Published in final edited form as:

Psychiatry Res. 2017 January ; 247: 172–181. doi:10.1016/j.psychres.2016.11.027.

Examining *FKBP5* mRNA expression in human iPSC-derived neural cells

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Abstract

In peripheral blood leukocytes, *FKBP5* mRNA expression is upregulated following glucocorticoid receptor activation. The single nucleotide polymorphism rs1360780 in *FKBP5* is associated with psychiatric illness and has functional molecular effects. However, examination of *FKBP5* regulation has largely been limited to peripheral cells, which may not reflect regulation in neural cells. We used 27 human induced pluripotent stem cell lines (iPSCs) derived from 20 subjects to examine *FKBP5* mRNA expression following GR activation. Following differentiation into forebrain-lineage neural cultures, cells were exposed to 1 μ M dexamethasone and mRNA expression of *FKBP5* and *NR3C1* analyzed. Results from the iPSC-derived neural cells were compared with those from 15 donor matched fibroblast lines. Following dexamethasone treatment, there was a 670% increase in *FKBP5* expression in fibroblasts, mimicking findings in peripheral blood-derived cells, but only a 23% increase in iPSC-derived neural cultures. *FKBP5* rs1360780 genotype did not affect the induction of *FKBP5* mRNA in either fibroblasts or neural cells. These results suggest that iPSC-derived forebrain-lineage neurons may not be an optimal neural cell type in which to examine relationships between GR activation, *FKBP5* expression, and genetic variation in human subjects. Further, *FKBP5* induction following GR activation may differ between cell types derived from the same individual.

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Conflicts of interest

Dr. Kranzler has served as a consultant, CME speaker, or advisory board member for the following companies: Indivior, Lundbeck, and Pfizer. He is a member of the Alcohol Clinical Trials Group of the American Society of Clinical Psychopharmacology, which is supported by Abbvie, Alkermes, Ethypharm, Indivior, Lilly, Lundbeck, Otsuka, Pfizer, and Xenoport.

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Keywords

FK506 Binding Protein 51; neural differentiation; gene expression; glucocorticoid receptor; fibroblasts; induced pluripotent stem cells; rs1360780

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis response to stress involves the interaction of many signaling molecules. Dysregulation of the stress response could increase vulnerability to stress-related disease, particularly if the affected individual is genetically predisposed to the negative effects of stress (De Kloet, 2004). Differences in response to stress have been observed in individuals with a variety of psychiatric disorders including schizophrenia, bipolar disorder, depression, post-traumatic stress disorder, and alcohol use disorder. However, the molecular mechanisms that underlie dysregulation of the stress response, particularly those in the human brain, remain unclear.

The glucocorticoid receptor (GR) is a cytosolic transcription factor activated by the stress hormone cortisol (de Kloet et al., 2005; Schatzberg et al., 2014). Following cortisol binding, the GR translocates to the cell nucleus and functions as a regulator of transcription by binding to specific DNA sequences in promoter or enhancer regions called GR response elements (GREs) (de Kloet et al., 2005; Grad et al., 2007; Binder, 2009). GR translocation is a complex process involving a variety of molecular chaperones and co-chaperones including the FK506 binding protein 51 (FKBP5), which is encoded by the *FKBP5* gene. FKBP5 is part of a protein heteromeric-complex that represses the transcriptional activity of the GR by reducing cortisol binding affinity and inhibiting GR translocation to the nucleus (Grad et al., 2007; Stechschulte et al., 2011; Sinclair et al., 2013). Following GR activation, *FKBP5* mRNA is upregulated via GR binding to GREs located in promoter and intronic regions of *FKBP5*. Increased levels of FKBP5 protein inhibit GR activity, creating a short negative feedback loop whereby GR activation limits further GR activation (Binder, 2009; Zannas et al., 2014).

Polymorphisms within a 100 kb haplotype block in *FKBP5* that are tagged by the single nucleotide polymorphism (SNP) rs1360780 have been linked to a number of stress-related psychiatric conditions including PTSD, anxiety, depression, and addictive disorders (Binder et al., 2004; Binder et al., 2008; Lavebratt et al., 2010; Roy et al., 2010; Kang et al., 2012; Klengel et al., 2013; Huang et al., 2014; Levran et al., 2014; Jensen et al., 2015). For PTSD, childhood trauma, but not adult trauma, interacts with *FKBP5* genotype to predict greater self-reported symptoms on the PTSD Symptom Scale (Binder et al., 2008), while for other stress-related disorders *FKBP5* genotype has been reported to interact with both childhood and lifetime stressors (Zannas et al., 2014). These genetic variants may lead to differential expression of *FKBP5* mRNA and protein by altering chromatin structure and DNA methylation in response to environmental or developmental events (Binder et al., 2004; Binder, 2009; Klengel et al., 2013), which may have long-lasting functional consequences for an individual's response to stress. For example, in a study of 310 infants, carriers of the *FKBP5* rs1360780 minor T-allele had increased cortisol reactivity to minor stress (Luijk et

al., 2010). However, no genotype effect was observed on salivary cortisol levels in a sample of 368 healthy adults using the Trier Social Stress Test (Mahon et al., 2013), suggesting that genotype effects on stress response may vary during development. *In vitro* evidence for developmental state-specific regulation of *FKBP5* comes from the examination of human hippocampal progenitor cells, where dexamethasone exposure during the proliferation/differentiation phase, but not during the post-proliferation/differentiation phase, induced long-lasting DNA de-methylation within a CpG site in *FKBP5* (Klengel et al., 2013).

Studies examining *FKBP5* mRNA expression following GR activation or as a function of genotype have largely relied on easily accessible peripheral blood cells, immortalized human cell lines, or animal models. However, because gene expression profiles in peripheral cells are weakly correlated with expression in the central nervous system (Sullivan et al., 2006), expression studies using human neural cells are needed. The purpose of this study was to examine gene expression changes in iPSC-derived human neural cells *in vitro* following acute GR activation with the synthetic glucocorticoid dexamethasone. We reprogrammed fibroblasts generated from 20 donor subjects into 27 induced pluripotent stem cell lines (Takahashi et al., 2006; Takahashi et al., 2007) and used an established protocol to differentiate stem cells into a forebrain lineage. We then examined the effects of a 6-hr dexamethasone exposure on mRNA expression of the GR gene, *NR3C1*, and the gene encoding its co-chaperone FKBP5 in human neural cells and compared the results to those from dexamethasone-treated fibroblasts from a subset of 15 donor subjects.

2. Materials and methods

2.1 Human iPSCs

iPSCs were generated from fibroblasts obtained using a skin punch biopsy of the inner, upper arm from 20 participants enrolled in a human laboratory study examining effects of alcohol or a clinical trial at UCONN Health (UC, Farmington, CT (Kranzler et al., 2014; Milivojevic et al., 2014). Participants enrolled in the study examining effects of alcohol were non-alcoholics, while those enrolled in the clinical trial were diagnosed with an alcohol use disorder. Fibroblasts were obtained from 9 non-alcoholic and 11 alcoholic participants. Average age of the donor subjects was 40.9 years. Three of donors were female. Biopsy samples were minced and cultured in Dulbecco's modified eagles medium (DMEM, Life Technologies) supplemented with 20% fetal bovine serum (FBS, Life Technologies), 1x non-essential amino acids (Life Technologies) and 1x penicillin/streptomycin (Life Technologies). Fibroblast cultures were expanded and passaged using trypsin (Life Technologies) prior to being frozen or sent for reprogramming.

The UCONN Stem Cell Core (Farmington, CT) reprogrammed fibroblasts to pluripotency using either a retrovirus to express five factors (*OCT4*, *SOX2*, *KLF4*, *c-MYC*, and *LIN28*) (13 donors) or a sendai virus to express four factors (*OCT4*, *SOX2*, *KLF4*, and *c-MYC*) (7 donors). Two to four weeks after viral transduction, multiple pluripotent stem cell colonies for each subject were selected and expanded as individual clones. Pluripotency of selected colonies was verified by immunocytochemistry for SSEA-3/4 and NANOG by the UCONN Stem Cell Core. 27 iPSC lines generated from the 20 donor subjects were used in the current

experiments, with 2 clones examined from seven donor subjects and 1 clone examined from 13 subjects. 5 of the 27 lines examined were female.

As previously described (Lieberman et al., 2012), iPSCs were cultured on a feeder layer of irradiated mouse embryonic fibroblasts using human embryonic stem cell media containing DMEM with F12 (DMEM/F12, 1:1 ratio, Life Technologies) supplemented with 20% Knockout Serum Replacer (Life Technologies), 1x non-essential amino acids, 1 mM L-glutamine (Life Technologies), 0.1 mM β -mercaptoethanol (MP Biomedicals, Solon, OH), and 4 ng/mL of basic fibroblast growth factor (bFGF, Millipore). iPSCs were monitored daily and colonies exhibiting spontaneous differentiation were manually removed. Media was fully replaced daily and cells were cultured for 7 days, or to confluency, before being passaged using 1 mg/mL Dispase (Life technologies) in DMEM/F12.

2.2 iPSC neural differentiation

iPSCs were differentiated into neural cell cultures as previously described (Lieberman et al., 2012) using an established protocol developed by the WiCell Institute for the differentiation of human embryonic stem cells into neural cells of a forebrain lineage (#SOP-CH-207, REV A, www.wicell.org, Madison, WI). Briefly, we used an “Embryoid Body” (EB)-based protocol wherein iPSC colonies are removed from the feeder layer substrate and cultured in suspension before going through a neural induction phase to generate primitive neuroepithelial cells. Following 3 weeks of culture in neural induction media, cells were dissociated and cultured in 24-well plates on Matrigel (BD Biosciences, Bedford, MA) coated glass coverslips in neural differentiation media containing the neural growth factors 1x B27 supplement (Life Technologies), 1 μ g/mL laminin (Sigma-Aldrich), and 10ng/ μ L of each brain-derived neurotrophic factor (BDNF, Peprotech, Rocky Hill, NJ), glial-derived neurotrophic factor (GDNF, Peprotech), and insulin-like growth factor 1 (IGF-1, Peprotech). All cells were incubated at 37° in 5% CO₂.

2.3 Fibroblast culture

Fibroblasts available from 15 of the 20 donor subjects were thawed from liquid nitrogen storage and plated into 12-well culture dishes in fibroblast media containing DMEM (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies) and 1x non-essential amino acids (Life Technologies). Cells were cultured to confluency for 10–14 days with full media changes twice per week.

2.4 Immunocytochemistry

Eight weeks after being plated onto glass coverslips, neural cultures were fixed using 4% paraformaldehyde in PBS for 20 min at room temperature and permeabilized using 0.2% triton X-100 (Sigma-Aldrich) in PBS for 10 min. Following a 1-hr block using 5% donkey serum (Jackson ImmunoResearch, West Grove, PA), cultures were incubated for 24–48 hours at 4° with the following primary antibodies diluted in 5% donkey serum in PBS: mouse anti-beta III-tubulin (1:500, Covance, Dedham, MA), mouse anti-GFAP (1:500, Millipore, Billerica, CA), rabbit anti-MAP2 (1:500, Millipore), rabbit anti-glucocorticoid receptor (1:100, Life Technologies), and rabbit anti-TBR1 (a forebrain glutamatergic neuromarker; 1:1000, ProteinTech Group, Chicago, IL, incubation included 0.1% triton

X-100). Cells were then incubated at room temperature for 45 min in donkey anti-mouse alexa fluor 594 (1:1000, Life Technologies) and donkey anti-rabbit alexa fluor 488 (1:1000, Life Technologies) secondary antibodies diluted in 3% donkey serum in PBS for visualization.

2.5 Electrophysiology

Whole cell patch-clamp electrophysiology was performed on 48 neurons derived from one subject using previously described techniques (Lemtiri-Chlieh et al., 2010). Eight-week old neurons were selected for recording based on morphology, including pyramidal-shaped soma and presence of neurites. Artificial cerebrospinal fluid (aCSF) containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 1 mM MgCl₂·6H₂O, 25 mM NaHCO₃, 2 mM CaCl₂, and 25 mM dextrose was perfused through the recording chamber at 1 ml/min at room temperature. To examine peak sodium and potassium currents, generation of action potentials, excitatory spontaneous synaptic activity, spontaneous action potential firing, and resting membrane potential, an internal recording solution was used containing 4 mM KCl, 125 mM K-gluconate, 10 mM HEPES, 10 mM phosphocreatine, 1 mM EGTA, 0.2 mM CaCl₂, 4 mM Na₂-ATP, and 0.3 mM Na-GTP (pH 7.3). Upon break-in, neurons were noted for their resting membrane potential by injection with 0 current and corrected post-hoc for liquid junction potential. Action potentials were evoked in current clamp mode at ~-70 mV by applying 500 ms duration current steps from -20 pA to +40 pA in 5 pA intervals. Spontaneous action potential firing was recorded at ~-40 mV. Inward and outward currents were elicited in voltage clamp mode by applying 300 ms steps from -100 mV to +40 mV in 10 mV increments with the membrane potential held at -70 mV. Spontaneous excitatory synaptic activity was monitored while holding the cell at -70 mV in voltage-clamp mode. All electrophysiological recordings were performed using a HEKA EPC9 amplifier and PatchMaster software (version 2×67). The analysis and quantification of peak currents (inward sodium, outward potassium, and transient potassium), action potential properties (peak amplitude, threshold, and half-width), and excitatory spontaneous activity (frequency and amplitude) was performed using Axon Clampfit software (version 10.3.1.4).

2.6 Acute dexamethasone exposure

Six to seven week-old neural cells and ~95% confluent fibroblast primary cultures were exposed to 1 μM dexamethasone (Sigma-Aldrich) for 6 hr. This concentration of dexamethasone has previously been shown to increase expression of *FKBP5* in human peripheral blood mononuclear cells (Vermeer et al., 2004). Dexamethasone was reconstituted to a concentration of 100 mM in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and diluted to experimental concentrations in either neural differentiation media for use with neural cells or in fibroblast media for use with fibroblasts. On the day of the experiment, plates of cells were removed from the incubator and 100% of the media was aspirated and replaced with 500 μL of neural differentiation media or 1 mL of fibroblast media containing either vehicle (DMSO) or dexamethasone. For neural cells, 4–6 wells of a 24-well plate were used per condition. For fibroblasts, 3 wells of a 12-well plate were used per condition. Cells were returned to the incubator during the 6-hr exposure.

2.7 Quantitative polymerase chain reaction (qPCR)

Following a 6-hr dexamethasone exposure, RNA was extracted from the neural cell and fibroblast cultures using TRIzol reagent (Life Technologies) according to the manufacturer's protocol. RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fischer Scientific, Pittsburgh, PA) and 50 μ L of cDNA was synthesized from 2 μ g of RNA using a High Capacity cDNA Reverse Transcription Kit (Life Technologies) per the manufacturer's protocol. Additionally, the RNA samples for all subjects underwent the same preparation without the addition of reverse transcriptase to test for potential DNA contamination.

cDNA was analyzed by qPCR using an Applied Biosystems 7500 instrument (Life Technologies) and FAM-labeled TaqMAN Assay on Demand (Life technologies) probe and primer sets for *NR3C1* (Hs00353740_m1), *FKBP5* (Hs01561006_m1), *FKBP4* (Hs00427038_g1), *BAG1* (Hs00185390_m1), *SLC17A7* (Hs00220404_m1), *SLC12A5* (Hs00221168_m1), *GAD2* (Hs00609534_m1), and *RBFOX3* (Hs01370653_m1).

Expression of these genes was normalized to the VIC-labeled housekeeping gene *GUSB* (beta-glucuronidase, 4326320E, Life Technologies). *GUSB* was selected as a control due to robust, detectable expression using qPCR in our neural cells and fibroblasts, and our prior demonstration of its reliability in studies of alcohol and genetic variation in iPSC-derived neural cultures (Lieberman et al., 2012; Lieberman et al., 2015). cDNA synthesized from RNA extracted from each culture well was assayed in triplicate 20 μ L reactions using Gene Expression Master Mix (Life Technologies) per the manufacturer's protocol, with the addition of 0.1% bovine serum albumin (BSA, Life Technologies).

PCR cycles were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. A standard curve consisting of a 4-level serial dilution of 100%, 50%, 25%, and 12.5% cDNA from neural cells differentiated from one subject was added to each plate to determine the relative mRNA expression among all subjects and across different qPCR plates. Data are displayed as mRNA abundance normalized to the housekeeping gene *GUSB* where a unit of 1 is equivalent to the abundance of the target gene relative to *GUSB* in the reference RNA sample.

2.8 Human post-mortem brain

Eleven frozen post mortem brain samples were obtained through the Harvard Brain Bank for comparison to iPSC-derived neural cells. RNA was extracted and cDNA synthesized as described above according to the manufacturer's protocols. The samples consisted of frontal cortex derived from 7 males and 4 females, all of which were Caucasian. The average age was 77.1 years (age information was missing from one donor subject), and the average post-mortem interval was 24.3 hours. mRNA expression of *FKBP5* and *NR3C1* was assayed using qPCR as described above, with quantitative analysis performed using the same standard curve reference RNA sample was used for iPSC-derived neural cells and fibroblasts to allow for comparison across qPCR reactions and between groups.

2.9 Statistical analysis

While the whole-transcriptome profiles of iPSC clones derived from the same donor subject are similar (Schuster et al., 2015), we previously identified differences in targeted gene

expression between clones (Lieberman et al., 2015). Therefore, we assumed for the statistical analysis that iPSC clones were separate lines even when derived from the same donor subject. Gene expression following dexamethasone exposure was analyzed for statistical significance using paired t-tests comparing vehicle- with drug-treated cells derived from the same iPSC line. Basal gene expression between neural cells differing by rs1360780 genotype was analyzed for statistical significance using unpaired t-tests. Basal expression of iPSC-derived neural cells, fibroblasts, and post-mortem brain was compared statistically using one-way ANOVA with a Bonferroni multiple comparison post-test. All statistical analysis was performed using GraphPad Prism software (V5.0f for Mac, GraphPad Software, San Diego, CA, www.graphpad.com). Statistical significance was identified as $p < 0.05$.

3. Results

3.1 iPSCs differentiate into mixed neural cultures enriched for functional glutamate neurons

The 27 iPSC lines derived from the 20 subjects were differentiated into mixed neural cultures containing MAP2-positive neurons and GFAP-positive astrocytes (Figure 1A). A majority (72.5%) of total cells ($N=371$ DAPI-positive counted) stained positive for the glutamatergic neuron marker TBR1, which co-localized with the neuron marker beta III-tubulin (Figure 1B). Importantly, expression of the glucocorticoid receptor was verified in iPSC-derived neural cultures, as observed in the cytosol of both beta III-tubulin-positive neurons (Figure 1C) and GFAP-positive astrocytes (Figure 1D). qPCR examining the abundance of *FKBP5* and *NR3C1* mRNA in cells harvested at four developmental time points (iPS Cells, neuroepithelial, 2-week neural cultures, and 6-week neural cultures) depicts the pattern of expression over neural differentiation and suggests that the genes are expressed at detectable levels in our culture system (Figure 1E–F).

Our prior report demonstrated the ability of the neural differentiation protocol that we used to generate neurons with functional voltage-gated sodium and potassium channels and excitatory (NMDA and AMPA) and inhibitory (GABA_A) ligand-gated ion channels (Lieberman et al., 2012). Additional functional characteristics of iPSC-derived neurons were assessed using whole cell patch-clamp electrophysiology on 8-week old cells derived from one donor. A total of 48 neurons were patched. Depolarizing voltage steps elicited inward sodium and outward potassium currents (Figure 2A), and the injection of depolarizing current evoked firing of action potentials (Figure 2B). Mature action potentials (either a single mature spike, or a train of mature spikes) were observed in 45/48 neurons. Presence of any spontaneous excitatory event over a 3-minute recording was observed in 25/48 cells, with an average frequency of 0.16 ± 0.05 Hz and average amplitude of $-16 \text{ pA} \pm 1.72$ (Figure 2C). Spontaneous action potentials recorded at -40 mV in current clamp mode were observed in 8/48 neurons (Figure 2D). The average resting membrane potential for the 48 cells was $-51.9 \text{ mV} \pm 1.28$ (Figure 2E). Taken together, these immunocytochemistry and electrophysiology results indicate that our differentiation protocol produced neural cells that express the glucocorticoid receptor and are enriched for glutamatergic forebrain neurons that

express voltage-sensitive ion channels, fire mature action potentials, and receive spontaneous excitatory synaptic inputs.

3.2 Comparing the expression of FKBP5 and NR3C1 for iPSC-derived neural cells, fibroblasts, and post-mortem brain

Recent studies have revealed dynamic regulation of the *FKBP5* and *NR3C1* genes in the dorsolateral prefrontal cortex during development (Sinclair et al., 2011; Weickert et al., 2015). The reports describe increasing mRNA expression with age, with *FKBP5* expression peaking in adulthood (>40 years) and *NR3C1* peaking in early adulthood (~20 years). The dynamic changes in expression may suggest a developmental increase in molecular stress-response components. To compare the relative maturity of our iPSC-derived neural cells with respect to *FKBP5* and *NR3C1* expression, we utilized RNA extracted from 11 frontal cortex samples (mean age = 77.1 years) to compare basal expression levels of *FKBP5* and *NR3C1* with that in human post-mortem brain. *FKBP5* mRNA expression was 4-fold higher in post-mortem brain than in iPSC-derived neural cells ($t=8.8$, $p<0.0001$) and fibroblasts ($t=8.6$, $p<0.0001$) (Figure 3A). There was no difference in the expression of *FKBP5* between iPSC-derived neural cells and fibroblasts ($t=0.9$, $p>0.05$). *NR3C1* mRNA expression was ~3-fold higher in post-mortem brain than iPSC-derived neural cells ($t=7.8$, $p<0.0001$) and ~5-fold higher in fibroblasts than iPSC-derived neural cells ($t=19$, $p<0.0001$), but was significantly lower in post-mortem brain than fibroblasts ($t=8.4$, $p<0.0001$) (Figure 3B).

3.3 Examining FKBP5 and NR3C1 mRNA following dexamethasone treatment in iPSC-derived neural cultures and fibroblasts

Acute dexamethasone treatment increased mRNA expression of *FKBP5* ($t=4.1$, $df=26$, $p<0.001$) (Figure 4A) in iPSC-derived neural cultures by an average of 23%, which was statistically significant. *FKBP5* induction was similar following a 24-hour dexamethasone exposure in neural cells derived from two subjects, with mRNA expression increasing an average of 28% (data not shown). There was no significant change in the expression of the glucocorticoid receptor gene *NR3C1* ($p=0.60$) (Figure 4B). Fibroblasts used for reprogramming were available from 15 of the 20 donor subjects. In contrast to neural cells, acute dexamethasone treatment produced a robust, 670% induction of *FKBP5* ($t=9.1$, $df=14$, $p<0.0001$) (Figure 4C) in fibroblast cultures. Expression of the glucocorticoid receptor gene *NR3C1* was reduced by 13% following dexamethasone treatment, a significant effect ($t=2.5$, $df=14$, $p<0.05$) (Figure 4D).

3.4 rs1360780 genotype did not moderate induction of FKBP5 mRNA in neural cells or fibroblasts

The 27 neural cell lines examined included 11 rs1360780*C homozygotes and 16 rs1360780*T-allele carriers (14 C/T and 2 T/T). There were no differences between rs1360780 C/C or T-allele carrier genotypes in basal expression of *FKBP5* ($p=0.12$), *NR3C1* ($p=0.64$), or additional glucocorticoid receptor regulators *FKBP4* ($p=0.59$) or *BAG1* ($p=0.51$), (Figure 5A–D). Furthermore, in neural cells from a subset of 5 C/C and 7 T-allele carriers, no significant differences were observed in the expression of the neural genes *RBFOX3* (NEUN, $p=0.87$), *GAD2* (GAD67, $p=0.38$), *SLC17A7* (VGLUT1, $p=0.34$), or *SLC12A5* (KCC2, $p=0.19$) (data not shown). These results reflect homogeneity in

the neural differentiation capacity of iPSCs derived from donor subjects of either *FKBP5* genotype group.

Acute exposure to 1 μ M dexamethasone non-significantly increased *FKBP5* expression in neural cells from rs1360780*C/C subjects ($t=2.2$, $df=10$, $p=0.055$), with a modest increase seen in T-allele carriers ($t=4$, $df=15$, $p<0.01$) and an average increase of 23% observed in neural cells from both genotypes (Figure 6A–B). The 15 fibroblast lines examined included 6 rs1360780*C homozygotes and 9 rs1360780*T-allele carriers (8 C/T and 1 T/T). Exposure to 1 μ M dexamethasone produced a robust, significant increase in *FKBP5* mRNA expression in fibroblasts from both rs1360780*C/C ($t=5.3$, $df=5$, $p<0.01$) and *T-allele carriers ($t=7.1$, $df=8$, $p<0.0001$), with average increases of 632% in C/C subjects and 700% in T-allele carriers (Figure 6C–D). There was no significant difference observed between C/C and T-allele carrier genotypes in the basal expression of *FKBP5* ($p=0.67$) or its induction by dexamethasone exposure ($p=0.96$).

4. Discussion

The differentiation of iPSCs makes it possible to examine difficult-to-obtain human cell types *in vitro*. In the current study, we examined mRNA expression of *FKBP5* and *NR3C1* after acute exposure to 1 μ M dexamethasone in 27 human neural cell cultures differentiated from iPSCs derived from 20 donor subjects. This concentration of dexamethasone was previously shown to induce *FKBP5* expression in human peripheral blood mononuclear cells (Vermeer et al., 2004), a cell type used in prior studies examining the effects of dexamethasone on gene expression due to its sensitivity to glucocorticoids. However, differences in the sensitivity to glucocorticoids can vary across cell types (Ebrecht et al., 2000) and little research has examined the effect of dexamethasone treatment on neural cells, particularly those from human donors.

We found that the expression of *FKBP5* mRNA changed by 23% in human iPSC-derived neural cells following dexamethasone treatment, which contrasts with findings obtained examining human peripheral blood-derived cells, in which a 15-fold increase in *FKBP5* mRNA in T- and B-lymphocytes following *in vitro* stimulation (Vermeer et al., 2004) and a 10-fold upregulation in whole blood following treatment of subjects with 1.5 mg of oral dexamethasone (Menke et al., 2012). While there are clearly limitations to the direct comparison of *in vitro* iPSC-derived neural cell responses to those that may occur *in vivo*, the similar magnitude of *FKBP5* mRNA induction following *in vitro* (Vermeer et al., 2004) and *in vivo* stimulation of white blood cells (Menke et al., 2012) suggests that physiologically relevant levels of GR stimulation of *FKBP5* expression can be modeled using *in vitro* systems despite the absence of an intact HPA-axis circuit *in vitro*.

Interestingly, we observed a robust upregulation of *FKBP5* mRNA in human fibroblasts following dexamethasone treatment. Comparing results from previous studies to the data here suggests that *FKBP5* mRNA expression is induced more robustly in peripheral cells than in neural tissue following GR activation. Additional support for this interpretation comes from an examination of the SH-SY5Y human neuroblastoma cell line, in which *FKBP5* expression increased by $\approx 25\%$ following exposure to cortisol (Tatro et al., 2009), a

finding that was mimicked in the mouse hippocampal HT-22 cell line, which showed an $\approx 50\%$ increase in *FKBP5* mRNA expression following a 6-hr corticosterone exposure, with a peak increase of $\approx 250\%$ after three days (Lee et al., 2010). Additional rodent studies have shown that levels of *FKBP5* mRNA vary by brain region, with the highest basal expression found in the hippocampal CA1 and dentate gyrus regions. Increases in *FKBP5* mRNA expression resulting from stimulation with dexamethasone or acute stress ranged from approximately 50–200% and differed by brain region, with larger increases in *FKBP5* mRNA expression in the central amygdala and paraventricular nucleus than in the hippocampal CA1 and dentate gyrus regions, with no change in expression observed in the CA2 and CA3 regions (Scharf et al., 2011). *FKBP5* expression, together with other GR-response element regulated genes, was increased in a dose-response fashion in the prefrontal cortex of DBA2/J mice following i.p. alcohol injections, which the authors propose promotes glucocorticoid release as observed in prior rodent studies (Ellis, 1966). In contrast, *FKBP5* mRNA (and that of the other GR-response genes examined) was not increased in the ventral tegmental area or nucleus accumbens (Kerns et al., 2005), providing further evidence for brain region-specific regulation of *FKBP5* and other GR-responsive genes. It is possible that we did not observe a significant change in *FKBP5* mRNA expression following dexamethasone exposure due to the forebrain-type neural differentiation protocol used. Future research should aim to differentiate human iPSCs into other neural cell types, such as hippocampal dentate gyrus-like neural cells (Yu et al., 2014; Mertens et al., 2015), in order to elucidate mechanisms that lead to differences by brain region in *FKBP5* mRNA induction following GR activation.

We also observed a significant decrease in *NR3C1* gene expression following dexamethasone exposure in fibroblasts, but not in iPSC-derived neural cells. A reduction in glucocorticoid receptor gene expression following dexamethasone has been reported previously, including in horses given oral dexamethasone (Ing et al., 2014). The autoregulatory loop whereby glucocorticoid receptor activation downregulates glucocorticoid receptor gene expression may serve to moderate hormone-activated responses, and is likely the result of both transcriptional repression of the *NR3C1* gene and posttranscriptional reduction in mRNA stability (Ing, 2005). This finding suggests that there are differences between glucocorticoid receptor-mediated signaling in iPSC-derived forebrain type neural cells and peripheral somatic cells, and that iPSC-derived neural cells may be less capable of functionally mature glucocorticoid receptor signaling.

Post-mortem analysis of human brain gene expression has implicated *FKBP5* in psychiatric disorders. *FKBP5* mRNA levels are more than 2-fold higher in the hippocampus of alcoholic individuals (McClintick et al., 2013), significantly elevated in the dorsolateral prefrontal cortex of individuals with bipolar disorder and schizophrenia (Sinclair et al., 2013), and significantly lower in the amygdala of suicide victims (Perez-Ortiz et al., 2013). At the genetic level, SNPs spanning promoter and intronic regions of the gene have been associated with anxiety and depression (Binder et al., 2004; Kang et al., 2012), post-traumatic stress disorder (Binder et al., 2008; Klengel et al., 2013), and most recently with the negative effects of nicotine (Jensen et al., 2015) and the severity of alcohol withdrawal symptoms (Huang et al., 2014). *FKBP5* genetic variation is associated with altered chromatin structure and differences in methylation in traumatized individuals (Klengel et al., 2013), leading to

“high induction” and “low induction” alleles and greater upregulation of *FKBP5* expression in trauma-exposed carriers of the rs1360780 T-allele (Binder, 2009). However, evidence for an *FKBP5* risk genotype moderating *FKBP5* expression is limited to a few studies and not yet conclusive. Using peripheral blood mononuclear cells, Binder et al. (2004) found no significant difference in *FKBP5* mRNA expression, but significantly higher FKBP5 protein levels in rs1360780 T-allele homozygotes (Binder et al., 2004). In post-mortem human brain from controls and subjects with bipolar disorder or schizophrenia, there was a significant effect of rs4713916, a SNP located in the promoter region of *FKBP5* and in moderate linkage disequilibrium with rs1360780 ($D' = 0.85$), on basal FKBP5 protein expression in homozygotes of the minor T-allele (Sinclair et al., 2013). Perhaps most relevant to our findings regarding *FKBP5* expression following GR activation, Hohne et al. (2015) found that psychosocial stress increased expression in the peripheral blood cells of rs1360780 C-allele homozygotes, while T-allele carriers showed a blunted induction of *FKBP5* mRNA that did not differ from unstressed controls (Hohne et al., 2015). Additionally, it may be that *FKBP5* genotype effects on gene expression following dexamethasone stimulation can only be seen in the setting of stress occurring at particular developmental time points, as suggested by a recent study that found a reduced induction of *FKBP5* mRNA following dexamethasone stimulation of lymphoblastoid cell lines derived from childhood trauma-exposed individuals harboring the rs1360780 T-allele compared with non-trauma exposed cell lines (Yeo et al., 2016). Furthermore, human hippocampal progenitor cells treated with dexamethasone during the early proliferation/differentiation phase, but not later in the post-proliferation/differentiating phase, exhibited long-lasting de-methylation of *FKBP5* DNA (Klengel et al., 2013). However, this study did not look at the effect of dexamethasone treatment on *FKBP5* mRNA expression and was limited to the use of a single genetically uncharacterized neural progenitor cell line. In contrast, human iPSC technologies allow for the examination of neural cells from multiple genetically characterized donor subjects. In the current study, we found no effect of *FKBP5* rs1360780 genotype on the magnitude of induction of *FKBP5* following acute dexamethasone exposure in either human iPSC-derived neural cells or fibroblasts. A limitation of our study is that our T-allele carrier group contained only a single rs1360780 T-allele homozygote, which could have obscured an association between genotype and *FKBP5* gene expression if dexamethasone response differences are limited to T/T individuals.

Another limitation is that iPSC-derived neural cultures model relatively immature neural cells. We observed that *FKBP5* and *NR3C1* mRNA levels were significantly higher in post mortem brain samples than in 6–7 week old iPSC-derived neural cells, which agrees with findings from Sinclair et al. (Sinclair et al., 2011) and Weickert et al. (Weickert et al., 2015) suggesting that these genes were upregulated in human brain over the course of development. These authors suggested that the developmental upregulation of *FKBP5* and the glucocorticoid receptor represent a functional maturation of stress-response machinery, such that frontal cortex neurons may become more stress-responsive coinciding with increases in these genes (Sinclair et al., 2011; Weickert et al., 2015). In comparisons of gene expression in 6-week-old iPSC-derived neural cells and human brain, the expression profile in iPSC-derived neural cultures is most similar to human first trimester forebrain tissue (Brennand et al., 2015). Taken together, these findings suggest that iPSC-derived neural cells

at the age examined in the current study were not mature enough to show genotype effects of *FKBP5* induction following glucocorticoid receptor activation, even though our immunocytochemistry and qPCR experiments revealed detectable expression of glucocorticoid receptor protein and mRNA, respectively. Future work should utilize different methods of differentiation so as to ensure that induced human neural cells are responsive to glucocorticoids, as recent work has shown that the reprogramming of fibroblasts to iPSCs resets aging-associated expression profiles while neurons directly induced from fibroblast harbor transcriptome profiles similar to those of aged brains (Mertens et al., 2015). The underlying mechanisms by which reprogramming of dexamethasone-responsive fibroblasts yields downstream dexamethasone-insensitive neural derivatives remain to be elucidated. A large body of research suggests that somatic cell reprogramming via expression of pioneer transcription factors results in pluripotency through modulation of the epigenetic landscape, resetting the cells to a near-embryonic stem cell-like state, although iPSCs do retain epigenetic marks of their tissue of origin (Bar-Nur et al., 2011; Carey et al., 2011; Lister et al., 2011; Mertens et al., 2016). Additional changes in epigenetic marks regulate neural cell fate and differentiation (Hsieh et al., 2004). The lower glucocorticoid receptor (*NR3C1*) gene expression in iPSC-derived neural cells compared to subject-matched fibroblasts suggests that there is a resetting of epigenetic markers that renders glucocorticoid receptor expression similar to that during early brain development, where *NR3C1* mRNA expression is low (Sinclair et al., 2011). It may be that iPSC-derived neural cells do not fully replicate all of the epigenetic changes observed during neural development *in vivo*.

When further considering the age of our neural cultures, a limitation of our study is that functional electrophysiological analysis was conducted on neurons from only one subject at a later developmental time (8 weeks old compared to 6–7 weeks old for gene expression analysis). Our previous publication using the same differentiation protocol yielded neurons that express functional voltage gated sodium and potassium channels, as well as AMPA, NMDA, and GABA_A ionotropic receptors (Lieberman et al., 2012). Furthermore, mature action potentials and spontaneous excitatory events were observed in unpublished recordings from our lab in 2–3 week old iPSC-derived neurons. Together, these results show that the established differentiation protocol used for this work effectively generates functional neurons. Future studies could compare neuronal properties and synaptic events over development in neurons derived from multiple *FKBP5* rs1360780 C/C and T/T subjects, possibly in the setting of glucocorticoid receptor activation, as recent work has shown differences in fMRI- and imaging-based studies by *FKBP5* genotype (White et al., 2012; Fani et al., 2013; Holz et al., 2014; Grabe et al., 2016), suggesting a developmental influence on brain structure/connectivity.

An additional limitation is that 14 of the 27 iPSC lines utilized in our study were obtained from donor subjects diagnosed with an alcohol use disorder. A possibility exists that these donors carry psychiatric disorder-associated genetic variants that may moderate dexamethasone effects on gene expression in the iPSC-derived neural culture system. Indeed, differences in the expression of HPA-axis genes have been reported in the post mortem brains of alcohol-dependent individuals (McClintick et al., 2013) and a blunted response to acute stress has been observed in abstinent alcoholics (Lovallo et al., 2000). Because iPSC-derived neural cultures recapitulate a transcription profile of early

development (Brennand et al., 2015), it would be of interest to utilize an iPSC model system to examine whether alcohol's effects on HPA axis genes are related to underlying genetic variation or the result of prolonged alcohol exposure.

In summary, we found that acute dexamethasone treatment produced a robust increase in *FKBP5* mRNA in fibroblasts, but only a modest induction in iPSC-derived forebrain-lineage neural cells. No significant effect of *FKBP5* rs1360780 genotype on *FKBP5* mRNA expression following dexamethasone treatment was observed in either fibroblasts or iPSC-derived neural cells. This work supports the potential of using iPSC neural differentiation to examine effects of genetic variation and drug exposure on mRNA expression in human neural cells, but suggests that future research aimed at exploring stress-response genes using iPSC technologies should use differentiation methods to generate neural cell types that express GR at higher levels and are more sensitive to GR activation than the default forebrain lineage. This work also suggests that changes in *FKBP5* gene expression following dexamethasone exposure observed in human peripheral cells may not fully reflect the *FKBP5* gene expression effects of GR activation in human neural cells even when derived from the same subjects.

Acknowledgments

Funding

Supported by NIH grants P60 AA03510 (Alcohol Research Center), AA23192 (HRK), AA015606 (JC), M01 RR06192 (University of Connecticut General Clinical Research Center) and by a grant from the CT Department of Public Health (JC).

We thank Chris Abreu at the UC Clinical Research Center Core Lab for assistance in developing subject-specific fibroblast lines and Leann Crandall at the UC Stem Cell Core for her valued assistance in generating iPSC lines. Human brain samples were obtained from The Harvard Brain Tissue Resource Center at the McLean Hospital in Belmont, MA, supported by federal grant R24 MH/NS068855.

References

- Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell*. 2011; 9:17–23. [PubMed: 21726830]
- Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology*. 2009; 34(Suppl 1):S186–195. [PubMed: 19560279]
- Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, et al. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA*. 2008; 299:1291–1305. [PubMed: 18349090]
- Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet*. 2004; 36:1319–1325. [PubMed: 15565110]
- Brennand K, Savas JN, Kim Y, Tran N, Simone A, Hashimoto-Torii K, et al. Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. *Mol Psychiatry*. 2015; 20:361–368. [PubMed: 24686136]
- Carey BW, Markoulaki S, Hanna JH, Faddah DA, Buganim Y, Kim J, et al. Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. *Cell Stem Cell*. 2011; 9:588–598. [PubMed: 22136932]

- De Kloet ER. Hormones and the stressed brain. *Stress: Current Neuroendocrine and Genetic Approaches*. 2004; 1018:1–15.
- de Kloet ER, Joels M, Holsboer F. Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*. 2005; 6:463–475. [PubMed: 15891777]
- Ebrecht M, Buske-Kirschbaum A, Hellhammer D, Kern S, Rohleder N, Walker B, et al. Tissue specificity of glucocorticoid sensitivity in healthy adults. *J Clin Endocrinol Metab*. 2000; 85:3733–3739. [PubMed: 11061532]
- Ellis FW. Effect of ethanol on plasma corticosterone levels. *J Pharmacol Exp Ther*. 1966; 153:121–127. [PubMed: 4288396]
- Fani N, Gutman D, Tone EB, Almli L, Mercer KB, Davis J, et al. FKBP5 and Attention Bias for Threat: Associations With Hippocampal Function and Shape. *JAMA Psychiatry*. 2013; 70:392–400. [PubMed: 23407841]
- Grabe HJ, Wittfeld K, Van der Auwera S, Janowitz D, Hegenscheid K, Habes M, et al. Effect of the interaction between childhood abuse and rs1360780 of the FKBP5 gene on gray matter volume in a general population sample. *Hum Brain Mapp*. 2016; 37:1602–1613. [PubMed: 26813705]
- Grad I, Picard D. The glucocorticoid responses are shaped by molecular chaperones. *Mol Cell Endocrinol*. 2007; 275:2–12. [PubMed: 17628337]
- Hohne N, Poidinger M, Merz F, Pfister H, Bruckl T, Zimmermann P, et al. FKBP5 genotype-dependent DNA methylation and mRNA regulation after psychosocial stress in remitted depression and healthy controls. *Int J Neuropsychopharmacol*. 2015; 18:1–9.
- Holz NE, Buchmann AF, Boecker R, Blomeyer D, Baumeister S, Wolf I, et al. Role of FKBP5 in emotion processing: results on amygdala activity, connectivity and volume. *Brain Struct Funct*. 2014
- Hsieh J, Gage FH. Epigenetic control of neural stem cell fate. *Curr Opin Genet Dev*. 2004; 14:461–469. [PubMed: 15380235]
- Huang MC, Schwandt ML, Chester JA, Kirchhoff AM, Kao CF, Liang T, et al. FKBP5 moderates alcohol withdrawal severity: human genetic association and functional validation in knockout mice. *Neuropsychopharmacology*. 2014; 39:2029–2038. [PubMed: 24603855]
- Ing NH. Steroid hormones regulate gene expression posttranscriptionally by altering the stabilities of messenger RNAs. *Biol Reprod*. 2005; 72:1290–1296. [PubMed: 15728791]
- Ing NH, Forrest DW, Riggs PK, Loux S, Love CC, Brinsko SP, et al. Dexamethasone acutely down-regulates genes involved in steroidogenesis in stallion testes. *J Steroid Biochem Mol Biol*. 2014; 143:451–459. [PubMed: 25010478]
- Jensen KP, Herman AI, Morean ME, Kranzler HR, Gelernter J, Sofuoglu M. FKBP5 variation is associated with the acute and chronic effects of nicotine. *Pharmacogenomics J*. 2015; 15:340–346. [PubMed: 25532758]
- Kang JI, Chung HC, Jeung HC, Kim SJ, An SK, Namkoong K. FKBP5 polymorphisms as vulnerability to anxiety and depression in patients with advanced gastric cancer: a controlled and prospective study. *Psychoneuroendocrinology*. 2012; 37:1569–1576. [PubMed: 22459275]
- Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, et al. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci*. 2005; 25:2255–2266. [PubMed: 15745951]
- Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, et al. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci*. 2013; 16:33–41. [PubMed: 23201972]
- Kranzler HR, Covault J, Feinn R, Armeli S, Tennen H, Arias AJ, et al. Topiramate treatment for heavy drinkers: moderation by a GRIK1 polymorphism. *Am J Psychiatry*. 2014; 171:445–452. [PubMed: 24525690]
- Lavebratt C, Aberg E, Sjöholm LK, Forsell Y. Variations in FKBP5 and BDNF genes are suggestively associated with depression in a Swedish population-based cohort. *J Affect Disord*. 2010; 125:249–255. [PubMed: 20226536]
- Lee RS, Tamashiro KL, Yang X, Purcell RH, Harvey A, Willour VL, et al. Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. *Endocrinology*. 2010; 151:4332–4343. [PubMed: 20668026]

- Lemtiri-Chlieh F, Levine ES. BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. *J Neurophysiol.* 2010; 104:1923–1932. [PubMed: 20719932]
- Levrán O, Randesi M, Li Y, Rotrosen J, Ott J, Adelson M, et al. Drug addiction and stress-response genetic variability: association study in African Americans. *Ann Hum Genet.* 2014; 78:290–298. [PubMed: 24766650]
- Lieberman R, Kranzler HR, Joshi P, Shin DG, Covault J. GABRA2 Alcohol Dependence Risk Allele is Associated with Reduced Expression of Chromosome 4p12 GABAA Subunit Genes in Human Neural Cultures. *Alcohol Clin Exp Res.* 2015; 39:1654–1664. [PubMed: 26250693]
- Lieberman R, Levine ES, Kranzler HR, Abreu C, Covault J. Pilot study of iPS-derived neural cells to examine biologic effects of alcohol on human neurons in vitro. *Alcohol Clin Exp Res.* 2012; 36:1678–1687. [PubMed: 22486492]
- Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature.* 2011; 471:68–73. [PubMed: 21289626]
- Lovallo WR, Dickensheets SL, Myers DA, Thomas TL, Nixon SJ. Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcohol Clin Exp Res.* 2000; 24:651–658. [PubMed: 10832906]
- Luijk MP, Velders FP, Tharner A, van Ijzendoorn MH, Bakermans-Kranenburg MJ, Jaddoe VW, et al. FKBP5 and resistant attachment predict cortisol reactivity in infants: gene-environment interaction. *Psychoneuroendocrinology.* 2010; 35:1454–1461. [PubMed: 20547006]
- Mahon PB, Zandi PP, Potash JB, Nestadt G, Wand GS. Genetic association of FKBP5 and CRHR1 with cortisol response to acute psychosocial stress in healthy adults. *Psychopharmacology (Berl).* 2013; 227:231–241. [PubMed: 23274505]
- McClintick JN, Xuei X, Tischfield JA, Goate A, Foroud T, Wetherill L, et al. Stress-response pathways are altered in the hippocampus of chronic alcoholics. *Alcohol.* 2013; 47:505–515. [PubMed: 23981442]
- Menke A, Arloth J, Putz B, Weber P, Klengel T, Mehta D, et al. Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients. *Neuropsychopharmacology.* 2012; 37:1455–1464. [PubMed: 22237309]
- Mertens J, Marchetto MC, Bardy C, Gage FH. Evaluating cell reprogramming, differentiation and conversion technologies in neuroscience. *Nat Rev Neurosci.* 2016; 17:424–437. [PubMed: 27194476]
- Mertens J, Paquola AC, Ku M, Hatch E, Bohnke L, Ladjevardi S, et al. Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects. *Cell Stem Cell.* 2015; 17:705–718. [PubMed: 26456686]
- Mertens J, Wang QW, Kim Y, Yu DX, Pham S, Yang B, et al. Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature.* 2015; 527:95–99. [PubMed: 26524527]
- Milivojevic V, Feinn R, Kranzler HR, Covault J. Variation in AKR1C3, which encodes the neuroactive steroid synthetic enzyme 3 α -HSD type 2 (17 β -HSD type 5), moderates the subjective effects of alcohol. *Psychopharmacology (Berl).* 2014; 231:3597–3608. [PubMed: 24838369]
- Perez-Ortiz JM, Garcia-Gutierrez MS, Navarrete F, Giner S, Manzanares J. Gene and protein alterations of FKBP5 and glucocorticoid receptor in the amygdala of suicide victims. *Psychoneuroendocrinology.* 2013; 38:1251–1258. [PubMed: 23219223]
- Roy A, Gorodetsky E, Yuan Q, Goldman D, Enoch MA. Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology.* 2010; 35:1674–1683. [PubMed: 20090668]
- Scharf SH, Liebl C, Binder EB, Schmidt MV, Müller MB. Expression and regulation of the Fkbp5 gene in the adult mouse brain. *PLoS One.* 2011; 6:e16883. [PubMed: 21347384]
- Schatzberg AF, Keller J, Tennakoon L, Lembke A, Williams G, Kraemer FB, et al. HPA axis genetic variation, cortisol and psychosis in major depression. *Molecular Psychiatry.* 2014; 19:220–227. [PubMed: 24166410]

- Schuster J, Halvardson J, Pilar Lorenzo L, Ameer A, Sobol M, Raykova D, et al. Transcriptome Profiling Reveals Degree of Variability in Induced Pluripotent Stem Cell Lines: Impact for Human Disease Modeling. *Cell Reprogram*. 2015; 17:327–337. [PubMed: 26348590]
- Sinclair D, Fillman SG, Webster MJ, Weickert CS. Dysregulation of glucocorticoid receptor co-factors FKBP5, BAG1 and PTGES3 in prefrontal cortex in psychotic illness. *Sci Rep*. 2013; 3:3539. [PubMed: 24345775]
- Sinclair D, Webster MJ, Wong J, Weickert CS. Dynamic molecular and anatomical changes in the glucocorticoid receptor in human cortical development. *Mol Psychiatry*. 2011; 16:504–515. [PubMed: 20308989]
- Stechschulte LA, Sanchez ER. FKBP51-a selective modulator of glucocorticoid and androgen sensitivity. *Curr Opin Pharmacol*. 2011; 11:332–337. [PubMed: 21565552]
- Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. *Am J Med Genet B Neuropsychiatr Genet*. 2006; 141B:261–268. [PubMed: 16526044]
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–872. [PubMed: 18035408]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
- Tatro ET, Everall IP, Kaul M, Achim CL. Modulation of glucocorticoid receptor nuclear translocation in neurons by immunophilins FKBP51 and FKBP52: implications for major depressive disorder. *Brain Res*. 2009; 1286:1–12. [PubMed: 19545546]
- Vermeer H, Hendriks-Stegeman BI, van Suylekom D, Rijkers GT, van Buul-Offers SC, Jansen M. An in vitro bioassay to determine individual sensitivity to glucocorticoids: induction of FKBP51 mRNA in peripheral blood mononuclear cells. *Mol Cell Endocrinol*. 2004; 218:49–55. [PubMed: 15130510]
- Weickert CS, Webster MJ, Boerrigter D, Sinclair D. FKBP5 Messenger RNA Increases After Adolescence in Human Dorsolateral Prefrontal Cortex. *Biol Psychiatry*. 2015
- White MG, Bogdan R, Fisher PM, Munoz KE, Williamson DE, Hariri AR. FKBP5 and emotional neglect interact to predict individual differences in amygdala reactivity. *Genes Brain Behav*. 2012; 11:869–878. [PubMed: 22979952]
- Yeo S, Enoch MA, Gorodetsky E, Ahktar L, Schuebel K, Roy A, et al. The influence of FKBP5 genotype on expression of FKBP5 and other glucocorticoid-regulated genes, dependent on trauma exposure. *Genes Brain Behav*. 2016
- Yu DX, Di Giorgio FP, Yao J, Marchetto MC, Brennand K, Wright R, et al. Modeling hippocampal neurogenesis using human pluripotent stem cells. *Stem Cell Reports*. 2014; 2:295–310. [PubMed: 24672753]
- Zannas AS, Binder EB. Gene-environment interactions at the FKBP5 locus: sensitive periods, mechanisms and pleiotropism. *Genes Brain Behav*. 2014; 13:25–37. [PubMed: 24219237]

Highlights

- iPSCs differentiate into neurons that express the glucocorticoid receptor and *FKBP5*
- Dexamethasone treatment increased *FKBP5* mRNA by 23% in iPSC-derived neural cells
- Dexamethasone treatment increased *FKBP5* mRNA by 670% in donor-matched fibroblasts
- No effect of rs1360780 genotype on *FKBP5* mRNA levels were observed

Figure 1. iPSCs Differentiate into Mixed Neural Cultures that Express the Glucocorticoid Receptor

(A) 8 weeks post-plating iPSC-derived neural cultures contain a mixture of MAP2-positive neurons (green) and GFAP-positive astrocytes (red). (B) The forebrain glutamatergic marker TBR1 (green) is co-localized in the nucleus of neurons marked by the neural marker Beta III-Tubulin (red). Nuclei are labeled with DAPI (blue). (C) Expression of the glucocorticoid receptor (green) can be seen in the cytosol of a beta III-tubulin-positive neuron (red, indicated by red arrow), but also in an unlabeled cell (indicated by white arrow). (D) The glucocorticoid receptor (green) is expressed in the cytosol of GFAP-positive astrocytes (red, indicated by red arrows). (E–F) Patterns of *FKBP5* and *NR3C1* expression over neural development in 3 iPSC, 6 neuroepithelial, 5 2-week old neural cell, and 27 6-week old neural cell lines indicate detectable levels of these genes in our culture system.

Figure 2. iPSCs Differentiate into Functional Neurons

Whole cell patch-clamp electrophysiology was performed on 48 neurons derived from one subject at 8 weeks-post neural plating. (A) Inward sodium, outward potassium, and transient potassium currents were evoked by 10 mV voltage step increases. Average size of currents (normalized for cell size, pA/pF) is illustrated in the graph (outward = $53.9 \text{ pA} \pm 3.47$, transient = $71.7 \text{ pA} \pm 4.32$, inward = $67.6 \text{ pA} \pm 6.15$). (B) Ability of iPSC-derived neurons to fire action potentials was probed by 10-pA current injection steps. The number of neurons able to fire a single mature, a mature train, or an immature action potential is indicated in the pie chart, and properties of each cell's action potential are illustrated in the graphs (average amplitude = $55.4 \text{ mV} \pm 2.53$, average threshold = $-25.6 \text{ mV} \pm 0.87$, average half-width = $3.68 \text{ ms} \pm 0.20$). (C) 25/48 neurons patched demonstrated spontaneous activity (presence of any spontaneous synaptic event over the course of a 3-minute recording). Of the 25 cells with a synaptic event, the average frequency was $0.16 \text{ Hz} \pm 0.05$ and the average amplitude of events was $-16 \text{ pA} \pm 1.72$. (D) Representative trace of a neuron firing spontaneous action potentials, which was recorded at -40 mV . Eight of 48 neurons demonstrated any spontaneous action potential over a 3-minute recording. (E) Average resting membrane potential was $-51.9 \text{ mV} \pm 1.28$, corrected for liquid junction potential.

Figure 3. Characterizing Basal *FKBP5* and *NR3C1* mRNA Expression in iPSC-Derived Neural Cells, Fibroblasts, and Human Post-Mortem Brain

(A) mRNA expression of *FKBP5* was significantly higher in post-mortem brain than in iPSC-derived neural cell cultures ($t=8.8$, $p<0.0001$) or fibroblasts ($t=8.6$, $p<0.0001$). There was no difference in basal expression between iPSC-derived neural cells and fibroblasts ($t=0.9$, $p>0.05$) (B) mRNA expression of *NR3C1* was significantly higher in post-mortem brain than in iPSC-derived neural cells ($t=7.8$, $p<0.0001$) or in fibroblasts compared to iPSC-derived neural cells ($t=7.8$, $p<0.0001$), while expression was significantly lower in postmortem brain than in fibroblasts ($t=8.4$, $p<0.0001$). Values of basal *FKBP5* and *NR3C1* expression in iPSC-derived neural cells and fibroblasts were taken from data used to generate Figure 3. Expression in post-mortem brain was analyzed using the same standard curve of reference RNA, allowing for comparison across qPCR reactions and between groups. (**** $p<0.0001$, NS=non-significant)

Figure 4. *FKBP5* and *NR3C1* mRNA Expression in Fibroblasts and iPSC-Derived Neural Cultures Following 6 Hr Dexamethasone Exposure

(A–B) A modest but significant effect of dexamethasone exposure was observed on *FKBP5* mRNA expression in iPSC-derived neural cells ($t = 4.1$, $df = 26$, $p < 0.001$), while no effect was observed on *NR3C1* mRNA expression ($p = 0.60$) (C–D) In fibroblasts, dexamethasone exposure resulted in a robust increase of *FKBP5* mRNA ($t = 9.1$, $df = 14$, $p < 0.0001$) and a modest but significant reduction in *NR3C1* mRNA ($t = 2.5$, $df = 14$, $p < 0.05$). (**** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$, NS=non-significant)

Figure 5. Basal Expression of Glucocorticoid Receptor-Related Genes Does not Differ by *FKBP5* rs1360780 Genotype

No difference was observed in the basal expression of genes encoding the glucocorticoid receptor or its regulators in 6–7 week old neural cells from 11 rs1360780*C homozygotes and 16 rs1360780*T-allele carriers (14 C/T and 2 T/T). (A–D) *FKBP5* ($p = 0.12$); *NR3C1* ($p = 0.64$); *FKBP4* ($p = 0.59$); *BAG1* ($p = 0.51$).

Figure 6. Genetic Variation in *FKBP5* Does Not Associate with Differences in *FKBP5* mRNA Induction Following Dexamethasone Exposure

(A–B) Acute dexamethasone increased *FKBP5* mRNA by 23% in neural cells derived from both rs1360780 genotypes (C/C: $t = 2.2$, $df = 10$, $p = 0.055$; T-allele carrier: $t = 4$, $df = 15$, $p < 0.01$). (C–D) A significant upregulation of *FKBP5* mRNA was observed in fibroblasts derived from donor subjects of both rs1360780 genotypes (C/C $t = 5.3$, $df = 5$, $p < 0.01$; T-carrier: $t = 7.1$, $df = 8$, $p < 0.0001$), while no significant differences were observed by genotype in the basal expression of *FKBP5* ($p = 0.67$) or its induction by dexamethasone exposure ($p = 0.96$). (*** $p < 0.001$, ** $p < 0.01$)