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Title: Determinants of psychosis vulnerability : focus on MEF2 - and glucocorticoid signaling
Issue Date: 2013-11-28

Chapter 4 | Depolarization-induced binding of MEF2 to the promoter region of NR4A1 is prevented by GR activation

ABSTRACT

Glucocorticoid Receptor (GR) and Myocyte Enhancer Factor 2 (MEF2) are transcription factors with important functions in synaptic plasticity. MEF2 activity is strongly induced by neuronal depolarization, resulting in increased expression of target genes. The most well-known MEF2 target gene regulated in this way is the immediate-early-gene Nuclear Receptor subfamily 4, group A, member 1 (NR4A1), known to play a role in neurite outgrowth. We previously showed in neuronal PC-12 cells that GR activation by dexamethasone leads to phosphorylation of MEF2, the transcriptionally repressive form of MEF2. Here we show in the same cell line that GR activation significantly attenuates NR4A1 expression underKCl-induced neuronal depolarization. While neuronal depolarization resulted in increased MEF2-binding to the NR4A1 promoter region, concomitant GR activation reduced MEF2-binding to levels observed under baseline non-depolarizing conditions. This points to a contextual action mediated by GR, in which activation of GR under depolarizing conditions abrogates the depolarization-induced effects of MEF2 on regulation of its target gene NR4A1, likely by interfering with MEF2 DNA binding. A similar modulatory effect via GR under depolarizing conditions could also be demonstrated for other MEF2 target genes, including immediate-early response 2 (IER2) and c-FOS. In conclusion we show that in a depolarizing environment, the modulatory activity of GR has direct consequences for MEF2-DNA binding and expression of its target genes. Counterbalancing MEF2-effects on gene expression upon neuronal depolarization may represent a novel mechanism via which glucocorticoids regulate neuronal plasticity.

INTRODUCTION

Neuronal activity plays a major role in neuronal plasticity, survival and long-term potentiation (LTP) (Wiegert & Bading 2011, Leslie & Nedivi 2011). Several processes such as learning, memory and behavioral sensitization depend on neuronal activity and its downstream effects, including induction of dendritic outgrowth and enhancement of synaptic strength.

Upon neuronal activation, neurons depolarize, leading to influx of Ca^{2+}-ions and activation of the phosphatase calcineurin (CaN) (Flavell *et al.* 2006, Lam *et al.* 2009, Youn *et al.* 2000). In turn CaN dephosphorylates the transcription factor myocyte enhancer factor 2 (MEF2) (Blaeser *et al.* 2000, Flavell & Greenberg 2008, Youn et al. 2000, Mao & Wiedmann 1999). It has been shown that dephosphorylation at serine 408 by CaN changes the transcriptional potential of MEF2 from repressive to active (Gregoire *et al.* 2006, Gong *et al.* 2003, Mao & Wiedmann 1999). Depolarization also decreases the activity of cyclin-dependent kinase 5 (CDK5), which is responsible for the phosphorylation of MEF2 at serine 408 (Schuman & Murase 2003). A recent genome-wide analysis in a depolarizing context, revealed that many MEF2 target genes have important functions in synaptic plasticity and neurotransmitter release (Flavell *et al.* 2008, Cole *et al.* 2012, Akhtar *et al.* 2012). One of these MEF2 target genes is the immediate-early gene nuclear receptor family 4, group A, member 1 (NR4A1) (also known as NGFI-B or NUR77) (Youn & Liu 2000) known to play a key role in neurite outgrowth (14,15).

The glucocorticoid receptor (GR), like MEF2, has also been implicated in neuronal plasticity (Liston & Gan 2011, Karst *et al.* 2010). GR acts as a ligand-activated transcription factor and is widely expressed throughout the brain. GR regulates a wide variety of functional gene classes including plasticity-related genes, in a context-dependent manner. GR mediates opposite effects of corticosterone on neuronal plasticity (Joels & Krugers 2007). In amygdala slices for instance, it was observed blockade of GR by mifepristone corticosterone enhances LTP via voltage-dependent calcium channels (VDCC) while it suppresses LTP via NMDA receptors (Krugers *et al.* 2005, Karst *et al.* 2000). These results suggest that a delicate balance exists between pathways that strengthen and pathways that weaken plasticity and that GR plays a key role in controlling this process that is so instrumental for neuronal functioning in a changing environment.

Using PC12 cells we recently showed for the first time that GR has a modulatory effect on MEF2 under baseline non-depolarizing conditions (Speksnijder *et al.* 2012). Since GR activation modulates depolarization-induced Ca^{2+}-influx, which is itself the activator of MEF2, the aim of this study was to investigate the effect of GR activation on MEF2 under depolarizing conditions. For this purpose we used NR4A1 as target gene because it is significantly induced following treatment with depolarizing

concentrations of KCl, while it is blocked when MEF2 is knocked down, both in PC-12 cells and hippocampal neurons [12, 16]. Constitutively active MEF2 was found to enhance NR4A1 expression in PC-12 cells via binding to two MEF2 binding sites, upstream of the transcription start site [17, 18]. In the current study we find in neuronally-differentiated PC12 cells that GR activation attenuates the expression of MEF2 target gene NR4A1 and interferes with MEF2-DNA binding to the NR4A1 promoter region in the context of KCl-induced neuronal depolarization.

MATERIALS & METHODS

Cell culture and treatment Rat pheochromocytoma (PC-12) cells (passage # 15-29) were cultured as described earlier (Morsink *et al.* 2006). In short, cells were grown in DMEM medium, supplemented with 0-10% fetal bovine serum and 0-10% horse serum, dependent on the stage of neuronal differentiation. For mRNA analysis, cells were seeded at a confluency of 30-50% in pre-coated 6-well plates (356400, BD Biosciences, San Jose, CA, USA). For chromatin immunoprecipitation (ChIP) experiments the cells were seeded at 50% confluency in pre-coated 175 cm^2 plates (356478, BD Biosciences). Neuronal differentiation was achieved by giving 50 ng/ml NGF-ß (N2513, Sigma-Aldrich, St. Louis, MO, USA) every other day for 10 days. At day 9 of the differentiation stage the culture medium was supplemented with charcoal stripped serum to remove endogenous steroids (Sarabdjitsingh *et al.*). At day 10 the cells were treated for 60 minutes (ChIP) or 90 minutes (mRNA expression), dependent on the experiment, with either vehicle (VEH) (0.1% ethanol), 100 nM dexamethasone (DEX) (D1756, Sigma-Aldrich), 55mM KCl (60128, Sigma-Aldrich) or a combination of these treatments.

Chromatin Immunoprecipitation (ChIP) At the end of the treatment time, the cells were fixed with 1% formaldehyde (344198, Merck, Darmstadt, Germany) for 10 minutes at room temperature (RT) to crosslink DNA-protein interactions. Cells were scraped from the plates in 10 ml ice-cold phosphate-buffered saline containing protease inhibitors (PI; 11836153001, Roche). The DNA was sonicated for 15-25 pulses (Bioruptor UCD-200, Diagenode, Sparta, NJ, USA). For immunoprecipitation, 20-60 µg of input DNA was used per antibody. Pre-cleared DNA was incubated overnight with 6 µg of anti-GR (H-300) antibody (sc-8992X, Santa Cruz, CA, USA), 6 µg of anti-MEF2 (C-21) antibody (sc-313X, Santa Cruz) or normal rabbit IgG (sc-2027, Santa Cruz). The next day, sepharose A beads were added to the DNA-protein-antibody complexes and incubated for 90 minutes at 4°C. The samples were subsequently washed at RT 1x with low salt buffer (0.1% SDS; 2mM EDTA pH 8.0; 20 mM Tris-HCl pH 8.0; 150 mM NaCl; 1% Triton-X-100), 1x with high salt buffer (as low salt except 500 mM NaCl), 1x with LiCL buffer (0.25 M LiCl; 1 mM EDTA pH 8.0; 20 mM Tris-HCl pH 8.0; 1% NP-40; 1% NaDOC) and 2x with TE buffer (1mM EDTA pH 8.0; 10 mM Tris-HCl pH 8.0) respectively. The DNA complexes were subsequently eluted from the beads and the DNA was reverse-crosslinked overnight in 0.2 M NaCl solution at 65°C. The next day the samples were treated with RNAse and the DNA purified using nucleospin columns (740609, Machery-Nagel, Duren, Germany) according to the manufacturer's protocol. The DNA was eluted in 50 µl of TE buffer for RT-qPCR analysis.

RT-qPCR primer design Primers were designed as described before (Speksnijder et al. 2012). In short, primers for RT-qPCR of immunoprecipitated DNA fragments were designed surrounding DNA regions

bound by GR as shown in glucocorticoid (GC) treated neuronally differentiated PC-12 cells (unpublished results by Polman et al). Primer-BLAST (NCBI, Bethesda, USA) was used to obtain primer specific sequences and an additional check for primer hairpins was performed using Oligo 7.0 (MBI Inc. Cascade, USA). Primers were tested with RT-qPCR on a standard curve to check the efficiency of the PCR reaction. Primer sequences are listed in supplementary table S1.

Real time quantitative PCR (RT-qPCR) Total RNA was isolated using Trizol (15596, Invitrogen) according to the manufacturer's instructions. RNA was diluted to 50 ng/ul and cDNA was synthesized using the iScript cDNA synthesis kit (170-8897, Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. RT-qPCR was performed on a Lightcycler 2.0 Real-Time PCR System (Roche Applied Science, Basel, Switzerland) in combination with the Lightcycler FastStart DNA Master^{PLUS} SYBR Green I Kit (03515885001, Roche). As a control for genomic contamination, samples without reverse transcriptase were used. The standard curve method was applied to quantify the expression differences (Livak and Schmittgen, 2001). Expression of TUBB2a (Tubulin, beta 2a) was used to normalize the RNA input. Primer sequences used are listed in supplementary table S1.

Western Blotting Protein was harvested in ice-cold RIPA buffer containing Protease Inhibitors (#04693124001, Roche) and phosphatase inhibitors ($NaVO_3$ and B-glycerophosphate). The cell lysate was incubated on ice for 30 minutes, spun down and the supernatant transferred to a new tube. Protein concentration was measured using the Pierce BCA Protein Assay kit (23225, Thermo Scientific, Rockford, IL, USA), according to the manufacturer's protocol. Diluted samples were supplemented with 1:2 volume of sample buffer (including 2.5% ß-mercaptoethanol and BromoPhenol Blue). Twenty µg of each sample was loaded on a 10% polyacrylamide gel. After sufficient separation of the proteins, they were transferred overnight at 4°C to a PVDF (Polyvinylidene fluoride) membrane. The membrane was subsequently blocked in 5% low fat milk for 1 hour at RT or 5 hours at 4°C for phospho-proteins. Primary antibodies were added in the blocking buffer and incubated for 1 hour at RT or at 4°C overnight for phospho-proteins with either one of the following primary antibodies: Anti-phospho S408 MEF2a rabbit monoclonal (ab51151, Abcam, Cambridge, UK) or anti-α-Tubulin DM1A mouse monoclonal antibody (T6199, Sigma). Blots were incubated for 1 hour at RT with the appropriate secondary antibody: goat-anti rabbit IgG HRP secondary antibody (sc-2054, Santa Cruz) or goat-anti mouse IgG HRP secondary antibody (sc-2055, Santa Cruz). Signals were quantified using ImageJ (v1.42; National Institute of Health, USA).

RESULTS

MEF2 is dephosphorylated under depolarizing conditions Neuronal PC-12 cells were treated with 55 mM KCl and proteins were harvested after 60 minutes to measure MEF2 s408 phosphorylation, the transcriptionally inactive form of MEF2. In agreement with the literature on other cell lines and *in vivo*, we observed that MEF2 phosphorylation at serine 408 is significantly decreased by KCl treatment (Fig. 1), thus enhancing the transcriptional potential of MEF2.

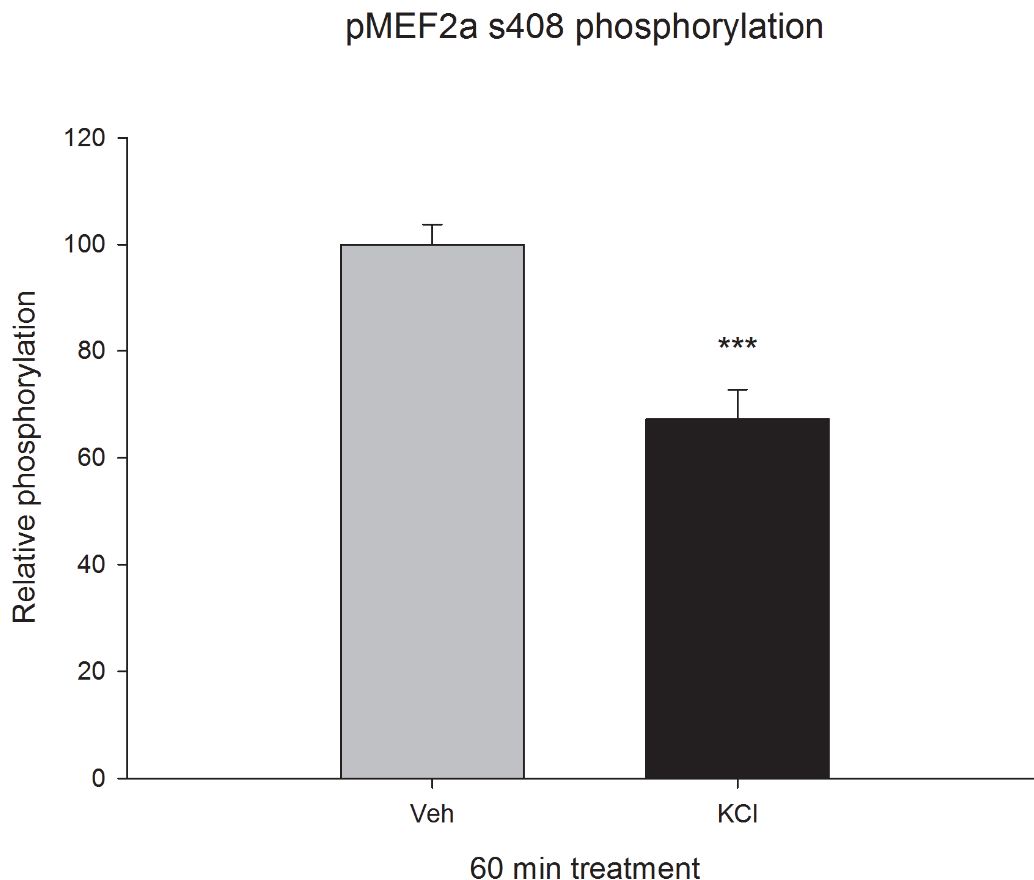

Figure 1 | MEF2a phosphorylation level at serine 408 after 60 minutes of KCl treatment (n=6 per group). A) Phosphorylation level is relative to 60 minutes VEH treatment, set at 100%. Expression is normalized to total MEF2a and alpha-tubulin. (* p<0.001 sign. vs VEH treatment).**

NR4A1 mRNA induction by KCl is attenuated by GR activation To study the effect of GR on mRNA expression of the MEF2 target gene NR4A1 under depolarizing conditions, neuronal PC-12 cells were treated with KCl, dexamethasone (DEX), or a combination of KCl and DEX for 90 minutes. This time point, 30 minutes after measuring MEF2 phosphorylation, was chosen since it typically takes 30 minutes to detect changes in gene expression of immediate-early genes (IEG). DEX treatment alone did not affect NR4A1 expression (Fig. 2), also not after 180 minutes of treatment (data not shown). Ninety minutes of a depolarizing concentration of KCl induced a large increase in NR4A1 expression

(~200-fold p<0.001) compared to the VEH condition. Surprisingly, DEX significantly attenuated this induction by 2-fold to a ~100-fold increase compared to the VEH condition (p<0.001 vs all other conditions) (Fig. 2).

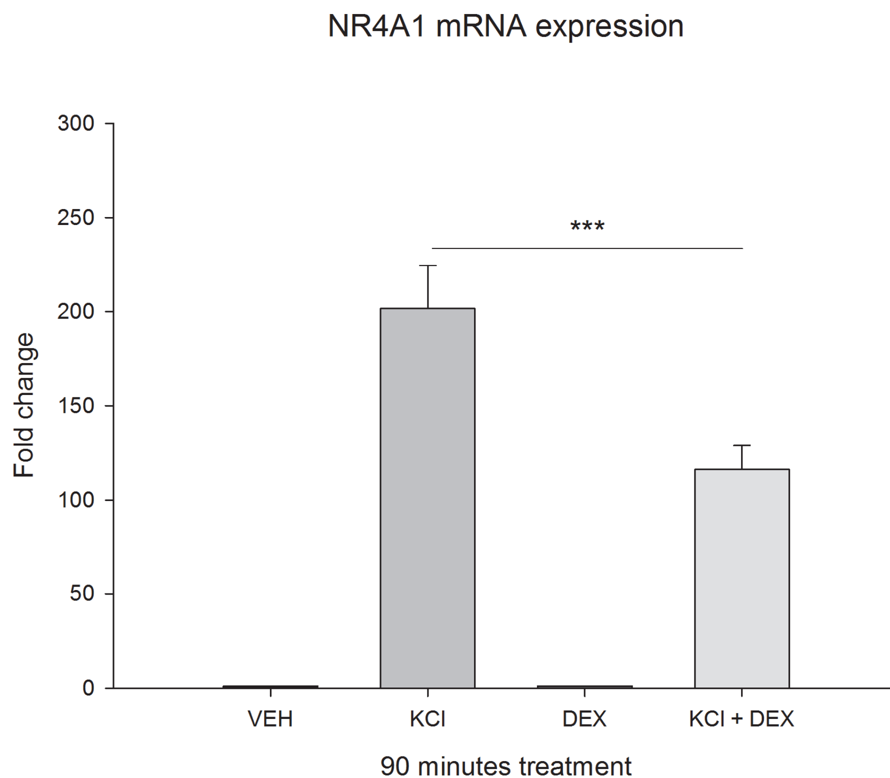

Figure 2 | NR4A1 mRNA expression at 90 minutes of KCl, DEX or KCl+DEX treatment (n=6 per group). Expression level is depicted as fold change relative to the VEH condition which is set at 1. Expression is normalized to TUBB2a. (ANOVA p<0.001; Tukey's post hoc test *** p<0.001).

MEF2 binding upstream and GR binding downstream NR4A1 is prevented by GR activation under depolarizing conditions Since KCl-induced depolarization resulted in both an increase in activity of MEF2 as well as a large induction of NR4A1, we next studied whether binding of MEF2 in the vicinity of NR4A1 was increased using chromatin immunoprecipitation of MEF2-bound genomic regions. Two MEF2 binding sites were identified in literature at -309 and -275 bp upstream of the transcription start site (TSS) of the NR4A1 gene (Lam *et al.* 2010). We did not differentiate between both sites, since they lie within 34 bp of each other and sonication of the DNA does not result in adequate discrimination between both sites. Therefore a DNA sequence harboring both sites was amplified, designated MBS1 (Fig. 3A) (Pulipparacharuvil *et al.* 2008). Following KCl-induced depolarisation, MEF2 binding to MBS1 was significantly enhanced (Fig. 3B). Although DEX treatment on its own did not result in an expression change of NR4A1, significant binding to MBS1 was observed (Fig. 3B). When both treatments were combined, MEF2-DNA binding was reduced by 3-fold compared to KCl treatment alone and was similar to VEH levels after 60 minutes of treatment (Fig. 3B).

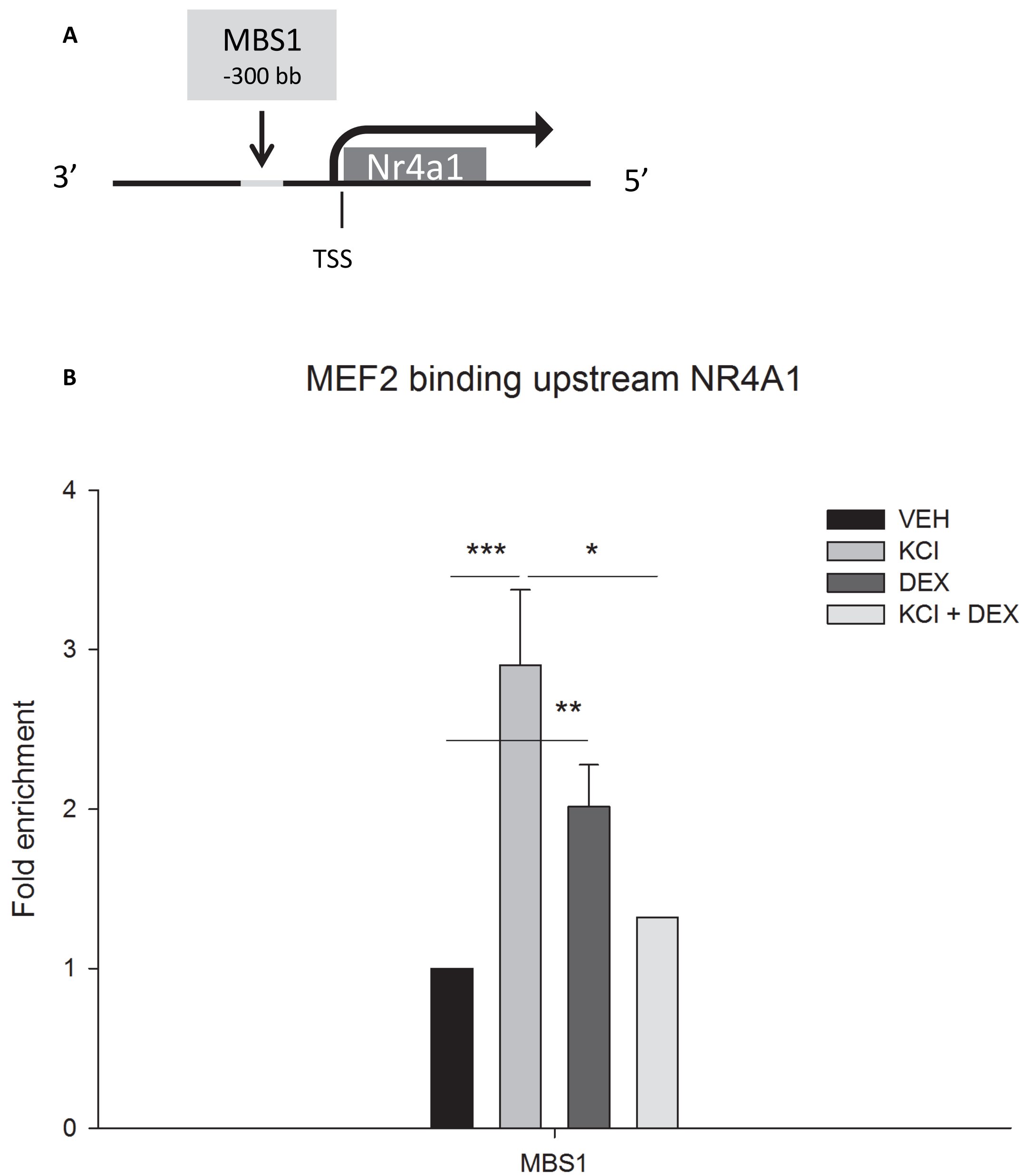

Figure 3 | Binding of MEF2 upstream NR4A1. A) Scheme representing the site of MEF2 binding upstream NR4A1, actually consisting of two neighboring MEF2 response elements. The number inside the box indicates the distance of the binding site to the TSS. B) Result of ChIP experiments representing DNA-binding of MEF2 upstream NR4A1 after VEH, KCl, DEX and KCl+DEX treatment (ANOVA $p<0.01$; Tukey’s post hoc test * $p<0.05$, ** $p<0.01$, *** $p<0.001$). Results are fold changes in binding vs VEH treatment and normalized against IgG immunprecipitated fractions.

MEF2 target gene expression is modulated by GR activation in depolarized neurons Many IEGs are known to be regulated by MEF2, including c-JUN, c-FOS, activity-regulated transcript (ARC), and immediate-early response 2 (IER2) (Flavell et al. 2008, Flavell et al. 2006, Han & Prywes 1995, Lam et al. 2010). Since we observed a significant DEX-effect on KCl-mediated expression of NR4A1, we hypothesized that DEX may also attenuate the neuronal activity-dependent expression of several other MEF2 target genes. First we identified IER2 and c-FOS as genes that are significantly upregulated by KCl treatment in neuronally differentiated PC-12 cells (Fig. 4). Similar to NR4A1, GR activation by DEX also significantly attenuated the KCl-induced expression of both these genes (Fig. 4).

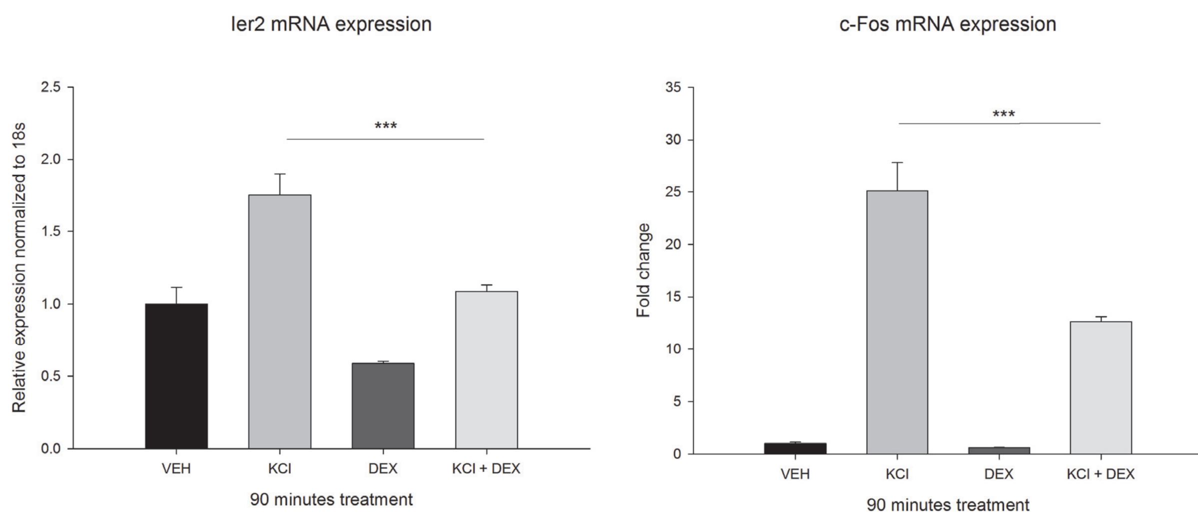

Figure 4 | Immediate-early genes Ier2 and c-FOS expression after 90 minutes of KCl, DEX and KCl+DEX treatment (n=6 per group). Expression levels are depicted as fold change relative to the VEH condition which is set at 1. Expression is normalized to TUBB2a. (ANOVAs $p<0.05$; Tukey's post-hoc *** $p<0.001$).

DISCUSSION

The MEF2 transcription factor is an important factor in synaptic plasticity and one of the target proteins of neuronal activation. We previously found that MEF2 and GR cooperate in the control of target gene expression in neuronally differentiated PC-12 cells under basal conditions. However, since MEF2 is activated by neuronal depolarization, we subsequently studied what the effect of GR is on MEF2 phosphorylation and DNA binding under depolarizing conditions.

NR4A1 mRNA is induced by KCl treatment We chose to investigate the effect of dexamethasone-activated GR on MEF2 in neuronally differentiated PC-12 cells, a cell model often used to study the effects of (neuronal) depolarization (Lam et al. 2010, Sheng *et al.* 1988, Ahn *et al.* 1998, Hansen *et al.* 2003). We show, in agreement with earlier reports, that MEF2 is dephosphorylated by KCl treatment. KCl-induced depolarization leads to calcium influx, which activates Calcineurin (CaN), resulting in dephosphorylation of MEF2 and a concomitant increase in its transcriptional potential. A well-known MEF2 target gene is NR4A1 and dephosphorylation of MEF2 is known to enhance NR4A1 expression. Conversely, blocking CaN, which prevents dephosphorylation of MEF2 was found to interfere with depolarization-induced NR4A1 expression (Lam et al. 2010, Ahn et al. 1998, Enslen & Soderling 1994, Tian *et al.* 2010, Blaeser et al. 2000). In agreement with the literature we show here that NR4A1 mRNA expression is induced by KCl. However, the magnitude of induction (~200-fold) is far greater compared to other studies in PC-12 cells. One study showed a 20-fold increase in NR4A1 expression using Northern blot analysis (Machado *et al.* 2008). Other studies using Northern blot analysis of KCl-induced induction of NR4A1 did not quantify the fold changes (Yoon & Lau 1993, Yoon & Lau 1994), or did not focus on direct mRNA expression of NR4A1 but instead used luciferase reporter assays to test activity of reporter constructs containing parts of the promoter region of NR4A1 (Lam et al. 2010)

Activation of GR in depolarizing neurons prevents MEF2-DNA binding to NR4A1 DEX treatment alone had no effect on NR4A1 expression, however, a clear attenuating effect of GR on depolarization-induced NR4A1 mRNA expression was observed. DNA binding of MEF2 is a prerequisite for MEF2-regulation of NR4A1. Two MEF2 binding sites were previously shown to reside just upstream of the TSS of NR4A1. MEF2 binding is enhanced by neuronal depolarization, which is consistent with a recent report by Lam et al, showing that constitutively active MEF2 attenuates KCl-induced expression of NR4A1 (Lam et al. 2010). Moreover, in unstimulated PC-12 cells, MEF2 actively represses NR4A1 expression, which is relieved by depolarization due to dephosphorylation of MEF2. In addition, DEX enhances binding of MEF2 to the DNA, which we previously also found to be the case for c-JUN (Speksnijder et al. 2012). Interestingly, we observed that MEF2-binding was

diminished to VEH levels when GR activation by DEX was combined with KCl-induced neuronal depolarization. Since we showed that treatment resulting in either GR activation or depolarization enhances binding of MEF2, this result was surprising.

Since MEF2-GR cooperation is a newly observed phenomenon, not much is known about this (Speksnijder et al. 2012). In the pituitary, it was observed that the MEF2 target gene NR4A1 and GR can antagonize each other. NR4A1 expression is enhanced by stress through induction of corticotrophin-releasing hormone (CRH). NR4A1 then binds to a negative glucocorticoid response element (nGRE) in the pro-opiomelanocortin (POMC) gene, preventing GR to block its expression and exert negative feedback of the hypothalamus-pituitary-adrenal (HPA)-axis (Okabe *et al.* 1998). In vitro, in AtT-20 cells, this antagonistic property was even found to result in decreased physical DNA binding of both GR and NR4A1 to a minimal POMC promoter. So, NR4A1 diminished GR-DNA binding while GR prevented NR4A1-DNA binding (Philips *et al.* 1997). This antagonistic mechanism might also play a role when both MEF2 and GR are activated and would explain why both MEF2-DNA and GR-DNA binding are diminished when they are activated at the same time by DEX and neuronal depolarization. Another possible explanation comes from the observation that the GR is able to

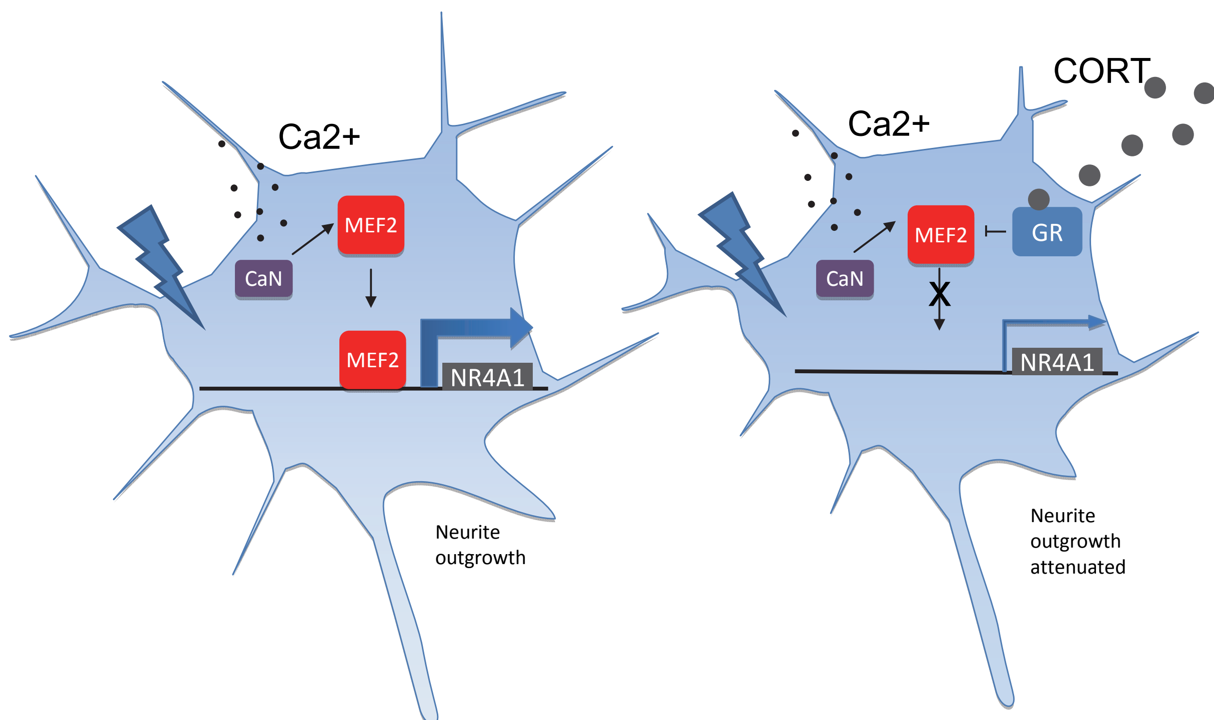

Figure 5 | Schematic overview of the obtained results. Depolarization by KCl leads to Ca^{2+}-ion influx (black dots) and activation of CaN. This would lead to dephosphorylation and transcriptional activation of MEF2 resulting in enhanced expression of NR4A1. GR activation by DEX leads to is known to enhance phosphorylation of MEF2, leading to transcriptional repression g. As depicted in this figure, combined depolarization and activation of GR leads to reduced binding of MEF2 to its binding site and attenuated NR4A1 expression compared to depolarization alone.

inhibit the action of nuclear factor of activated T cells (NFAT), a protein which is an activator of MEF2. NFAT is known to travel to the nucleus upon Ca^{2+} influx and bind to MEF2 to enhance transcription (Youn et al. 2000, Vacca *et al.* 1992, Blaeser et al. 2000). Simultaneous activation of GR by DEX and subsequent binding of GR to NFAT might prevent enhanced activation of MEF2 (Fig 5).

Following this hypothesis, another transcription factor might be responsible for the expression of NR4A1. Indeed, neuronal depolarization is also known to enhance the activity of cAMP response element binding protein (CREB) and NR4A1 is target gene of this transcription factor as well (Fass *et al.* 2003, Flavell & Greenberg 2008). This might indicate that expression of NR4A1 under the specific condition of both activated GR and depolarization is regulated solely by CREB. Since depolarization leads to dephosphorylation of MEF2, resulting in recruitment of transcriptional enhancers, the inability of MEF2 to bind upstream NR4A1 may lead to attenuated expression of NR4A1. NR4A1 expression gives rise to neurite outgrowth in PC-12 cells, one of the prerequisites for synaptic plasticity (Maruoka *et al.* 2010). Inhibition of NR4A1 expression by GR through preventing MEF2-DNA binding might pose a new mechanism of regulation by GR and might be an interesting new avenue of research on GR influencing plasticity.

Attenuating effect of GR on MEF2-regulated IEG expression In this study we mainly focused on IEGs since we previously identified these genes to be common target genes of GR and MEF2 (Datson *et al.* 2011). MEF2 is a neuronal activity-induced transcription factor and regulates transcription within a very short time span. Multiple IEGs apart from NR4A1 are known to contain binding sites for MEF2 such as c-JUN, ARC, IER2, c-FOS etc (Kawashima *et al.* 2009, Lam et al. 2010, Knoll & Nordheim 2009, Han *et al.* 1992, Han & Prywes 1995, Flavell et al. 2008). GR binding sites in the vicinity of these IEGs were found by GR ChIP-sequencing in PC-12 cells and rat hippocampus (unpublished data).

In PC-12 cells we found that KCl also induced the expression of IER2 and c-FOS but not of ARC or c-JUN. In both IER2 and c-FOS we also observed the attenuating effect of DEX on KCl-induced gene expression, comparable to NR4A1. Both IEGs showed lower mRNA expression after DEX + KCl treatment compared to KCl treatment alone. However, for IER2, GR activation by DEX by itself decreased expression of IER2. c-FOS expression was not changed by GR activation alone, but it significantly attenuated c-FOS expression induced by KCl. These results, are in line with in vivo observations where absence of endogenous glucocorticoids by adrenalectomy significantly enhanced IEG expression induced by kainate, a NMDA receptor agonist, resulting in Ca^{2+}-influx and hence neuronal activation (Li *et al.* 1992). Whether the obtained results can be extrapolated to other genes apart from IEGs remains to be studied.

CONCLUSION

The results clearly show an attenuating effect of GR activity on depolarization-induced NR4A1 expression. More importantly we show that under this condition MEF2-DNA binding upstream of NR4A1 is reduced to VEH levels. Depolarization-induced NR4A1 expression is an important mediator of synaptic plasticity, by facilitating neurite outgrowth. One of the known ways in which GR modulates synaptic plasticity is to balance calcium influx. Based on the presented results we propose another, more downstream, mechanism of modulation where GR attenuates NR4A1 expression by preventing MEF2-DNA binding, possibly leading to inhibited neurite outgrowth.

ACKNOWLEDGEMENTS

This work was supported by the Netherlands Organization for Scientific Research (NWO) grant 836.06.010 (MEERVOUD) to N.A. Datson and TI Pharma grant T5-209. ERdK was supported by the Royal Netherlands Academy of Science. All authors declare that they have no conflicts of interest.

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SUPPLEMENTARY DATA

Table S1 Primer sequences used for gene expression measurements or immuno-precipitated DNA fragments bound by GR or MEF2.

NCBI accession #	Gene nam e	Gene Symbol	purpose	BP from TSS	Forward primer	Reverse primers
NC_005106.3	Nuclear Receptor, family 4, group A, member 1	NR4A1	MEF2 DNA binding	-271	GTGCAGCGGGCGAGAGGAAA	CGCGGGGTTCCATTGACGCA
NC_005106.2	Myoglobin	Mb	Negative control GR and MEF2 DNA-binding	3504	TAGTGTGCATCCAGCAGAGG	ACACTGTGGCCTTTTTGTCC
NM_024388.2	Nuclear Receptor, family 4, group A, member 1	NR4A1	Expression	NA	GCTTGGGTGTTGATGTTCCT	ACAGCTAGCAATGCGGTTCT
NM_022197.2	FBJ osteosarcoma oncogene	c-FOS	Expression	NA	GGGACAGCCTTTCCTACTACC	TGGCACTAGAGACGGACAGA
NM_001009541.1	Immediate-early response 2	IER2	Expression	NA	AACGTGCTGGTGCGAACCGT	CTTCGCCTCGGGTGTGCGTT
NM_001109119.1	Tubulin beta 2A	Tubb2a	Expression	NA	GAGGAGGGCGAGGATGAGGCTT	GACAGAGGCAAACTGAGCACCAT