (vehicle) levels; # $p < 0.05$ vs. CORT + NMDA (B). Representative images of PI uptake in co-cultures of the NAcc and VTA pretreated with either DMSO, CORT, DMSO + mifepristone, or CORT + mifepristone and exposed to NMDA or NMDA + APV for 24 hours.

Figure 3. Effects of CORT and NMDA on NeuN immunoreactivity

(A) Pre-treatment with CORT prior to NMDA exposure resulted in a significant loss of NeuN immunoreactivity, an effect which was not seen in vehicle pretreated cultures. Co-exposure to the competitive NMDA receptor antagonist APV or pre-treatment with the glucocorticoid receptor antagonist mifepristone significantly attenuated the ability of CORT to potentiate NMDA-induced toxicity. Dashed line represents DMSO-treated value. Data represented as percentage of DMSO-treated cultures (mean $\pm$ SEM). * $p < 0.05$ vs. DMSO (vehicle) levels; # $p < 0.05$ vs. CORT + NMDA. (B) Representative images of NeuN immunoreactivity in co-cultures of the NAcc and VTA pretreated with either DMSO, CORT, DMSO + mifepristone, or CORT + mifepristone and exposed to NMDA or NMDA + APV for 24 hours.