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Psychological Stress, Cocaine and Natural Reward Each Induce Endoplasmic Reticulum Stress Genes in Rat Brain

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Abstract

Our prior research has shown that the transcription of endoplasmic reticulum (ER) stress transcription factors Activating Transcription Factor 3 (ATF3) and ATF4 are induced by amphetamine and restraint stress in rat striatum. However, presently it is unknown the full extent of ER stress responses to psychological stress or cocaine, and which of the three ER stress pathways is activated. The current study examines transcriptional responses of key ER stress target genes subsequent to psychological stress or cocaine. Rats were subjected to acute or repeated restraint stress or cocaine treatment and mRNA was isolated from dorsal striatum, medial prefrontal cortex and nucleus accumbens brain tissue. ER stress gene mRNA expression was measured using quantitative PCR and RNA sequencing. Restraint stress and cocaine induced transcription of the classic ER stress-induced genes (BIP, CHOP, ATF3 and GADD34) and of two other ER stress components XBP1 and ATF6. In addition, rats living in an enriched environment (large group cage with novel toys changed daily) exhibited rapid induction of GADD34 and ATF3 after 30 min of exploring novel toys, suggesting these genes are also involved in normal non-pathological signaling. However, environmental enrichment, a paradigm that produces protective addiction and depression phenotypes in rats, attenuated the rapid induction of ATF3 and GADD34 after restraint stress. These experiments provide a sensitive measure of ER stress and, more importantly, these results offer good evidence of the activation of ER stress mechanisms from psychological stress, cocaine and natural reward. Thus, ER stress genes may be targets for novel therapeutic targets for depression and addiction.

Keywords

Depression; Addiction; Cellular stress; Environmental enrichment; Differential rearing

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Introduction

Endoplasmic Reticulum (ER) stress is an adaptive cellular response to a number of cellular insults including excess unfolded protein accumulation, cellular calcium disruption, microbe infection, and other stresses in the cell (Hetz, 2012). ER stress sensor proteins detect cellular stress and then activate a number of basic region leucine zipper (B-zip) transcription factors in a coordinated fashion to initiate transcription of adaptive response genes that work to bring the cell back to homeostasis or to trigger apoptosis if the insult is too severe (Hetz, 2012).

There are three branches of the ER stress pathway: the inositol-requiring protein 1α (IRE1a) pathway, the protein kinase RNA-like ER kinase (PERK) pathway, and activating transcription factor 6 (ATF6) pathway that lead to activation of four B-zip transcription factors: x-box binding protein 1 (XBP1), ATF3/ATF4, and ATF6, respectively. The transcription factors then dimerize and activate the transcription of BIP, CHOP, GADD34, ATF3 and other adaptive response genes, which can trigger a number of cellular responses (Wek et al., 2006). Thus, transcriptional activation of these four adaptive response genes is seen as a *de facto* measure of ER stress response.

Activating Transcription Factors belong to the cAMP response element (CRE) binding protein (CREB) family of B-zip transcription factors. CREB expression in the nucleus accumbens and dorsal striatum has been shown to be involved in depression and addiction. For example, blockade of CREB activity decreases depression-like behavior and also decreases cocaine self-administration and cocaine seeking while simultaneously increasing cocaine sensitivity in rats (Green et al., 2006; Green et al., 2008; Green et al., 2010; Larson et al., 2011).

The CREB data described above were extended to include other CREB/ATF family members, among them being ATF3 and ATF4 (Green et al., 2008). Results showed that ATF3 and ATF4 also play a role in depression- and addiction-like behavior. ATF3 and ATF4 have CREB like effects when overexpressed and both are induced in the rat striatum by restraint stress or amphetamine administration. Because ATF3 and ATF4 are also well known as ER stress transcription factors, we hypothesized that restraint stress and drugs of abuse would also regulate other ER stress genes in the striatum.

The environmental enrichment paradigm is one where rats are assigned either to a singly-housed isolated control condition (IC) with no social contact or novelty, or a group-housed enriched condition (EC) with social contact and novelty (*i.e.* children's toys changed daily). Rats reared in the enriched condition exhibit protective phenotypes for depression and addiction (Green et al., 2002; Green et al., 2003; Laviola et al., 2008; Solinas et al., 2008; El Rawas et al., 2009; Thiel et al., 2009; Green et al., 2010; Thiel et al., 2010). Thus, we hypothesized that EC rats would exhibit a reduced stress- and cocaine-induced transcriptional response of ER stress genes compared to IC control rats.

Accordingly, the current studies assess ER stress induction after restraint stress or cocaine administration by measuring induction of transcription of well-characterized ER stress chaperone proteins BIP (aka GRP78 or HSPA5), CHOP (aka DDIT3 or GADD153), ATF3 and GADD34 (aka PPP1R15A). We also measured mRNA induction of other ER stress-related proteins (XBP1, ATF6 and ATF4). Lastly, we investigated the ability of environmental enrichment to blunt ER stress-induced transcriptional responses.

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Materials and Methods

Animals

Male Sprague Dawley rats (Harlan, Houston, TX), 250–350g, were used for all experiments except for environmental enrichment (see below). Rats were pair-housed in an Association for Assessment and Accreditation of Laboratory Animal Care- (AAALAC) approved colony, and all experiments conformed to the NIH *Guide for the Use and Care of Laboratory Animals* and the University of Texas Medical Branch Institutional Animal Care and Use Committee. Rats were housed on a 12 h light/dark cycle with lights on at 6:00 A.M. All procedures were conducted during the light phase of the cycle.

Environmental Enrichment

Male Sprague Dawley rats arrived at 21 days of age. Rats were assigned to an isolated condition (IC), singly housed with no novelty, or an enriched condition (EC) where rats were group housed in a large cage with conspecifics for social contact and novel hard plastic toys changed daily for maximal novelty. Rats were housed in these conditions for one month prior to any experimentation(Green et al., 2010).

Statistical analyses

All data were analyzed using ANOVA methods except as noted. Where appropriate, planned comparisons or Bonferroni's *post hoc* procedures were used. All statistical p values are for two-tailed tests unless otherwise noted.

Quantification of mRNA via qPCR

Rats were killed by rapid decapitation and the STR, mPFC and NAcc were dissected on an ice-cold plate. The mRNA was isolated using the RNA Stat-60 reagent (Teltest, Houston, TX) according to manufacturer's directions. Contaminating DNA was removed *via* DNase treatment (Turbo DNA-Free, catalog #1906; Ambion, Austin, TX). Purified RNA was reverse- transcribed into cDNA (Superscript First Strand Synthesis; catalog #12371-019; Invitrogen). ER stress-related transcripts were quantified using real-time PCR (SYBR Green; Applied Biosystems,Foster City, CA) on an Applied Biosystems 7500fast thermocycler. Primers for rat ATF3 (forward:GCCATCCAGAACAAGCACC; reverse: ACTTGGCAGCAGCAATTT), ATF4 (forward: CCTTCGACCAGTCGGGTTTG; reverse: CTGTCCCGGAAAAGGCATCC), BIP (forward: AACCAAGGATGCTGGCACTA; reverse: ATGACCCGCTGATCAAAGTC), CHOP (forward: GGAAGTGCATCTTCATACACCACC; reverse: TGACTGGAATCTGGAGAGAGCGAGGGC), GADD34 (forward: TGAATGTTGAGAGAAGAACC; reverse: TTGTTTAGAAGTCGCTCTG), XBP1 (forward: TTACGAGAGAAAACTCATGGGC; reverse: GGGTCCAACTTGTCCAGAATGC), and ATF6 (forward: AAGTGAAGAACCATTACTTTATATC; reverse: TTTCTGCTGGCTATTTGT) were validated for linearity and specificity prior to experiments. All PCR data were normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA levels (forward: AACGACCCCTTCATTGAC; reverse TCCACGACATACTCAGCAC), which were not altered by stress or cocaine treatments.

Restraint stress

Rats were placed in a plastic conical sleeve (Decapi-Cone; model DC200; Braintree Scientific, Braintree, MA) for up to 60 min.

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Non-contingent cocaine administration

Cocaine HCl (National Institute on Drug Abuse, Bethesda MD) was administered at a dose of 20 mg/kg (i.p.). Rats received 9 daily injections of saline or cocaine. Thus, for example, "acute" cocaine animals received eight daily injections of saline, followed by a cocaine injection on day 9, whereas "repeated" cocaine animals received nine daily cocaine injections. Animals were analyzed at varying times after the last injection. Because cocaine has a short half life *in vivo*, rats were injected again with 20 mg/kg one hour after the first injection (2 X 20 mg/kg; for the 3hr time point).

Cocaine self-administration

To prevent differential acquisition of cocaine self-administration between EC and IC groups (Green et al., 2002), rats were first trained to press a lever for sucrose pellets. To train EC and IC rats to press the lever, rats were regulated for food intake to bring them down to 85% of their free-feed body weight over a period of 4 days. Rats were acclimated to Noyes sucrose pellets (45 mg) to alleviate neophobia. Rats were then trained to lever press for sucrose pellets in 15-min sessions in standard two-lever operant chambers (Med-Associates ENV-007) beginning with a Fixed Ratio 1 (FR1), incrementing the ratio each day until rats were pressing under an FR5 schedule of reinforcement. After completion of the FR5 session, Rats were allowed to return to 100% free-feed body weight before catheter surgery. For the catheter surgery, rats were anesthetized with ketamine (100 mg/kg, IP) and xylazine (20 mg/kg, IP) before implantation of an indwelling Silastic (Fisher Scientific) catheter exiting at the nape of the neck. Rats were allowed to recover for 1 week prior to cocaine self-administration. For self-administration, rats were placed into the operant chambers and allowed to self-administer 1 mg/kg/infusion cocaine HCl (NIDA, Bethesda MD) under an FR1 schedule for 2 hrs daily for a total of 14 days. To prevent overdose, each infusion was accompanied by a 60 second timeout signaled by illumination of two cue lights above the levers. To prevent differential intake of cocaine within and between EC and IC groups (Green et al., 2002) that could produce undue variance in transcript levels, the sessions were capped at 30 infusions maximum per session. Tissue was collected 3 hrs after the beginning of the last session.

RNA Sequencing

RNA from rat NAcc was purified using the RNeasy lipid tissue mini kit (Qiagen) according to the manufacturer's instructions. RNA was sequenced using the Illumina HiSeq system. Double-stranded cDNA libraries were created by first reverse-transcribing the RNA and then generating the second strand. Blunt ends were 5'-phosphorylated and "A-tailed" so that adapters could be ligated to each end. The cDNA was added to HiSeq flow cells using 4 samples per lane. Adapters hybridize to immobilized oligonucleotides and amplified using "bridge" amplification. Base calls were made via fluorescent-tagged nucleotides. Reads were 50bp and double-ended. Over 100 million reads per sample were mapped and counted using Tophat and Bowtie packages. The EdgeR package was used for analysis using trimmed mean of M-values (TMM) method for normalization and tag-wise dispersion. A likelihood ratio F-test was used for generating P-values.

Results

Effects of psychological stress on ER stress gene expression

Our previous research showed that psychological stress induces ATF3 and ATF4 mRNA expression, so we measured mRNA levels of downstream ER stress-response genes three hours after restraint stress. Bip ($F(2,14) = 21.1$, $P < 0.001$), GADD34 ($F(2, 14) = 5.8$, $P < 0.05$) and XBP1 ($F(2, 14) = 11.5$, $P < 0.005$) were all induced in the STR (Fig 1, top panel)

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3hrs after acute and chronic restraint stress, but CHOP ($F(2,15) = 9.7$, $P < 0.005$) and ATF6 ($F(2,13) = 3.6$, $P < 0.05$; one tailed test) were only induced by repeated stress. In the mPFC none of the ER stress genes was induced, but GADD34 was decreased after acute and repeated stress ($F(2, 15) = 13.9$, $P < 0.001$; Fig 1, middle panel). In the NAcc, GADD34 was only decreased after repeated stress ($F(2,15) = 5.4$, $P < 0.05$; Fig 1, bottom panel).

The results depicted in Figure 1 show that restraint stress induces ER stress gene induction at 3 hrs in the STR but not in the mPFC or NAcc.

Effects of cocaine on ER stress gene expression

Three hours after cocaine administration BIP ($F(2, 9) = 12.5$, $P < 0.005$) and XBP1 ($F(2, 9) = 8.4$, $P <0.01$) were induced by acute and repeated cocaine in the STR (Fig 2, top panel) while CHOP ($F(2, 9) = 12.3$, $P < 0.005$) and ATF6 ($F(2, 9) = 40.2$, $P < 0.001$) were only induced by acute cocaine. However, ATF6 was unexpectedly decreased after repeated cocaine.

In the mPFC (Fig 2, middle panel) CHOP ($F(2, 15) = 7.8$, $P < 0.05$) and XBP1 ($F(2, 15) = 8.9$, $P < 0.05$) were induced by acute and repeated cocaine while BIP ($F(2, 15) = 16.38$, $P< 0.001$) was only induced by repeated cocaine. The induction of GADD34 ($F(2, 15) = 4.5$, $P < 0.05$) was only statistically significant with acute cocaine. For the NAcc, only BIP ($F(2,15) = 10.8$, $P < 0.005$) and ATF4 ($F(2,15) = 4.5$, $P < 0.05$) were increased, and only with repeated cocaine (Fig 2, bottom panel).

The results in Figure 2 show that, in contrast to restraint stress, cocaine induces ER stress response genes in the STR, mPFC and to a lesser degree in the NAcc.

Effect of cocaine on rapid response ER stress genes

To study rapid ER stress responses to cocaine we examined a one-hour timecourse of induction of GADD34 and ATF3. In the STR, GADD34 produced a main effect of Time ($F(3, 23) = 3.8$, $P < 0.05$) but no main effect of Acute/Repeated or the interaction of the two factors. The results were similar for the mPFC (main effect of Time ($F(3, 23) = 13.3$, $P < 0.001$)). Thus, cocaine induced GADD34 mRNA in the STR and mPFC peaking at 30 min for acute and 15 minutes for repeated cocaine (Fig 3). However, the NAcc was less responsive to cocaine-induced GADD34 (only main effect of Time ($F(3,23) = 7.9$, $P < 0.005$)).

For ATF3, the STR tissue produced a main effect of Time ($F(3, 21) = 6.3$, $P < 0.005$) but no main effect of Acute/Repeated or interaction. In the mPFC, cocaine produced a main effect of Time ($F(3, 21) = 28.9$, $P < 0.001$), a main effect of Acute/Repeated ($F(3, 21) = 29.9$, $P < 0.001$) and a significant interaction ($F(3, 21) = 7.8$, $P < 0.005$). In the NAcc, cocaine only yielded a main effect of Time ($F(3,23) = 3.9$, $P < 0.05$). Although ATF3 was induced in a similar manner by acute or repeated cocaine in the STR, the induction in the mPFC was significantly greater for repeated cocaine than for acute. This was unexpected.

Overall, the results of Figure 3 demonstrate rapid induction of GADD34 and ATF3 in the STR, mPFC and to a lesser degree in the NAcc by cocaine.

Environmental enrichment and restraint stress

Because environmental enrichment produces a protective antidepressant-like phenotype in rats (Green et al., 2008), we hypothesized that EC rats would show less transcriptional induction of GADD34 and ATF3 30 min after restraint stress than IC rats. In the STR, GADD34 produced a main effect of Stress ($F(1, 26) = 8.8$, $P < 0.01$; Fig 4, top left) but not

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Enrichment ($F(1, 26) = 0.009$, n.s.). The interaction was also not significant ($F(1, 26) = 0.02$, n.s.). For ATF3 in the STR, there was a main effect of Stress ($F(1, 25) = 36$, $P < 0.001$; Fig 4, top right) and of Enrichment ($F(1, 25) = 10.1$, $P < 0.005$). The interaction was also significant ($F(1, 25) = 10.2$, $P < 0.005$).

For GADD34 in the mPFC there was a significant main effect of Enrichment ($F(1, 24) = 9.7$, $P < 0.005$; Fig 4, middle left) and Stress ($F(1, 24) = 27.5$, $P < 0.001$) and an interaction of those two factors ($F(1, 24) = 13.4$, $P < 0.005$). For ATF3 in the mPFC there was also a significant main effect of Enrichment ($F(1, 25) = 23.4$, $P < 0.001$; Fig 4, middle right) and Stress ($F(1, 25) = 145$, $P < 0.001$) and the interaction ($F(1, 25) = 23.3$, $P < 0.001$).

In the NAcc, there was a significant increase in GADD34 subsequent to stress (Main effect: $F(1, 16) = 6.0$, $P < 0.05$; Fig 4, bottom left), but the Enrichment by Stress interaction was not quite significant ($F(1, 16) = 3.6$, $P = 0.08$). For ATF3, there was a significant main effect of Stress ($F(1, 16) = 23.1$, $P < 0.001$; Fig 4 bottom right) and Enrichment ($F(1, 16) = 7.5$, $P < 0.05$), and the interaction was also significant ($F(1, 16) = 8.4$, $P < 0.05$).

The results in Figure 4 demonstrate that restraint stress induces GADD34 and ATF3 in all three regions at 30 min and further demonstrates that environmental enrichment attenuates ATF3 induction in all three regions and GADD34 induction only in the mPFC.

Effect of environmental factors on GADD34 and ATF3 induction

Each day when the toys are changed the rats exhibit a burst of locomotor activity (e.g. exploring and playing) higher than anything seen after cocaine or amphetamine (personal observation). We hypothesized that this high level of play activity would induce GADD34 and ATF3. However, since environmental enrichment decreases transcriptional responses (see Fig 4 above) we further hypothesized that EC rats given toys after being withdrawn from environmental enrichment for 4 days would display larger induction than EC rats not withdrawn. Our results on tissue collected 30 min after toy change mostly support these hypotheses. For the STR, GADD34 was induced by toy change (*i.e.* main effect of Toy Change ($F(1, 19) = 28$, $P < 0.001$); Fig 5, top left), but there was no main effect of Isolation or interaction. However, for ATF3, the Toy Change main effect ($F(1, 19) = 52.5$, $P < 0.001$) was accompanied by a main effect of Isolation ($F(1, 19) = 17$, $P < 0.005$) and a significant interaction of these two variables ($F(1, 19) = 17.1$, $P < 0.005$; Fig 5, top right).

In the mPFC, GADD34 analysis yielded a main effect of Toy Change ($F(1, 19) = 247$, $P < 0.001$), main effect of Isolation ($F(1, 19) = 78$, $P < 0.001$) and an interaction of the two ($F(1, 19) = 90$, $P < 0.001$; Fig 5, middle left). For ATF3 (Fig 5, middle right) there was also a significant main effect of Toy Change ($F(1, 19) = 44.7$, $P < 0.001$), Isolation ($F(1, 19) = 28.4$, $P < 0.001$) and interaction ($F(1, 19) = 28.4$, $P < 0.001$).

In the NAcc, GADD34 yielded a main effect of Toy Change ($F(1, 20) = 12.9$, $P < 0.005$), main effect of Isolation ($F(1, 20) = 14.4$, $P < 0.001$) and a significant interaction ($F(1, 20) = 9.3$, $P < 0.01$; Fig 5 bottom left). For ATF3, the analysis also revealed main effects of Toy Change ($F(1,20) = 70.1$, $P < 0.001$), Isolation ($F(1, 20) = 31.4$, $P < 0.001$) and an interaction of the two ($F(1,20) = 33.4$, $P < 0.001$).

Overall, Figure 5 shows that daily toy changes induce GADD34 and ATF3 in the STR and mPFC, and that 4 days of isolation before the toy change increases the induction of GADD34 (in the mPFC and NAcc) and ATF3 (in all three regions).

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Orthogonal validation of ER stress-regulated transcription in NAcc of EC and IC rats after chronic self-administration of cocaine or saline

We discovered significant main effects of cocaine self-administration increasing expression of BIP ($P < 0.005$; Figure 6, top panel), GADD34 ($P < 0.05$) and XBP1 ($P < 0.005$), but a statistically significant decrease in ATF6 ($P < 0.05$). Transcriptional induction of CHOP did not quite reach statistical significance ($P = 0.08$).

In addition to cocaine main effects, BIP ($P < 0.01$), XBP1 ($P < 0.05$) and ATF6 ($P < 0.001$) produced main effects of enrichment. In each case EC rats had less expression than IC rats. Finally, ATF6 also produced a cocaine X enrichment interaction ($P < 0.01$). The nature of this interaction was such that IC rats decreased expression after cocaine whereas EC rats trended upward.

In addition to the six transcripts listed above, an Ingenuity Pathways Analysis found regulation of IRE1 and MBTPS, two additional constituents of the ER stress pathway. In fact, a total of 6 of the 18 constituents of the canonical pathway were regulated by cocaine ($–\log(\text{P-value}) = 3.527$, $P < 0.001$; Fig 6 bottom).

These results confirm cocaine-induced transcription of BIP, and XBP1 and also confirm decreases in ATF6 transcript after repeated cocaine (compare to Figure 2) using a different method of mRNA detection. Moreover, these results extend the earlier data by demonstrating these changes in the NAcc, in differentially reared (EC and IC) rats and after volitional self-administered cocaine rather than merely non-contingent IP drug administration.

Discussion

The major findings of these experiments are: (1) psychological stress induces ER stress genes at 3hrs in the STR but not mPFC and NAcc, (2) cocaine induces ER stress genes at 3hrs in all three brain regions, (3) GADD34 and ATF3 are rapidly induced in the STR, NAcc and mPFC by cocaine or stress, (4) environmental enrichment attenuates the rapid GADD34 (in mPFC and NAcc) and ATF3 (in all three regions) induction from psychological stress, (5) the mPFC is more sensitive in the rapid transcriptional response of GADD34 and ATF3 than the STR and NAcc, and (6) a non-pathological naturally rewarding stimulus (novelty) robustly induces ER stress response genes.

Further, these experiments provide a sensitive and reliable array of qPCR targets that can be used to determine ER stress induction in rat brain tissue *ex vivo*. These specific targets were chosen because they represent three well-characterized ER stress-response genes (BIP, GADD34 and CHOP) as well as transcripts that code for proteins involved in each of the three ER stress pathways. ATF3 and ATF4 measure transcription of genes involved in the PERK pathway, XBP1 in the IRE1a pathway and ATF6 in the ATF6 pathway.

In the addiction literature, some studies have drawn a link between stimulants and ER stress. For example, the Cadet laboratory has demonstrated multiple times that methamphetamine neurotoxicity involves ER stress pathways (Jayanthi et al., 2009; Beauvais et al., 2011). Our own prior research has shown amphetamine induces ATF3 and ATF4 (Green et al., 2008). More relevant to the current experiments, the Choe laboratory has reported that cocaine increases indices of ER stress proteins (Ahn et al., 2007; Shin et al., 2007). Thus, the mRNA results of the current project are supported by protein data. These published results along with the current observations are interesting in light of the fact that cocaine does not have the reputation for neurotoxicity methamphetamine has.

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Although protein levels are *functionally* more important than mRNA, the innate quantitative limitations of the Western blot technique provide a niche for other techniques such as qPCR. Thus, one goal of the current project was to develop a qPCR panel to confirm and extend the cocaine results published before. In a sense, Figure 2 (top panel) combined with the published papers serves as a validation of the assay. These experiments extend upon the published cocaine literature by showing that ER stress-related genes are similarly induced in the mPFC.

Our prior research showed that ATF3 and ATF4 mRNA were induced by psychological stress (Green et al., 2008). Being that psychological stress is a trigger for depression, those and the current results suggest ER stress genes may be important for depression. Indeed, an XBP1 polymorphism has been associated with depressive disorders and poor response to anti-depressants (Grunebaum et al., 2009). Further, several studies have suggested that malfunctioning ER stress genes may be involved in other mood disorders like bipolar disorder and schizophrenia (Carter, 2007; So et al., 2007; Hayashi et al., 2009). Research has also suggested that some mood-stabilizer drugs like valproate, may work by alleviating ER stress (Kim et al., 2009). The current data (Figures 1 and 4) add good evidence for psychological stress causing ER stress.

Environmentally enriched rats have been shown to exhibit an antidepressant-like behavioral phenotype (Green et al., 2010). Because psychological stress is a major trigger for depression we hypothesized EC rats to have less of an ER stress response than IC rats. Figures 4 and 5 support this hypothesis for GADD34 in the mPFC and NAcc, and ATF3 in all three brain regions.

Figures 1 – 3 suggest that the NAcc is less sensitive to cocaine and psychological stress than the closely-related dorsal striatum. In fact, the NAcc showed little ER stress response to investigator-delivered non-contingent cocaine injections (Fig 2). However, the RNA sequencing data suggest that the NAcc is indeed sensitive to cocaine self-administration (Fig 6). These differences are likely a function of differential dosing regimens: self-administering rats get more cocaine into the brain, more rapidly and the exposure time is greater for self-administering rats. Thus, the differences between Figs 2 and 6 are most likely a matter of degree and route of exposure.

The results of Figure 5 are interesting from the standpoint that the rapidly induced ER stress genes (GADD34 and ATF3) are robustly induced by novelty, a non-pathological naturally-rewarding stimulus (Bardo et al., 1989; Bevins and Bardo, 1999). This raises the question whether naturally-rewarding stimuli put brain cells into a compromised ER stress state or if these ER stress pathways are also signaling pathways that participate in normal cellular functioning and regulation. The fact that other apoptotic signaling pathways (*e.g.* calcium dysregulation, MAP kinase pathways, and NFκB) have non-apoptotic signaling functions (Berhow et al., 1996; Krishnan et al., 2008; Russo et al., 2009), the latter option is likely.

Taken as a whole, the results of these experiments point to a dynamic and sensitive ER stress system induced *in vivo* by a multitude of stimuli—some pathological and some non-pathological. A more full understanding of the role of ER stress pathways in addiction and major depression could lead to some novel and much needed therapeutic targets.

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Highlights

1. Psychological stress induces ER stress genes at 3hrs in the STR but not mPFC
2. Cocaine induces ER stress genes at 3hrs in both the STR and mPFC
3. GADD34 and ATF3 are rapidly induced in the STR and mPFC by cocaine or stress
4. Enrichment attenuates the GADD34 and ATF3 induction from psychological stress
5. A naturally rewarding stimulus (novelty) robustly induces ER stress response genes.

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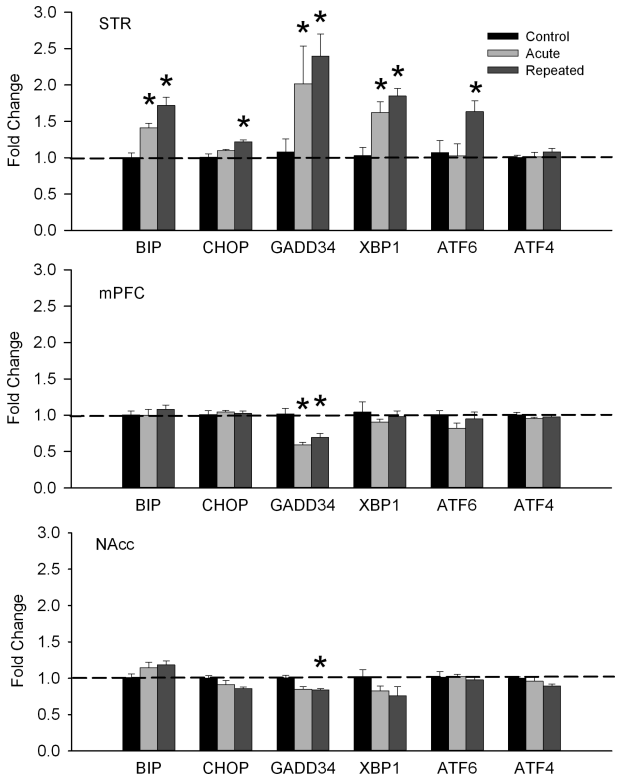

Figure 1. Restraint stress induces ER stress response genes in the STR

Bars represent mean (±SEM) fold change mRNA for acute and repeated (9 days) restraint stress in STR (top), mPFC (middle) and NAcc (bottom). Stress was given for 1 hr and tissue harvested at 3hrs after the beginning of the stress. N = 5-6. Asterisk denotes statistically significant from Control.

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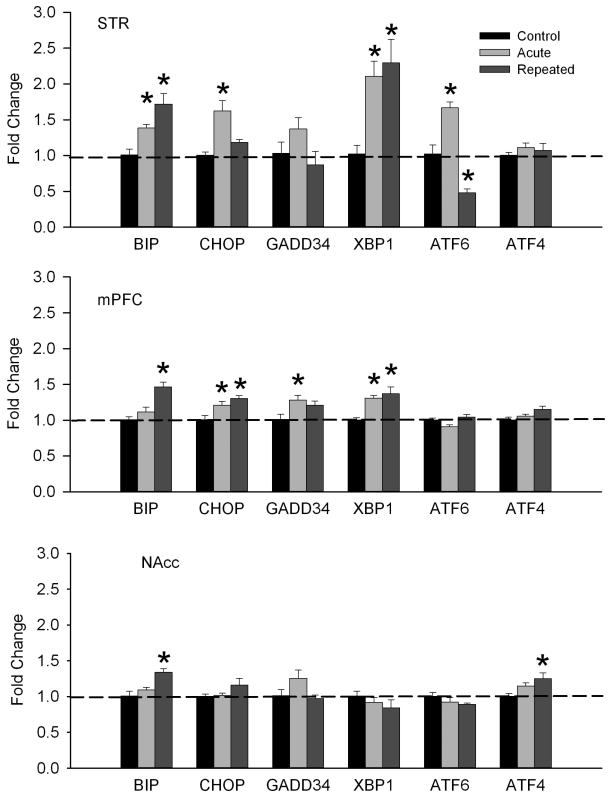

Figure 2. Cocaine induces ER stress response genes in the STR, mPFC and NAcc

Bars represent mean (±SEM) fold change mRNA for acute and repeated cocaine (2 X 20 mg/kg; Repeated = 9 days) in STR (top), mPFC (middle) and NAcc (bottom). Cocaine was injected at 0 and 1 hr and tissue harvested at 3hrs after the first injection. N = 5-6. Asterisk denotes statistically significant from Saline Control.

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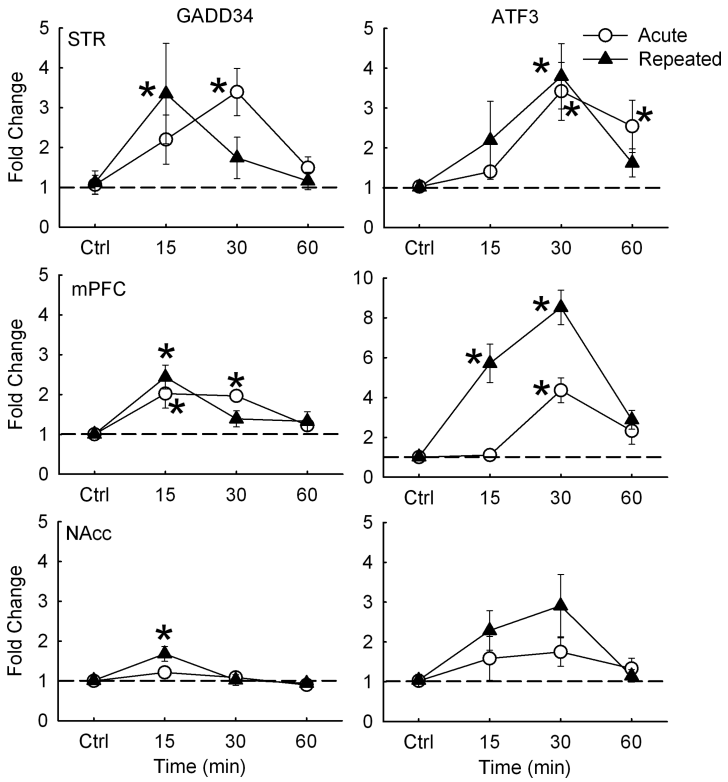

Figure 3. Acute and repeated cocaine rapidly induce GADD34 and ATF3

Points represent mean (±SEM) fold induction of mRNA for GADD34 (left panels) and ATF3 (right panels) in STR (top panels), mPFC (middle panels) and NAcc (bottom panels). Cocaine was administered at a dose of 20 mg/kg (IP). Control rats were immediately sacrificed at 0 min. Asterisks represent significant difference from Control. N = 5-6.

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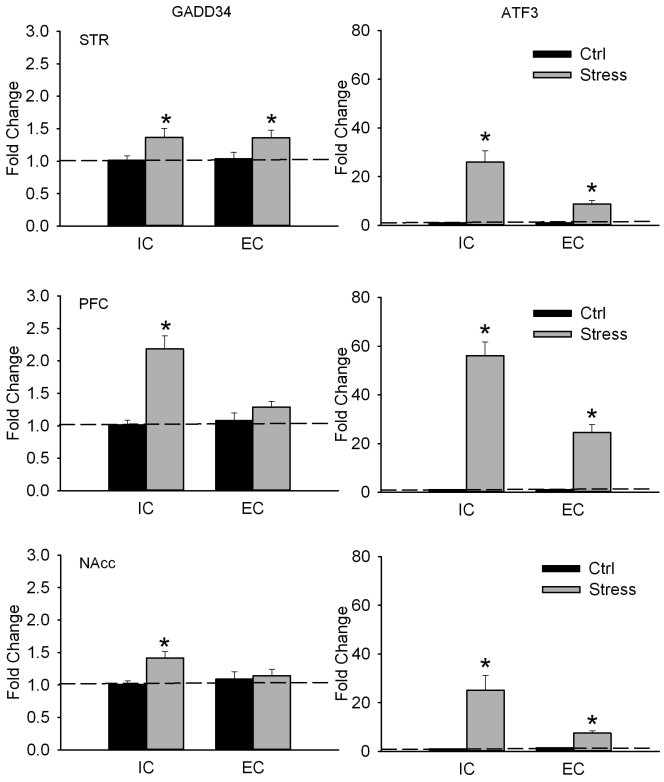

Figure 4. Environmental enrichment attenuates restraint stress-induced GADD34 and ATF3 mRNA induction

Bars represent mean (±SEM) fold induction of GADD34 (left panels) and ATF3 (right panels) mRNA in STR (top panels),mPFC (middle panels) and NAcc (bottom panels) of enriched (EC) and isolated (IC) rats after 30 min of restraint stress. Asterisks represent significant differences from control group of same condition. N = 5-6.

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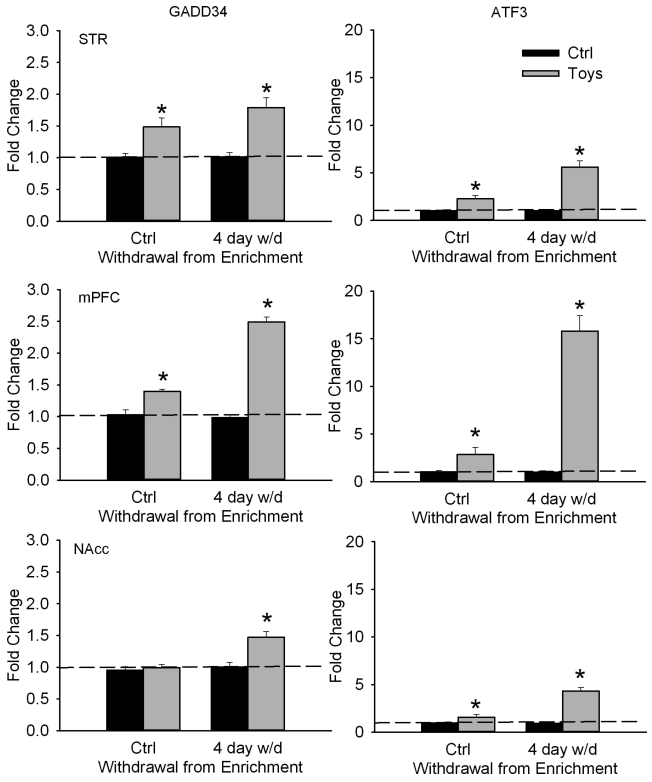

Figure 5. Four days of enrichment withdrawal increases GADD34 and ATF3 induction after toy change

Bars represent mean (±SEM) mRNA induction of GADD34 (left panels) and ATF3 (right panels) in STR (top panels), mPFC (middle panels) and NAcc (bottom panels) in EC Control rats versus EC rats given 30 min of novel toys. Additionally, half of the animals were isolated for 4 days prior to novelty. Asterisks denote difference from Control of same condition. N = 5-6.

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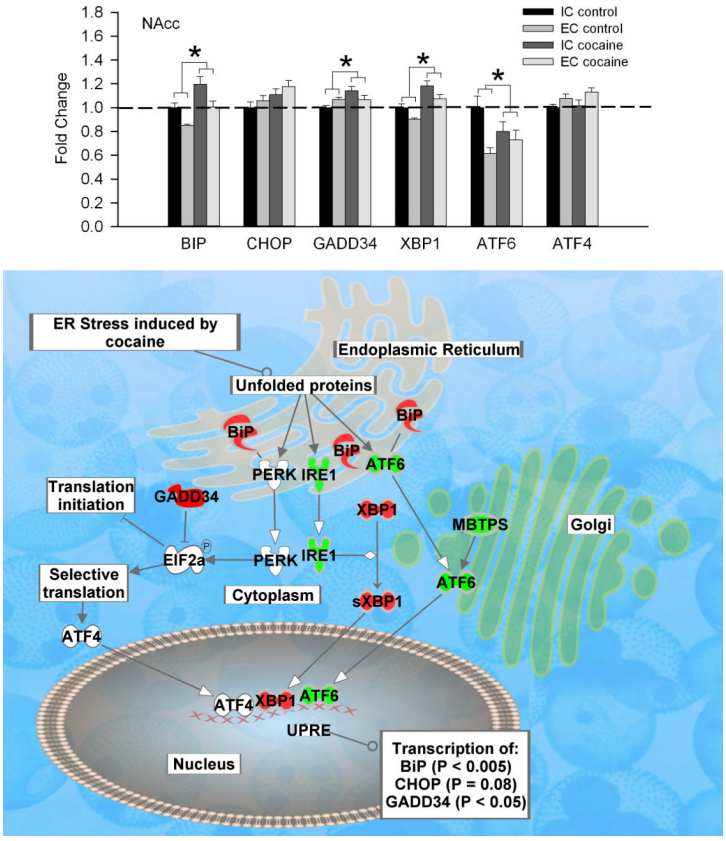

Figure 6. Orthogonal validation of ER stress-induced transcription *via* RNA sequencing

Bars represent mean (± SEM) fold change in transcript expression of ER stress-related genes in NAcc of EC and IC rats 3 hrs after chronic self-administration of cocaine or saline (N = 7 - 8). Asterisks represent statistically significant differences in Cocaine vs. Saline groups (i.e. main effect of Cocaine). The bottom panel depicts the canonical ER stress pathway with regulated transcripts. Red symbols denote upregulation of mRNA and green symbols denote downregulation. MBTPS is the enzyme that cleaves unfolded ATF6, allowing it to activate transcription.