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Time-course of immediate early gene expression in hippocampal subregions of adrenalectomized rats after acute corticosterone challenge

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Abstract

Corticosterone hormones mediate the stress response and function in the survival of hippocampal neurons via activation of gluco- (GR) and mineralocorticoid (MR) receptors. Activated GR and MR couple the corticosterone signal through immediate early genes (IEGs) to the late expression of downstream genes, such as neurotrophic factors. The potential importance of IEGs in GR/MR-dependent plasticity in the brain is largely unknown. We examined the region- and time-dependent transcriptional profiles of six IEGs (*c-fos*, *fosB*, *fra-1*, *junB*, *c-jun* and *egr-1*) by in situ hybridization after acute corticosterone challenge in the hippocampus and the primary somatosensory cortex (S1). Adrenalectomized rats and subsequent hormone injections were used as a model system to eliminate interference of endogenous corticosterone on IEG expression.

In the hippocampus, a single corticosterone dose (10 mg/kg, s.c.) caused a widespread and transient reduction of *fosB* mRNA after 0.8 h, whereas changes in both *c-fos* and *fra-1* mRNA levels were restricted to the dentate gyrus region. Corticosterone treatment gave rise to a delayed and significant reduction of *junB* mRNA signals after 2 h in all hippocampal regions, which reversed to increase at 4 h. *C-jun* and *egr-1* mRNA levels were unaffected by corticosterone treatment. On the contrary, in the S1, IEG expression seems to be unaffected by corticosterone treatment, with the exception of a transient increase of *junB* transcripts at 0.8 h.

The early reduction in *c-fos* family and *junB* transcripts may contribute to the GR/MR-dependent changes on hippocampal plasticity and may be dependent on rapid corticosteroid signaling.

Keywords

glucocorticoid hormone; transcription factors; *fos* family proteins; neuroplasticity; in situ hybridization; rat brain

1. Introduction

The adrenocorticosteroid hormone corticosterone (CORT) is a potent modulator of brain function and a critical factor in the mediation of the stress response. The hippocampus is a brain region for ongoing plasticity and functionally important for declarative/spatial learning and memory. Stress, depletion or sustained high levels of CORT affect the morphology and survival of hippocampal neurons (Gould et al., 1990; Sloviter et al., 1989; Watanabe et al., 1992) and have been shown to influence the expression of genes coding for trophic and

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protective proteins, e.g. basic fibroblast growth factor (bFGF) and brain derived neurotrophic factor (BDNF) (Chao et al., 1998; Chao and McEwen, 1994; Hansson et al., 2000; Hansson et al., 2003; Riva et al., 1995; Schaaf et al., 1998; Smith et al., 1995).

CORT acts via its low affinity receptors (glucocorticoid receptor, GR) and high affinity receptors (mineralocorticoid receptor, MR), which bind as transcription factors to glucocorticoid response elements within promoter regions, thereby modulating the expression of specific genes, e.g. neurotrophic factors (Parrelli et al., 1998). Both receptors are highly prevalent and co-expressed in the majority of the hippocampal neurons (Cintra et al., 1994; Van Eekelen and De Kloet, 1992). Emerging evidence suggests that the inducible transcription factor and immediate early gene (IEG) *c-Fos* is essential for neuronal cell survival through its regulation of downstream genes such as BDNF (Zhang et al., 2002). The expression of *c-fos* and the IEG early growth response gene (*egr-1*, alternatively known as *zif268*, *krox24*, *NGFI-A* or *TIS8*) have both been used as functional markers of brain activity and neuroplasticity (James et al., 2005; Pfenning et al., 2007; Sheng and Greenberg, 1990). When induced by physiological or pharmacological stimulation, *c-Fos* proteins dimerize with proteins from the Jun family to form the activator protein-1 (AP-1), which subsequently interacts with AP-1 recognition sequences in the regulatory regions of various genes (Herdegen and Waetzig, 2001). Interactions between GR and the AP-1 complex independent from DNA-binding have been previously described in tissue culture studies (Schule et al., 1990; Yangyen et al., 1990). Using the *c-fos* antisense oligodeoxynucleotide approach, we recently demonstrated in vivo an antagonistic *c-Fos*/GR interaction on both *bFGF* and *BDNF* gene expression in the CA1 region of the hippocampus (Hansson et al., 2003). Interactions between GR/MR and IEGs other than *c-Fos* and their potential importance in GR/MR-dependent changes on brain plasticity are largely unknown.

Several laboratories found that stress increases both the protein and mRNA levels of *c-fos* in the brain (Cullinan et al., 1995; Herrera and Robertson, 1996; Hyder SM, 1994; Kononen et al., 1992; Kovacs and Sawchenko, 1996), resulting in changes in the composition and activity of the transcription factor AP-1 complex (Autelitano, 1998). Notable, however, is that the stress-induced increase in *c-fos* seems to be independent of circulating levels of CORT (Hansson et al., 2003; Helmreich et al., 1996; Melia et al., 1994; Ryabinin et al., 1999). Depletion of adrenocorticosteroid hormones by adrenalectomy (ADX) increases *c-Fos* immunoreactivity in the paraventricular nucleus 3 to 6 h after ADX; at 12 h, *c-Fos* levels have diminished, and are no longer detectable after 24 h (Brown and Sawchenko, 1997).

We previously used the acute 24 h ADX animal model with no detectable levels of endogenous adrenocorticosteroid hormones, under the assumption that GR and MR are largely unoccupied at this time point. Subsequent treatment with low and high doses of CORT hormone allowed us to separately analyze the consequences of GR and MR activation on gene expression profiles without interference from endogenous adrenocorticosteroid hormones potentially pre-occupying GR and MR (Hansson et al., 2000; Hansson et al., 2001; Hansson et al., 2003; Hansson et al., 2006; Hansson and Fuxe, 2002). In a previous paper we established that in the present acutely adrenalectomized rat model there exists an upregulation of GR and MR mRNA levels in the dorsal hippocampus (Hansson et al., 2000). In the study presented here, a high dose of CORT (10 mg/kg, s.c.) was chosen in order to fully activate both GR and MR over several hours (Hansson et al., 2003; Hansson and Fuxe, 2002). This allowed us to analyze the GR/MR-dependent changes on the temporal and regional expression patterns of IEGs (*c-fos*, *fosB*, *fos related antigen-1* or *fra-1*, *junB*, *c-jun*, *egr-1*) of ADX rats by in situ hybridization. The aim of the paper was to study the impact of CORT on IEG expression in order to understand how CORT may contribute to stress induced influences on the neuronal networks. The CORT actions on regional hippocampal *c-fos* mRNA levels have previously been reported in this model (Hansson et al., 2003).

2. Results

The time course of mRNA levels for six IEGs in the hippocampus and primary somatosensory barrelfield cortex (S1, see Fig. 1) of ADX rats in response to 10 mg/kg CORT (s.c.) as well as plasma CORT levels of the experimental groups are shown in Tables 1 and 2. The regional and cellular expressions for *c-fos*, *fosB*, *fra-1*, *junB*, *cjun* and *egr-1* mRNA levels are illustrated in Figure 2. IEG expression was mainly linked to nerve cell bodies as revealed in bright-field emulsion autoradiograms counterstained with cresyl violet. In the granular cell layer of the dentate gyrus, we observed a scattered population of nerve cell bodies strongly labelled for all IEG mRNAs with the exception of *c-jun* (Fig. 2).

In a second experiment we addressed the effects of injection stress on the expression of IEG and compared mRNA levels at different time points (0.8 h, 4 h and 24 h) in the above mentioned regions by comparing solvent-injected and non-injected ADX rats. Overall, saline-injection had no effects on *fosB*, *junB*, *c-jun* and *egr-1* mRNA levels as compared to non-injected ADX control rats at any time-interval in each analyzed regions (see Table 3). In the same animal experiment injection stress had also no effects on *c-fos* mRNAs as previous reported in Hansson et al., 2003.

Time-dependent changes of immediate early gene expression after CORT treatment in ADX rats

C-fos mRNA levels—The expression of *c-fos* in the granule cell layer (DG) and the polymorph layer (CA4) of the dentate gyrus and the S1 demonstrated a time-dependent and significant decrease after CORT treatment as demonstrated by a two-way ANOVA for interaction of time and treatment (DG: $F[1, 38] = 3.3$, $p < 0.05$; CA4: $F[1, 40] = 2.7$, $p < 0.05$; S1: $F[1, 40] = 2.8$, $p < 0.05$). The corrected post-hoc test showed significant decreases of *c-fos* mRNA levels in the DG between 0.8 h and 2 h (38 % to 46 %, corrected $p < 0.05$, Table 2, Fig. 3), in the CA4 after 2 h (39 %, corrected $p < 0.05$, Table 2, Fig. 3) and in the S1 after 24 h (24 %, corrected $p < 0.05$, Table 2, Fig.3). There was no interaction effect in the CA1 and CA3 hippocampal regions.

Furthermore, two-way ANOVA demonstrated a time effect in all subregions in both CORT and solvent treated rats (CA1: $F[1, 39] = 33$, $p < 0.0001$; CA3: $F[1, 40] = 16.6$, $p < 0.0001$; CA4: $F[1, 40] = 12.9$, $p < 0.0001$; DG: $F[1, 38] = 16.1$, $p < 0.0001$; S1: $F[1, 40] = 62.2$, $p < 0.0001$, Table 2, Fig. 3).

FosB mRNA levels—Two-way ANOVA for interaction of time and treatment demonstrated effects in all regions (CA1: $F[1, 40] = 5.2$, $p < 0.01$, CA3: $F[1, 40] = 2.8$, $p < 0.05$, CA4: $F[1, 40] = 4.2$, $p < 0.01$, DG: ($F[1, 40] = 5.0$, $p < 0.01$, S1: $F[1, 40] = 3.3$, $p < 0.05$). The corrected post-hoc test showed that CORT (10 mg/kg, s.c.) caused a significant and widespread reduction of *fosB* mRNA levels in the CA1 (39 %, corrected $p < 0.01$), CA3 (34 %, corrected $p < 0.05$), and DG (49 %, corrected $p < 0.01$) regions after 0.8 h; levels had disappeared as soon as 2 h, as shown in Figure 3 and Table 2. After 24 h, a significant reduction was again observed in the CA1 region (57 %, corrected $p < 0.05$), and trends towards reduction were also observed at this time point in all other regions, including CA4 and S1 (Table 2).

Fra-1 (fos related antigen-1) mRNA levels—As shown in Table 2 and Fig. 3, CORT produced an early and transient reduction of *fra-1* mRNA levels exclusively in the DG (38 %, corrected $p < 0.05$). The CA1 to CA4 and the S1 did not show any detectable levels of *fra-1* mRNA at any time point (see Table 2).

JunB mRNA levels—Two-way ANOVA showed an interaction of time and treatment on *junB* mRNA in the CA1 ($F[1,40] = 4.1$, $p < 0.01$), CA3 ($F[1,40] = 4.4$, $p < 0.01$), CA4 ($F[1,40]$

= 8.0, $p < 0.001$), DG ($F[1,40] = 6.7$, $p < 0.001$) and S1 ($F[1,40] = 6.1$, $p < 0.001$). In the S1, an early significant rise in *junB* mRNA levels at the 0.8 h time-interval (49 %, corrected $p < 0.05$) was detected, which disappeared at later time points. Further statistical analysis showed a delayed significant reduction of *junB* mRNA signals after 2 h in the CA1 (33 %, corrected $p < 0.05$), CA3 (32 %, corrected $p < 0.05$) and CA4 (25 %, corrected $p < 0.05$) regions but not in the DG and the S1 (Table 2 and Fig. 4). This reduction was followed by a rise of transcripts after 4 h in all regions except for the S1; this increase was only statistically significant in the CA3 after correction (48 %, corrected $p < 0.05$, Table 2 and Fig. 4) and was also transient, having disappeared at the 8 h time interval. In the CA4 and DG regions, a late reduction of *junB* mRNAs was seen at the 24 h time interval (CA4: 24 %, corrected $p < 0.05$, DG: 12 %, corrected $p < 0.05$, Table 2).

Overall, there was a strong effect on *junB* mRNA levels in the CA1 (two-way ANOVA: $F[1,40] = 16.1$, $p < 0.0001$), CA3 (two-way ANOVA: $F[1,40] = 7.6$, $p < 0.0001$), CA4 (two-way ANOVA: $F[1,40] = 7.0$, $p < 0.001$), DG (two-way ANOVA: $F[1,40] = 6.7$, $p < 0.001$) and S1 (two-way ANOVA: $F[1,40] = 27.4$, $p < 0.0001$, see Table 2) for both CORT and solvent treated ADX rats over time.

C-jun mRNA levels—No changes in *c-jun* mRNA levels were detected in the hippocampal regions and the S1 at any given time point after CORT treatment as compared to the solvent treated control group (Table 2).

Egr-1 mRNA levels—No changes in *egr-1* mRNA levels were observed in the hippocampal regions and the S1 at the different time intervals after CORT treatment, with the exception of the DG region (two-way ANOVA for time and treatment: $F[1,39] = 3.2$, $p < 0.05$) at the 24 h interval. In this case, a significant *egr-1* mRNA increase was observed (25 %, corrected $p < 0.01$, Table 2).

The two-way ANOVA showed an overall time effect for both CORT and solvent treated rats in the CA1 ($F[1, 39] = 13.4$, $p < 0.0001$) CA4 ($F[1, 39] = 6.6$, $p < 0.001$), DG ($F[1, 39] = 2.7$, $p < 0.05$) and S1 ($F[1, 39] = 25.1$, $p < 0.0001$, Table 2).

3. Discussion

The present paper focused on the differential effects of GR/MR activation on the regional and temporal expression of six IEG genes in the hippocampus and the primary somatosensory cortex of ADX rats to understand the role of corticosterone in mediating the effects of stress on the neuronal networks.

As expected and illustrated in Table 1, 24 hours after ADX, no plasma CORT was found by radioimmunoassay. The plasma levels of CORT were pharmacologically high at 0.8 h after corticosterone injection, while at 2 h after injection they represented levels found at high stress, reaching levels found at the diurnal peak at the 4 h time point (Hansson et al., 2003; Sapolsky et al., 1986). However, we have no evidence for the view that these supramaximal doses produce antagonistic effects in the present model. Thus, in a previous paper (Hansson et al., 2000) it was found that in the present adrenalectomized model a low dose (2 mg/kg, giving similar levels to nadir) and a high dose of corticosterone (10 mg/kg) give a similar rise of bFGF mRNA levels in the dorsal hippocampus and a similar reduction of BDNF mRNA levels in this region. Thus, a full saturation and activation of intracellular GR and MR (Reul et al., 1987a; Reul et al., 1987b) likely occurs after treatment with this dose of CORT in this time period; by comparison, the plasma levels at the 8 h time point are similar to those as seen at nadir in normal and untreated rats, and may lead most likely to predominant MR activation, but also to some GR activation (Sapolsky et al., 1986; Spencer et al., 1991).

In the hippocampus, a high dose of CORT that activates both GR and MR reduced *c-fos*, *fosB* and *fra-1* transcripts at an early time-point (0.8 h) followed by a decrease and an increase of *junB* transcripts after 2 h and 4 h, respectively. The effect of CORT on regional hippocampal *c-fos* expression as well as the lack of influence of the injection stress have previously been reported in this model (Hansson et al., 2003). Hippocampal *c-jun* and *egr-1* transcript levels seemed to be unaffected at an early time point by CORT treatment.

In the S1, IEG expression seemed to be relatively unaffected by CORT treatment. Surprisingly, at 0.8 h CORT stimulated *junB* transcript levels in the S1. Notably, the S1 expresses predominantly GR and low or no detectable levels of MR (Ahima et al., 1991; Cintra et al., 1994), thus the effects of CORT on IEG expression may be mainly mediated by GR. This suggests that *junB* may have a special role in mediating GR-induced changes in cortical vs hippocampal plasticity.

Overall, heterogeneity among the hippocampal subregions was observed in the present study in terms of GR/MR-induced changes in IEG expression. The dentate gyrus was the only region in which CORT caused a decrease in the expression level of all analyzed *fos* family transcripts. These are interesting observations, as they demonstrate for the first time that despite the presence of GR and MR in most of the hippocampal subregions (Cintra et al., 1994; Joels and Dekloet, 1994), CORT can induce a differential early decrease of *c-fos*, *fosB* and *fra-1* transcripts which may lead to unique plasticity changes in this region compared to the other hippocampal subregions.

Depending on brain region and/or cell population, our data may suggest differential mechanisms underlying the effects of GR and MR on IEG regulation. The mechanisms involved in the GR/MR-dependent dynamics of the *fos* family and *junB* expression are unclear. Regulation of IEG transcription occurs very rapidly and is independent of de novo protein synthesis. The ability of GR/MR to decrease IEG transcripts at an early time point may be mediated by post-transcriptional processes (e.g., decreased RNA stability and RNA half-life), post-translational processes (e.g., phosphorylation) and rapid changes in neuronal excitability. Such alterations in neuronal excitability may be brought by rapid CORT signaling via membrane-associated receptors (Tasker et al., 2006). In fact, CORT may act as allosteric modulator of distinct G protein-coupled receptors leading to changes in the intracellular signaling cascades with changes in the expression of the IEG transcripts as observed in the present paper. Alternatively, *fos* family and *junB* expression can be reduced by specific self-inhibition processes through the AP-1 complex (Sassone-Corsi et al., 1988).

Under normal or stimulated conditions, Fos family members form heterodimers with Jun family proteins, and the resulting AP-1 complexes bind to AP-1 responsive sequences within promoters to regulate the transcription of a wide panel of genes. A repression of AP-1 by glucocorticoids was previously demonstrated in tissue culture studies (Diamond et al., 1990; Konig et al., 1992; Yangyen et al., 1990) and the interaction of GR with Jun/Fos heterodimers led to both reduced AP-1 activity and *c-fos* expression (Herdegen and Leah, 1998; Reichardt et al., 1998; Teurich and Angel, 1995). The *c-fos* antisense oligodeoxynucleotide approach also demonstrated the existence of an antagonistic interaction of c-Fos and GR/MR in the hippocampus on the expression of bFGF and BDNF (Hansson et al., 2003). In line with these studies, our results in the hippocampus of ADX rats may suggest that activation of GR and MR reduces the availability of Fos family and JunB proteins, which may affect the composition and activity of AP-1 complexes to further impact changes in neurotrophic factor gene expression and plasticity (Barbany and Persson, 1992; Chao et al., 1998; Hansson et al., 2000; Hansson et al., 2003).

In the ADX model, CORT (at 10 mg/kg) substantially reduces GR and MR mRNA levels in all hippocampal subregions starting at the 2 h time point and persisting up to 24 h (Hansson et al., 2003). It is possible that the proposed changed activity of the AP-1 complexes could also be involved in the CORT autoregulation of GR and MR gene expression. However, using the *c-fos* antisense oligodeoxynucleotide approach (Hansson et al., 2003), it was not possible to change the strong negative CORT-induced autoregulation of GR and MR gene expression in the hippocampus. Again, this may be explained by the observations presented here indicating that *fosB* mRNA levels are rapidly and broadly reduced in the hippocampus by this high dose of CORT, thus reducing the possible FosB containing AP-1 complexes. It should also be considered that the possible modulatory influence of such AP-1 complexes on GR/MR autoregulation of their genes may become more pronounced with a lower dose of CORT, leading to less activated GR/MR.

In conclusion, the present study demonstrated that a high dose of CORT that activates both GR and MR produces discrete and specific patterns of IEG expression over time and across multiple brain regions in ADX animals. The major changes after CORT treatment are found in *c-fos*, *fosB*, *fra-1* and *junB* mRNA levels within the hippocampus, while *c-jun* and *egr-1* expression is more resistant to the CORT treatment. The CORT-induced early reduction of *c-fos*, *fosB*, *fra-1* at 0.8 h, which may involve rapid CORT signaling via membrane associated receptors, and delayed reduction of *junB* transcripts at 2 h may lead to changes in the composition and activity of the AP-1 complexes containing these IEGs, and thus may play a role in the GR/MR mediated changes in regional hippocampal plasticity.

4. Experimental procedures

Animals

Male Sprague-Dawley rats (body weight 200 g), obtained from Alab/Stockholm one week before the start of the experiment, were kept under a standard light/dark cycle (lights on at 6:00 and off at 20:00) and constant room temperature (23 °C) with free access to tap water and food pellets. All animal experiments were approved by the local ethics committee (Stockholm Norra Försöksdjurs Etiska Kommittee). The ADX model used here has been previously described in detail (Hansson et al., 2000; Hansson et al., 2001; Hansson et al., 2003; Hansson et al., 2006; Hansson and Fuxe, 2002). All rats were bilaterally ADX under halothane anesthesia. After surgery, 0.9 % NaCl was added to the drinking water.

24 h after ADX, CORT (10 mg/kg, Sigma, Missouri, USA) or hormone solvent (propylene glycol, Sigma, Missouri, USA) was given subcutaneously (s.c.) and rats were killed 0.8 h, 2 h, 4 h, 8 h and 24 h after injection according to a previous study (Hansson et al., 2003). The experiments with solvent and corticosterone were run absolutely in parallel at the very same time points to avoid an influence of the circadian rhythm. In a second experiment 24 h ADX rats were either injected with hormone solvent alone (s.c., propylene glycol, Sigma, Missouri, USA) or received no injection but handled in the same way as the injected rats. The animal experiment 2 was performed under the same conditions as described above. Rats were killed at 0.8 h, 4 h and 24 h after injection. All surgical procedures and hormone injections (injection volume: 1ml/kg) were performed between 7:00 am and 9:00 am. At various times, trunk blood was collected for plasma CORT measurements and brains were snap-frozen in liquid isopentane (−40 °C) and stored at −70°C.

Radioimmunoassay for CORT

Trunk blood samples were collected in heparin-containing tubes and centrifuged at 4 x g for 20 min at 4°C. Plasma CORT levels were determined by radioimmunoassay (RIA; *Coat-a-count*, Diagnostic Products Corporation, Los Angeles, CA, USA). The RIA was performed

with rat [125 I]CORT with a detection limit of ~ 5.7 ng/ml. Plasma CORT values for all animal groups are summarized in Table 1.

In situ hybridization

The time- and CORT-dependent expression profiles of IEG were studied in the dorsal hippocampus, a classical region for the stress response, and in the primary somatosensory barrelfield cortex (S1), a region which is not primarily involved in the stress response (see Fig. 1). For in situ hybridization, 10 μ m coronal brain sections were cryo-sectioned at bregma level -3 mm (Paxinos and Watson, 1986). Rat and mouse specific riboprobes were used as follows: *c-fos* (position 306 bp to 864 bp on rat cDNA, gene reference sequence: **NM_022197.2**), *egr-1* (position 1384 bp to 1851 bp on rat cDNA, gene reference sequence: **NM_012551.1**), *fosB* (positions 1206 bp to 1912 bp and 2051 bp to 2220 bp on mouse cDNA, gene reference sequence: **X14897.1**, with 96 % sequence homology to rat *fosB* cDNA), *fra-1* (position 1020 bp to 1663 bp on rat cDNA, gene reference sequence: **M1965.1**), *junB* (position 457 bp to 931 bp on mouse *junB* cDNA, gene reference sequence: **NM_008416.1**, with 97 % sequence homology to rat cDNA) and *c-jun* (position 1902 bp to 2068 bp on mouse *cjun* cDNA, gene reference sequence: **NM_021835.3**, with 91 % sequence homology to rat cDNA). Plasmids for both *junB* and *c-jun* riboprobes were kindly provided by Dr. M. Yaniv, Department of Biotechnology, Institute Pasteur, France. Labeling of riboprobes and in situ hybridization have been recently described (Hansson et al., 2003). The hybridized sections were exposed to Kodak BioMax MR film (Eastman Kodak Company, UK). Afterwards, sections were coated with film emulsion (Ilford, Cheshire, UK), stored in desiccated light-tight boxes at 4°C until development, counterstained with cresyl violet and coverslipped with Mountex (Göteborgs Termometerfabrik). Sections were digitized and qualitatively evaluated using a Leica microscope connected with to a high-resolution digital CCD video camera.

Data analysis

Semiquantitative values of mRNA levels were obtained by measuring the gray values of the film autoradiograms according to figure 1 using an SAS Biovision image analyzing system (Avanzati, Milan, Italy). Three measurements were performed for each region: (1) the total value, i.e., measurements of the region in the sections hybridized with [35 S]UTP-labeled antisense RNA; (2) the unspecific value, i.e., measurements of the corresponding region in the control sections hybridized with [35 S]UTP-labeled sense RNA; and (3) the background value, i.e., measurements of the film background outside the sections. The transmittance percentage values (T%) of specific and unspecific labeling were obtained as described in (Benfenati et al., 1986) and (Zoli et al., 1991). From the T % values the optical density (O.D.) can be obtained ($O.D. = -\log T \%$).

All data are expressed as means \pm SEM. Data meet assumptions of normality and homogeneity of variances, and were analyzed using standard parametric ANOVA test. Region-wise two-way ANOVA for treatment and time followed by one-way ANOVA (treatment) per time point and Bonferroni's correction (Fisher's *p* value multiplied by number of brain regions analyzed) was used for the statistical analysis. Significance is indicated at levels for $\alpha < 0.05$, $\alpha < 0.01$ or $\alpha < 0.001$. Bonferroni's correction was used to demonstrate the most robust effects of CORT on expression of IEGs.

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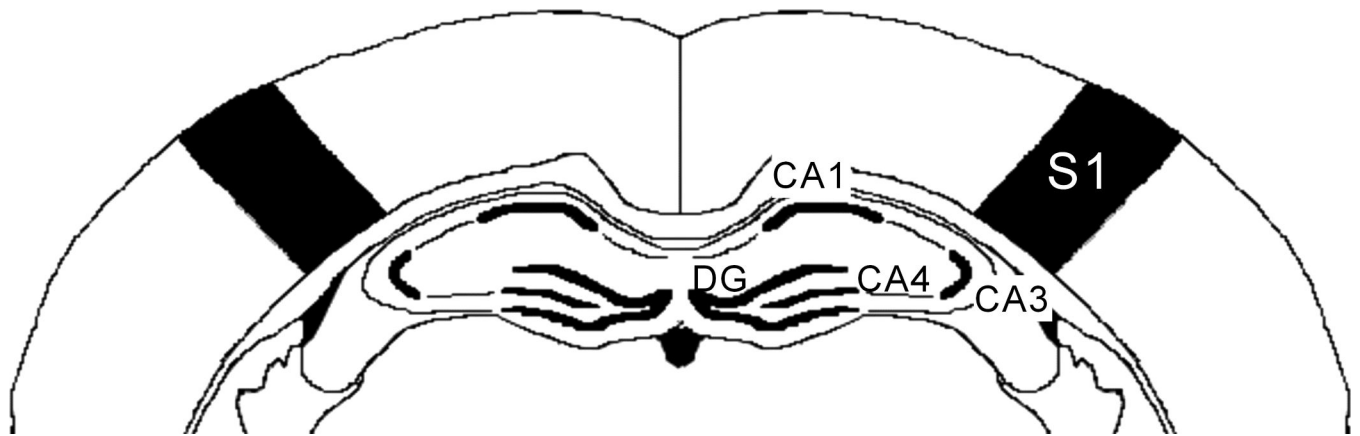


Fig.1. Schematic representation of the sampled areas for the densitometric evaluation of IEG mRNA in a coronal section through the dorsal hippocampus. (Bregma levels = -2.8 mm). CA1 – CA4: Cornus Ammon areas; DG: granular cell layer of the dentate gyrus; S1: primary somatosensory barrelfield cortex.

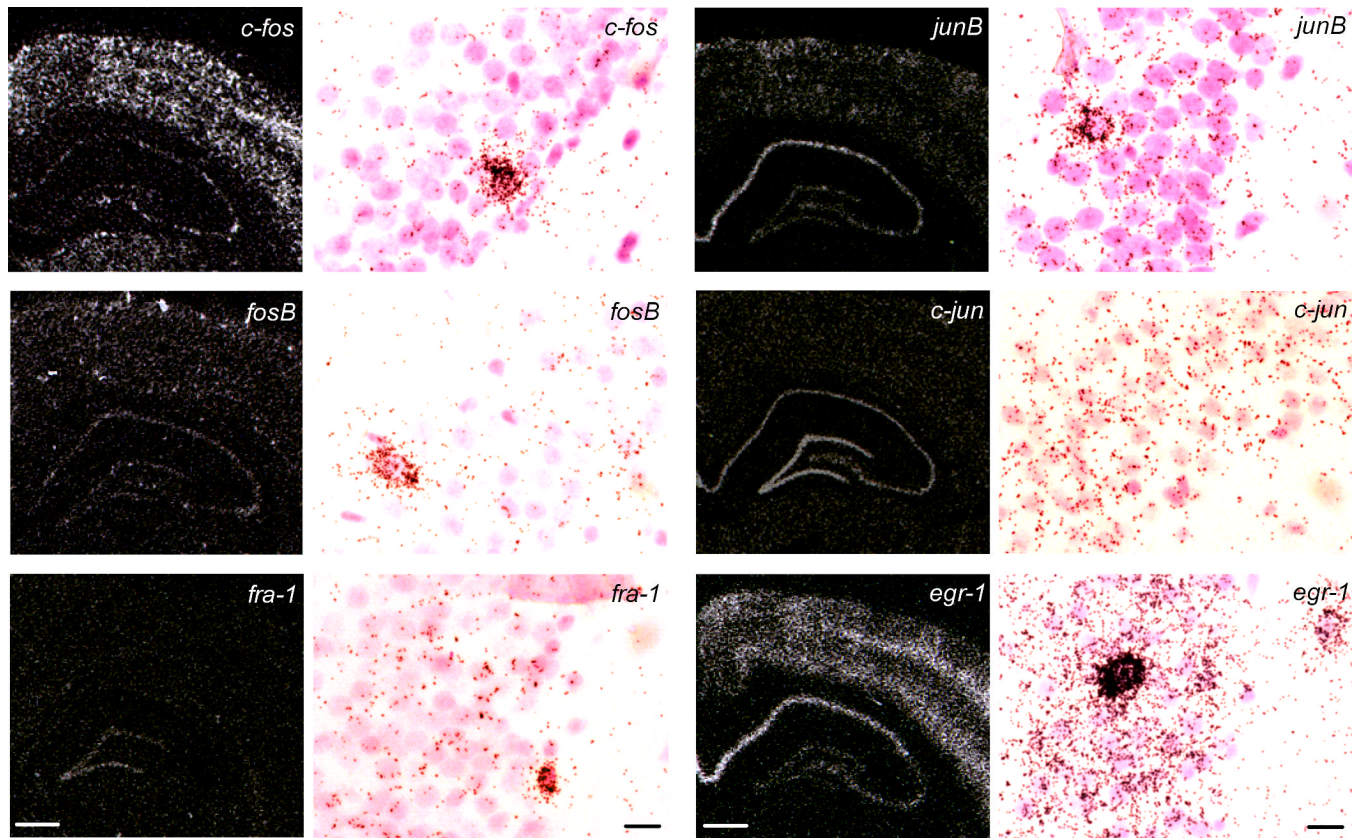


Fig. 2. Dark-field microphotographs from *in situ* hybridization of autoradiograms showing the regional distribution of *c-fos*, *fosB*, *fra-1*, *junB*, *c-jun* and *egr-1* mRNA in the dorsal hippocampus and S1 of saline injected (s.c.) 24 h ADX rats at the 0.8 h time point (left, Bregma = -3 to -4 mm, scale bar = 1 mm), and their respective cellular expression in the dentate gyrus in brightfield emulsion autoradiograms (right, scale bar = 15 μ m). CA1 – CA4: Cornus Ammon areas; DG: granular cell layer of the dentate gyrus; S1: primary somatosensory barrelfield cortex. For details on treatment, see *Material and Methods*.

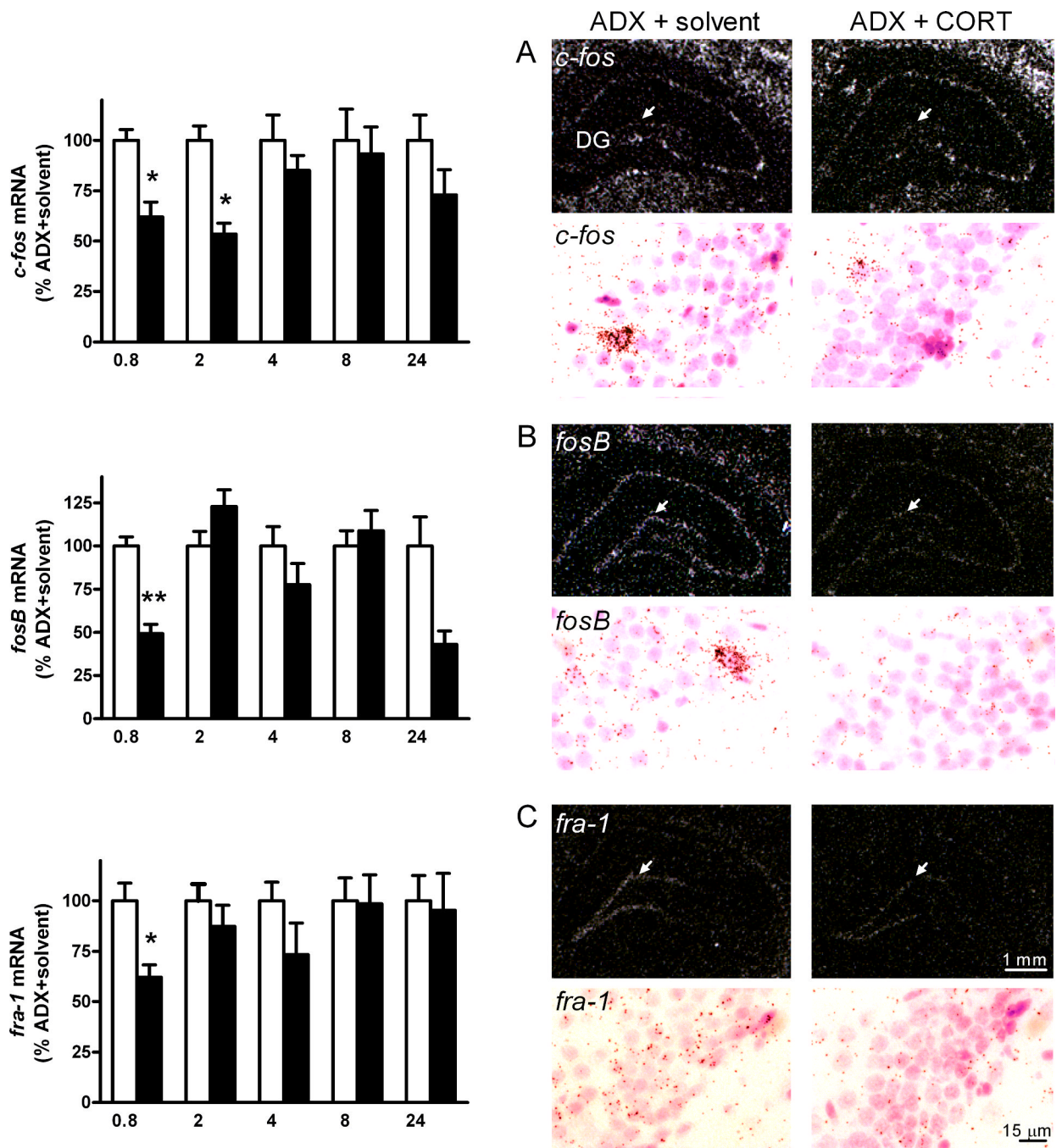


Fig. 3. Left: Densitometric evaluation from autoradiograms of *c-fos*-, *fosB*- and *fra-1* transcripts in the dentate gyrus of the dorsal hippocampus of CORT (black bars) and solvent (white bars) treated 24 h ADX rats. Values are given in percent of control group (% ADX + solvent, mean \pm S.E.M.). Statistical analysis was performed by two-way ANOVA (time x treatment) followed by Fisher's PLSD test and Bonferroni's correction. Corrected values: * $p < 0.05$, ** $p < 0.01$, $n = 4-5$ /group of CORT vs solvent treated ADX rats. Right: Dark-field microphotographs from in situ hybridization of autoradiograms (upper panel) and brightfield emulsion autoradiograms (lower panel) showing decreased (A) *c-fos*, (B) *fosB*, and (C) *fra-1* transcripts after acute CORT treatment (10 mg/kg, s.c.) in the granular cell layer of the dentate gyrus at the 0.8 h time interval

as compared to solvent treated ADX rats. Bregma = -3 to -4 mm. For abbreviations and details on treatment, see Figure 1.

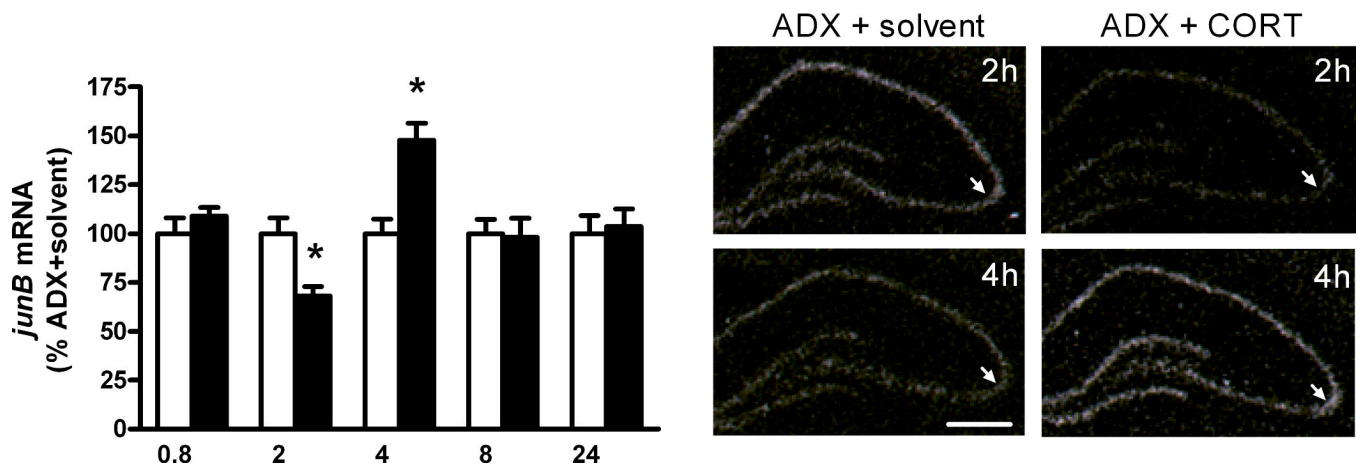


Fig. 4.

Left: Densitometric evaluation from autoradiograms of *junB* mRNAs in the CA3 subregion of the dorsal hippocampus of CORT (black bars) and solvent (white bars) treated 24 h ADX rats. Values are given in percent of control group (% ADX + solvent, mean \pm S.E.M.). Statistical analysis was performed by two-way ANOVA (time \times treatment) followed by Fisher's PLSD test and Bonferroni's correction. Corrected values: * $p < 0.05$, $n = 4-5$ /group of CORT vs solvent treated ADX rats. Right: Dark-field microphotographs from in situ hybridization of autoradiograms showing decreased *junB* mRNA levels after CORT treatment (10 mg/kg, s.c.) at the 2 h time interval followed by increased transcript levels at the 4 h time interval in the dorsal hippocampus as compared to the solvent treated ADX rats. Bregma = -3 to -4 mm, scale bar = 1 mm. Arrows point to the hippocampal CA3 subregion. For abbreviations and details on treatment, see Figure 1.

Table 1**Radioimmunoassay for plasma CORT**

24 h after ADX rats were injected with either a high dose of CORT (10 mg/kg, s.c.) or saline (s.c., experiment 1) or s.c. injected with saline alone (experiment 2). Data are expressed as CORT (ng/ml, mean \pm S.E.M.), n.d. = not detectable.

time (h)	treatment	(n)	plasma CORT (ng/ml)
<i>Experiment 1</i>			
0.8	ADX + solvent	4	n.d.
0.8	ADX + CORT	5	2638 \pm 244
2	ADX + solvent	5	n.d.
2	ADX + CORT	5	805 \pm 143
4	ADX + solvent	4	n.d.
4	ADX + CORT	5	147 \pm 27
8	ADX + solvent	5	n.d.
8	ADX + CORT	5	29 \pm 4
24	ADX + solvent	4	n.d.
24	ADX + CORT	5	n.d.
<i>Experiment 2</i>			
0.8	ADX	4	n.d.
0.8	ADX + solvent	4	n.d.
4	ADX	4	n.d.
4	ADX + solvent	4	n.d.
24	ADX	4	n.d.
24	ADX + solvent	4	n.d.

Table 2

Effects of CORT (10 mg/kg, s.c.) on IEG expression in ADX rats

Data are expressed as optical densities ($OD \times 10^{-3}$, mean \pm S.E.M.), statistically analyzed by two-way ANOVA (time and treatment) followed by Fisher's PLSD test and Bonferroni's correction; $n = 4-5$ /group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ CORT vs solvent treated ADX rats per time point; n.d.=not detected, CA1-CA4: Cornus of Ammon areas, DG: Dentate Gyrus, S1: primary somatosensory barrelfield cortex. For details of treatment, see *Material and Methods*.

	time	S1	CA1	CA3	CA4	DG
<i>c-fos</i> mRNA						
ADX + solvent	0.8	154 \pm 9	107 \pm 10	134 \pm 7	94 \pm 9	92 \pm 5
ADX + CORT	0.8	173 \pm 7	85 \pm 10	120 \pm 12	74 \pm 10	57 \pm 7 *
ADX + solvent	2	80 \pm 7	41 \pm 4	102 \pm 6	67 \pm 3	56 \pm 4
ADX + CORT	2	79 \pm 6	31 \pm 7	78 \pm 8	41 \pm 6 *	30 \pm 3 *
ADX + solvent	4	53 \pm 5	39 \pm 8	62 \pm 8	34 \pm 5	40 \pm 5
ADX + CORT	4	47 \pm 8	24 \pm 3	56 \pm 4	36 \pm 5	34 \pm 3
ADX + solvent	8	60 \pm 9	47 \pm 7	69 \pm 14	46 \pm 5	45 \pm 7
ADX + CORT	8	76 \pm 14	50 \pm 6	69 \pm 3	42 \pm 6	42 \pm 6
ADX + solvent	24	115 \pm 7	48 \pm 3	82 \pm 14	64 \pm 7	48 \pm 6
ADX + CORT	24	88 \pm 3 *	48 \pm 7	73 \pm 8	57 \pm 9	35 \pm 6
<i>fosB</i> mRNA						
ADX + solvent	0.8	75 \pm 7	89 \pm 5	103 \pm 4	59 \pm 5	75 \pm 4
ADX + CORT	0.8	59 \pm 4	54 \pm 5 **	68 \pm 7 *	45 \pm 5	37 \pm 4 **
ADX + solvent	2	88 \pm 7	96 \pm 3	113 \pm 8	74 \pm 9	83 \pm 7
ADX + CORT	2	96 \pm 7	118 \pm 10	125 \pm 8	98 \pm 9	102 \pm 8
ADX + solvent	4	80 \pm 5	103 \pm 6	128 \pm 11	93 \pm 9	98 \pm 11
ADX + CORT	4	75 \pm 9	85 \pm 13	105 \pm 24	71 \pm 15	76 \pm 12
ADX + solvent	8	57 \pm 8	76 \pm 6	86 \pm 13	59 \pm 6	68 \pm 6
ADX + CORT	8	46 \pm 5	68 \pm 11	88 \pm 11	58 \pm 5	74 \pm 8
ADX + solvent	24	76 \pm 12	89 \pm 14	121 \pm 20	85 \pm 18	65 \pm 11
ADX + CORT	24	33 \pm 6	38 \pm 5 *	57 \pm 10	33 \pm 6	28 \pm 5
<i>fra-1</i> mRNA						
ADX + solvent	0.8	n.d.	n.d.	n.d.	n.d.	79 \pm 7
ADX + CORT	0.8	n.d.	n.d.	n.d.	n.d.	49 \pm 5 *
ADX + solvent	2	n.d.	n.d.	n.d.	n.d.	95 \pm 8
ADX + CORT	2	n.d.	n.d.	n.d.	n.d.	83 \pm 10
ADX + solvent	4	n.d.	n.d.	n.d.	n.d.	64 \pm 6
ADX + CORT	4	n.d.	n.d.	n.d.	n.d.	47 \pm 10
ADX + solvent	8	n.d.	n.d.	n.d.	n.d.	69 \pm 8
ADX + CORT	8	n.d.	n.d.	n.d.	n.d.	68 \pm 10
ADX + solvent	24	n.d.	n.d.	n.d.	n.d.	87 \pm 11
ADX + CORT	24	n.d.	n.d.	n.d.	n.d.	83 \pm 16
<i>junB</i> mRNA						
ADX + solvent	0.8	97 \pm 6	192 \pm 9	112 \pm 9	90 \pm 6	98 \pm 2
ADX + CORT	0.8	145 \pm 12 *	194 \pm 10	122 \pm 5	110 \pm 7	98 \pm 6
ADX + solvent	2	63 \pm 3	151 \pm 6	125 \pm 10	96 \pm 2	109 \pm 3
ADX + CORT	2	48 \pm 4	101 \pm 10 *	85 \pm 6 *	72 \pm 6 *	91 \pm 5
ADX + solvent	4	62 \pm 4	122 \pm 6	94 \pm 7	72 \pm 9	104 \pm 9
ADX + CORT	4	75 \pm 11	157 \pm 16	139 \pm 8 *	100 \pm 6	141 \pm 7
ADX + solvent	8	81 \pm 7	195 \pm 15	152 \pm 11	108 \pm 6	125 \pm 9
ADX + CORT	8	90 \pm 8	186 \pm 10	149 \pm 15	111 \pm 9	116 \pm 9
ADX + solvent	24	74 \pm 2	175 \pm 12	143 \pm 13	124 \pm 7	121 \pm 3
ADX + CORT	24	72 \pm 4	180 \pm 6	148 \pm 13	94 \pm 5 *	107 \pm 2 *
<i>c-jun</i> mRNA						
ADX + solvent	0.8	65 \pm 10	95 \pm 17	132 \pm 20	111 \pm 11	197 \pm 30
ADX + CORT	0.8	58 \pm 6	88 \pm 11	108 \pm 13	95 \pm 9	135 \pm 13

	time	SI	CA1	CA3	CA4	DG
ADX + solvent	2	65 ± 6	112 ± 8	159 ± 9	119 ± 7	202 ± 11
ADX + CORT	2	74 ± 6	102 ± 12	140 ± 23	116 ± 6	156 ± 15
ADX + solvent	4	58 ± 5	87 ± 10	125 ± 11	98 ± 12	165 ± 12
ADX + CORT	4	66 ± 5	96 ± 6	122 ± 9	99 ± 8	150 ± 9
ADX + solvent	8	58 ± 9	111 ± 15	153 ± 12	106 ± 7	192 ± 15
ADX + CORT	8	75 ± 3	101 ± 9	128 ± 16	99 ± 11	155 ± 12
ADX + solvent	24	39 ± 3	65 ± 8	107 ± 6	66 ± 6	123 ± 12
ADX + CORT	24	35 ± 3	51 ± 14	109 ± 17	59 ± 11	100 ± 20
<i>egr-1 mRNA</i>						
ADX + solvent	0.8	176 ± 10	234 ± 14	125 ± 12	127 ± 5	78 ± 5
ADX + CORT	0.8	195 ± 14	281 ± 7	149 ± 7	133 ± 13	68 ± 6
ADX + solvent	2	180 ± 9	268 ± 10	155 ± 7	137 ± 9	97 ± 6
ADX + CORT	2	137 ± 13	218 ± 18	125 ± 11	115 ± 8	73 ± 5
ADX + solvent	4	89 ± 6	173 ± 8	124 ± 10	108 ± 10	83 ± 4
ADX + CORT	4	78 ± 13	195 ± 17	118 ± 4	92 ± 7	89 ± 4
ADX + solvent	8	99 ± 17	248 ± 20	129 ± 3	102 ± 3	74 ± 7
ADX + CORT	8	115 ± 15	238 ± 14	116 ± 7	97 ± 8	123 ± 29
ADX + solvent	24	191 ± 15	307 ± 9	146 ± 23	121 ± 2	59 ± 2
ADX + CORT	24	150 ± 8	297 ± 32	142 ± 9	124 ± 7	74 ± 2**

Table 3
Effects of injection stress on IEG expression in the dorsal hippocampus of ADX rats

Data are expressed as optical densities ($OD \times 10^{-3}$, mean \pm S.E.M.), statistically analyzed by two-way ANOVA (time and treatment) followed by Fisher's PLSD test and Bonferroni's correction; n=4/group; There are no statistically significant differences between hormone solvent-injected (ADX + solvent) and non-injected ADX rats at the same time point for each transcript. CA1-CA4: Cornus of Ammon areas, DG: Dentate Gyrus, S1: primary somatosensory barrelfield cortex. For details on treatment, see *Material and Methods*.

	time	S1	CA1	CA3	CA4	DG
<i>fosB</i> mRNA						
ADX	0.8	296 \pm 16	481 \pm 30	617 \pm 23	430 \pm 14	450 \pm 31
ADX + solvent	0.8	278 \pm 40	384 \pm 62	488 \pm 86	385 \pm 47	346 \pm 59
ADX	4	258 \pm 16	373 \pm 4	501 \pm 2	389 \pm 21	366 \pm 4
ADX + solvent	4	295 \pm 30	453 \pm 40	579 \pm 67	466 \pm 34	457 \pm 36
ADX	24	311 \pm 10	430 \pm 27	578 \pm 14	455 \pm 28	416 \pm 25
ADX + solvent	24	314 \pm 52	476 \pm 58	623 \pm 31	517 \pm 35	514 \pm 31
<i>junB</i> mRNA						
ADX	0.8	38 \pm 5	107 \pm 10	78 \pm 8	60 \pm 10	63 \pm 17
ADX + solvent	0.8	57 \pm 9	89 \pm 11	57 \pm 12	41 \pm 3	40 \pm 7
ADX	4	13 \pm 4	70 \pm 9	51 \pm 2	39 \pm 4	44 \pm 10
ADX + solvent	4	20 \pm 2	79 \pm 6	44 \pm 13	54 \pm 8	59 \pm 8
ADX	24	26 \pm 4	77 \pm 4	53 \pm 6	45 \pm 6	43 \pm 3
ADX + solvent	24	24 \pm 8	66 \pm 3	53 \pm 7	43 \pm 5	40 \pm 5
<i>c-jun</i> mRNA						
ADX	0.8	58 \pm 12	102 \pm 15	123 \pm 13	89 \pm 11	175 \pm 23
ADX + solvent	0.8	66 \pm 3	94 \pm 18	99 \pm 10	103 \pm 14	165 \pm 28
ADX	4	75 \pm 1	136 \pm 11	110 \pm 9	111 \pm 10	201 \pm 19
ADX + solvent	4	74 \pm 4	121 \pm 13	151 \pm 26	119 \pm 15	185 \pm 12
ADX	24	74 \pm 1	108 \pm 6	114 \pm 10	106 \pm 2	158 \pm 4
ADX + solvent	24	66 \pm 3	112 \pm 10	152 \pm 22	116 \pm 13	171 \pm 23
<i>egr-1</i> mRNA						
ADX	0.8	105 \pm 5	176 \pm 9	101 \pm 9	95 \pm 3	61 \pm 7
ADX + solvent	0.8	114 \pm 10	172 \pm 6	102 \pm 6	81 \pm 7	53 \pm 5
ADX	4	50 \pm 4	111 \pm 17	76 \pm 13	66 \pm 3	56 \pm 1
ADX + solvent	4	54 \pm 4	110 \pm 18	70 \pm 10	65 \pm 5	50 \pm 10
ADX	24	58 \pm 3	159 \pm 5	81 \pm 5	67 \pm 6	47 \pm 2
ADX + solvent	24	70 \pm 9	174 \pm 14	104 \pm 17	87 \pm 8	62 \pm 8