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The anxiolytics CI-988 and chlordiazepoxide fail to reduce immediate early gene mRNA stimulation following exposure to the rat elevated X-maze

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Abstract

This study uses immediate early transcription factor gene expression to map neuronal activation after a single exposure to the elevated X-maze. Exposure to this novel environment leads to widespread upregulation in the gene expression of c-*fos*, NGFI-A and NGFI-B (the nerve growth factor induced genes), but not c-*jun* nor *jun* B as shown by in situ hybridization and northern blot analysis. Changes in c-*fos* were evident after just 5 min exposure to the maze. The cholecystokinin$_B$ receptor antagonist, CI-988, given intraperitoneally at 1 mg/kg 40 min prior to exposure to the X-maze demonstrated an anxiolytic profile without affecting overall movement around the maze, however it did not reduce the increased levels of gene expression with the methodology used. Likewise the anxiolytic benzodiazepine, chlordiazepoxide at 3 mg/kg did not reduce gene expression. It is concluded that a reduction in an index of behavioural stress/anxiety produced by anxiolytic agents is not concomitantly followed by a detectable reduction in immediate early gene induction.

Keywords: Anxiety; Immediate early gene; Elevated X-maze; CCK (cholecystokinin); CI-988; Benzodiazepine

1. Introduction

The elevated X-maze, as first described by Pellow et al. (1985), is an ethological animal model based on the animals natural fear of heights/open spaces. It is frequently used to measure stress and anxiety, and to assess the potential anxiolytic/anxiogenic properties of administered substances in rodents. It is known that anxiolytic benzodiazepines are active on the elevated X-maze (Pellow et al., 1985). Recently the cholecystokinin$_B$ (CCK_B) receptor antagonist CI-988 has demonstrated an anxiolytic profile in this model (Hughes et al., 1990; Singh et al., 1991a), as well as in other ethological models such as the mouse black and white box (Singh et al., 1991a). However, it demonstrates limited activity in conflict models such as the squirrel monkey punished response test (Powell and Barrett, 1991). Additionally, other studies using CI-988 and the other CCK_B receptor antagonists, L-365,260 and LY 262691 demonstrate a reversal of the anxiogenic properties of CCK receptor agonists (Costall et al., 1991; Palmour et al., 1991; Powell and Barrett, 1991; Singh et al., 1991b), suggesting that CCK plays a role in responses to anxiety/stress via the CCK_B receptor subtype. Recent reports that both CI-988 and L-365,260 reduce the frequency and severity of CCK-4-induced panic attacks in humans (Bradwejn et al., 1992, 1994) has increased interest in the possible therapeutic potential of these agents, especially as these CCK_B receptor antagonists do not have the addictive and sedative problems associated with benzodiazepines which are frequently used therapeutically.

The recent discovery of a number of proteins responsible for the regulation of transcription such as Fos (Curran and Teich, 1982) has lead to new methods to study patterns of neuronal activation. Of importance to this particular study are the immediate early genes: c-*fos*, c-*jun*, *jun* B, NGFI-A and NGFI-B whose patterns of gene expression often agree with patterns of neuronal activation as determined by other methods such as positron emission tomography (Phelps et al., 1982; Sokoloff, 1984). Recently, Graeff et al. (1993) have reported that exposure to the elevated X-maze causes neuronal activation of brain areas associated with the limbic system as determined by changes in Fos immunoreactivity. Additionally, other rodent models of stress such as acute restraint, bright open

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field exposure and acute swim stress have been shown to increase the gene expression of a range of transcription factors (Watanabe et al., 1994; Handa et al., 1993; Cullinan et al., 1995). Further studies using restraint at later time points have shown changes in hypothalamic peptide gene expression (Bartanusz et al., 1993; Harbuz et al., 1994; Iglesias et al., 1992), indicating that a brief period of acute stress can lead to plastic neuronal responses.

This present study examines the effects of a single exposure to the elevated X-maze on changes in the immediate early gene mRNAs for: c-*fos*, c-*jun*, *jun* B, NGFI-A and NGFI-B in the rat brain using in situ hybridization and northern blot analysis. It then uses in situ hybridization to examine the effect of the anxiolytic agents CI-988 and chlordiazepoxide on the observed changes in immediate early gene expression.

2. Materials and methods

2.1. Animals

Male Hooded-Lister rats of weight 250–300 g were used (Interfauna, Huntingdon, Cambs, UK). Prior to experimentation animals were housed in groups of 5, maintained in constant 12 h light/12 h dark lighting cycle (on 07:00/off 19:00) with controlled temperature and humidity, and allowed access to food (laboratory chow) and water ad libitum. Animals were placed in the experimental room 24 h prior to the start of the experiment to allow them to acclimatize. [In total 72 animals were used in this study and all experiments were conducted in accordance with the animal scientific procedures act, 1986.]

2.2. Drug administration

For each experiment animals were separated into 2 groups. Within each group half the animals received drug and the other half vehicle intraperitoneally (i.p.). 40 min later one group was exposed to the X-maze for 5 or 30 min whilst the other stayed in their home cage.

2.3. Rat elevated X-maze

A standard elevated X-maze (Handley and Mithani, 1984; Pellow et al., 1985) was automated as previously described (Field et al., 1991). The animals were placed on the centre of the X-maze facing one of the open arms, and the number of entries and time spent on the end half of the open arms was measured for the first 5 min period (Costall et al., 1991; Singh et al., 1991a). Total arm entries were recorded as a measure of locomotor activity around the maze. Percentages are calculated as: (time or entries on ends of open arms)/(time or entries on ends of both open and closed arms).

Animals in the 5 min group were then removed from the maze and returned to their home cage for a further 25 min. The other experimental groups were allowed to explore the maze for a total of 30 min. Animals were killed by decapitation and the brains rapidly removed, frozen at -30°C in *n*-pentane and stored at -70°C prior to sectioning. Statistical analysis of behavioural parameters were performed using the Mann-Whitney U-test. $P < 0.05$ was taken as the minimum level of statistical significance.

2.4. In situ hybridization

In situ hybridization was carried out using the standard protocol described previously (Hinks et al., 1995). Briefly sections (10 μm) were cut at -20°C on a Bright cryostat at approximate bregma -1.8 mm or -7.3 mm (Paxinos and Watson, 1986) and thaw mounted onto sterile poly-L-lysine-coated slides. The sections were fixed using 4% paraformaldehyde in phosphate-buffered saline, rinsed twice in 1 × phosphate-buffered saline, dehydrated in 70% ethanol and stored in 95% ethanol at 4°C until use. Slides containing control and experimental sections were hybridized overnight in hybridization buffer and [^{35}S]deoxyadenosine 5′-triphosphate-labelled oligonucleotide probes diluted to a concentration of 3000 cpm/μl hybridization buffer. Sections were covered with parafilm and hybridized at 42°C in a humid atmosphere. Excess unbound probe was removed using the following stringency washes: 1 × standard sodium citrate buffer at room temperature for 30 min, then 1 × standard sodium citrate at 55°C for 30 min. Slides were finally rinsed in 1 × standard sodium citrate, dehydrated in ethanol and air dried. Sections from control and experimental animals were then apposed to the same sheet of Hyperfilm-Betamax for a predetermined time, prior to developing the film under standardized conditions. Non-specific binding was determined using 100-fold excess of unlabelled probe. The length of time that sections were opposed to film was dependent on the mRNA under study, but once determined this time was kept constant for that particular probe. Care was taken to standardize procedures for control and experimental sections at all stages.

2.5. RNA extraction

RNA was extracted from whole rat brain according to the method of Sambrooke et al. (1989). Brains were homogenized (Polytron homogenizer) in guanidium thiocyanate buffer, and the total RNA was pelleted by centrifugation on a caesium chloride cushion at 32 000 rpm for 24 h at 20°C (Beckman L8-70M ultracentrifuge with SW41Ti swinging-bucket rotor). After resuspension in Tris-EDTA buffer containing 0.1% sodium dodecyl sulphate, the RNA was reprecipitated in 100% ethanol and sodium acetate at 4°C overnight, and collected by a final centrifugation at 12 000 × *g* at 4°C for 10 min.

Table 1
Results of in situ hybridisation showing the effect of CI-988 on c-*fos* mRNA expression in different areas of the rat brain after 30 min exposure to the elevated X-maze

Treatment	ROD units				
	Piriform cortex	Cingulate cortex	Amygdala	Dentate gyrus	Hippocampus CA1
Control	20 ± 7	13 ± 2	10 ± 2	4 ± 1	4 ± 2
X-maze	42 ± 2 [a]	31 ± 7 [a]	22 ± 5 [a]	16 ± 7 [a]	5 ± 4
CI-988	20 ± 3	15 ± 1	11 ± 2	9 ± 2	10 ± 3
X-maze + CI-988	46 ± 3 [a]	54 ± 3 [b]	45 ± 6 [b]	43 ± 3 [b]	31 ± 4 [b]

Statistical analysis was performed using the Mann-Whitney U-test. $n = 5$. $P < 0.05$. [a] Different from control level, [b] different from CI-988 controls and X-maze groups.

2.6. RNA gel electrophoresis

The concentration of RNA was determined by spectrophotometer, diluted to 20 μg/4.5 μl in diethyl pyrocarbonate-treated water, and then 4.5 μl loaded per lane. This was separated using a denaturing 1% agarose/formaldehyde gel (Sambrooke et al., 1989) in 1 × 3-[*N*-morpholino]propanesulphonic acid buffer along with standard RNA markers. Ethidium bromide was added to the gel in order to visualize the RNA after electrophoresis. The samples were electrophoresed at 60 V for 2.5 h (Biorad submarine gel electrophoresis tank), and the resulting gel was photographed under ultraviolet light against a fluorescent ruler (Polaroid Land camera and Polaroid type 57 film).

2.7. Northern blotting

The separated RNA was transferred overnight from the gel onto a nitrocellulose membrane by capillary elution, using 10 × standard sodium citrate buffer. The membrane was baked under vacuum for 2 h at 80°C to fix the RNA to the membrane.

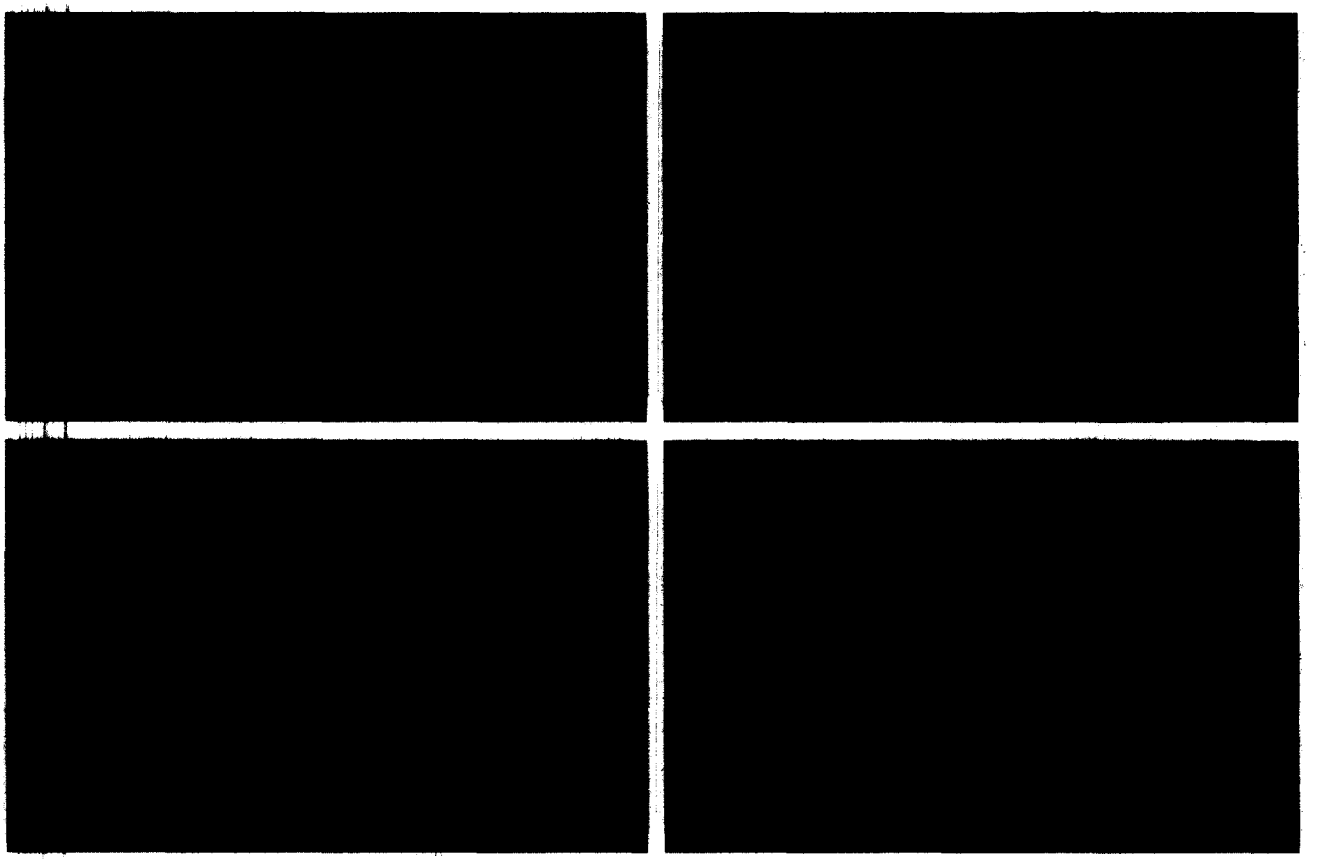

Fig. 1. Autoradiographs following in situ hybridization of mRNA encoding for c-*fos* mRNA in rat brain coronal sections taken at bregma −1.8 mm and −7.3 mm (Paxinos and Watson, 1986). Results are presented for naive and X-maze exposed rats as follows: (a) naive at bregma −1.8 mm, (b) X-maze at bregma −1.8 mm, (c) naive at bregma −7.3 mm, (d) X-maze at bregma −7.3 mm. Cg = cingulate cortex, Cx = cortex, DCG = dorsal central gray, DG = dentate gyrus, Hi = hippocampus, Pag = periaqueductal gray, Pir = piriform cortex. Scale bar = 2 mm.

Table 2
Changes in immediate early gene expression following 5 or 30 min exposure to the X-maze, measured from whole brain RNA extracts by northern blot analysis

mRNA	ROD units		
	Cage only	5 min on X-maze	30 min on X-maze
c-*fos*	0.07 ± 0.01	0.09 ± 0.01 [a]	0.13 ± 0.01 [a]
c-*jun*	0.11 ± 0.2	0.12 ± 0.02	0.13 ± 0.02

Statistical analysis was performed using the Mann-Whitney U-test. $n = 4$. $P < 0.05$. [a] Different from control level.

2.8. Hybridization of northern blots

After prehybridizing membranes in hybridization buffer overnight (Hybaid mini hybridization oven), [^{32}P]-labelled oligonucleotide probe was added at a concentration of 3×10^6 cpm/ml hybridization buffer and hybridized at 42°C overnight. Membranes were washed in 1 × standard sodium citrate/0.1% sodium dodecyl sulphate for 30 min at room temperature, followed by 1 × standard sodium citrate/0.1% sodium dodecyl sulphate for 30 min at 55°C.

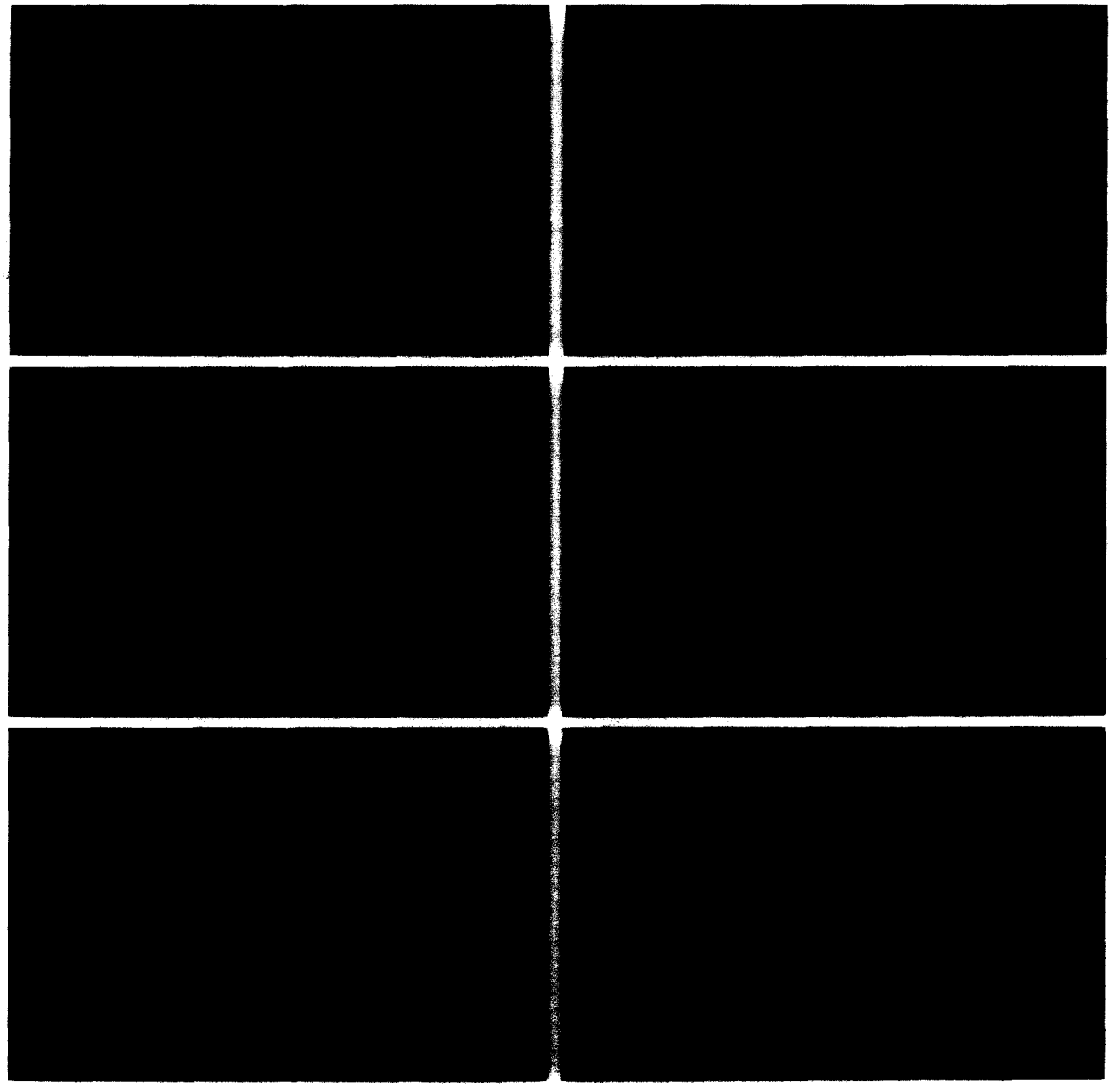

Fig. 2. Autoradiographs following in situ hybridization of mRNA encoding for c-*jun*, NGFI-A and NGFI-B in rat brain coronal sections taken at bregma −1.8 mm (Paxinos and Watson, 1986). Results are presented for naive and X-maze exposed rats as follows: (a) naive, c-*jun* mRNA, (b) X-maze, c-*jun* mRNA, (c) naive, NGFI-A mRNA, (d) X-maze NGFI-A mRNA, (e) naive, NGFI-B mRNA, (f) X-maze NGFI-B mRNA. ACe = central nucleus of the amygdala, Cx = cortex, DG = dentate gyrus, Hi = hippocampus, Pir = piriform cortex. Scale bar = 2 mm.

After wrapping in clingfilm, membranes were exposed to X-ray film (Kodak X-OMAT AR) at −70°C for 7 days.

2.9. Oligonucleotide probes

Oligonucleotide probes were made using an Applied Biosystems DNA synthesizer. After deprotection at 55°C for 16 h the probes were dried in a Speed Vac centrifuge and dissolved in sterile water to give a stock solution of 20 ng/μl. They were then labelled at the 3′ end using terminal deoxynucleotidyl transferase (New England Nuclear) and [^{35}S]deoxyadenosine 5′-triphosphate (1000 Ci/mmol) or [^{32}P]deoxyadenosine 5′-triphosphate (3000 Ci/mmol). The radiolabelled probe was purified using Sephadex columns (Biospin6) and dithiothreitol added to a final concentration of 50 mM for [^{35}S]deoxyadenosine 5′-triphosphate-labelled probes. The following oligonucleotide sequences were used: c-*fos*, complementary to nucleotides spanning amino acids 1–15 (Curran et al., 1987), c-*jun*, complementary to nucleotides spanning the last 20 amino acids (Angel et al., 1988), *jun* B complementary to nucleotides spanning the last 20 amino acids (Ryder et al., 1988), NGFI-A, complementary to nucleotides spanning amino acids 2–16 (Milbrandt, 1987) and NGFI-B, complementary to nucleotides spanning amino acids 1–15 (Milbrandt, 1988).

2.10. Analysis of autoradiographical data

Analysis of both in situ hybridization autoradiograms and northern blots were carried out using a MCID image analyzer (model M4) to measure relative optical density values (ROD). Measurements were taken within the linear range of optical density levels. In situ hybridization results are expressed in arbitrary units (ROD's X100). Measurements were taken in groups from at least 5 animals with 3–6 sections measured per animal. The data from each animal was processed independently on the MCID and then statistically analyzed using the non-parametric Mann-Whitney U-test. $P < 0.05$ was taken as the minimum level of statistical significance.

2.11. Drugs

The following drugs were used: CI-988:[*R*-(*R**,*R**)]-4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo-[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxobutanoic acid compound with 1-deoxy-1-(methylamino)-D-glucotil (1 : 1) was synthesised at Parke Davis Neuroscience Research Centre. Chlordiazepoxide hydrochloride was obtained from Sigma Chemical Co. CI-988 was dissolved in 0.9% saline, and chlordiazepoxide was suspended in 1.0% w/v carboxymethylcellulose with the aid of ultrasonification. CI-988 (1 mg/kg i.p.) and chlordiazepoxide (3 mg/kg i.p.) were administered 40 min before testing in a volume of 1 ml/kg. These doses are known to induce anxiolytic-like activity in the X-maze (Singh et al., 1991a). Previous indications are that any higher dose of chlordiazepoxide can have sedative effects on locomotor activity (Singh et al., 1991a).

3. Results

3.1. Effect of 5 and 30 min exposure to the elevated X-maze on immediate early gene expression in the rat

Rats allowed to explore the elevated X-maze for either 5 or 30 min showed significant increases in c-*fos* mRNA in a number of brain areas as seen by in situ hybridization (Table 1, Fig. 1). Sections taken at the level of the hypothalamus showed significant increases in the cingulate cortex, piriform cortex, thalamus, amygdala and dentate gyrus. Sections taken more caudally also demonstrated increases in the dorsal central gray and periaqueductal gray (Fig. 1). Although these changes were apparent after a 5 min exposure they were much greater after a 30 min exposure (Table 2). In addition to c-*fos*, in situ hybridization was performed for c-*jun*, *jun* B, NGFI-A and NGFI-B

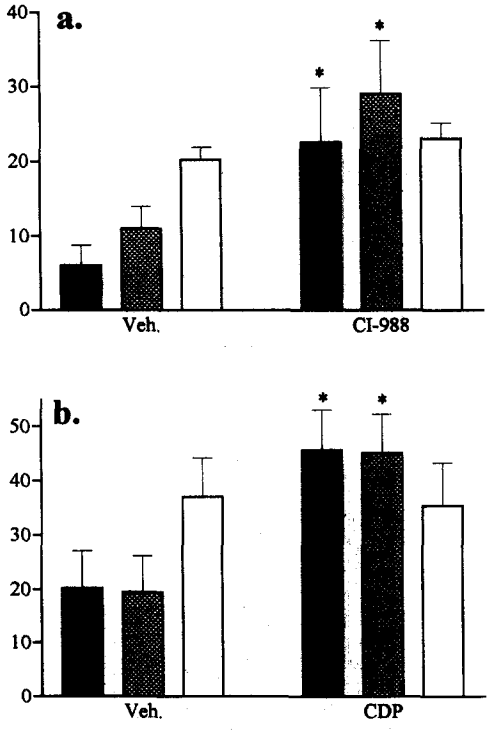

Fig. 3. Effect of (a) CI-988 and (b) CDP in the rat elevated X-maze. CI-988 (1 mg/kg), CDP (3 mg/kg) or their respective vehicles (veh) were administered i.p. 40 min before testing. The percentage time spent (black columns) and entries (shaded columns) on to the end half sections of the open arms, and the total number of entries (open columns) were measured during a 5 min test. Results are shown as the mean (vertical bars represent ± S.E.M.) of 9–10 animals per group. * Significantly different from vehicle-treated controls. $P < 0.05$ (Mann-Whitney U-test).

mRNAs. Of these NGFI-A and NGFI-B mRNAs showed a similar pattern of upregulation to c-*fos*, with increases observed after a 30 min exposure to the X-maze (Fig. 2). However, no significant increases were observed in these mRNAs after just 5 min exposure. No changes in c-*jun* (Fig. 2) or *jun* B expression was observed at either time point. This anatomical pattern of expression is in agreement with that seen for Fos immunoreactivity after 15 min exposure to the X-maze as reported by Graeff et al. (1993). Additionally, significant increases in c-*fos* expression was observed when RNA from whole brain extracts were examined by northern blot analysis (Table 2). Again changes were not detected in c-*jun* mRNA by this method in agreement with the in situ hybridization studies.

3.2. Anxiolytic-like effects of CI-988 in the rat elevated X-maze

CI-988 (1 mg/kg i.p.) was given 40 min prior to a 5 min period of automated behavioural scoring as described by Field et al. (1991). The percentage of time spent on the ends of the open arms and the percentage of entries on to the ends of the open arms of the maze were significantly increased compared with vehicle-injected controls (Fig. 3a). This agrees with previous reports of anxiolytic effects of CI-988-injected rats on the elevated X-maze (Hughes et al., 1990; Singh et al., 1991a,b). Further, the number of total entries into any arm of the maze was unchanged by CI-988, indicating that there was no change in overall locomotor activity (Fig. 3a).

3.3. Anxiolytic-like effects of chlordiazepoxide in the rat elevated X-maze

Likewise chlordiazepoxide produced an anxiolytic profile during the first 5 min on the X-maze with a significant increase in the percentage of time and percentage of entries on to the ends of the open arms (Fig. 3b), in agreement with previous studies (Pellow et al., 1985). Again there was no evidence of a change in overall activity around the maze in response to the compound at the 3 mg/kg dose as compared with vehicle-treated animals, indicating that at the dose used animals did not exhibit sedation.

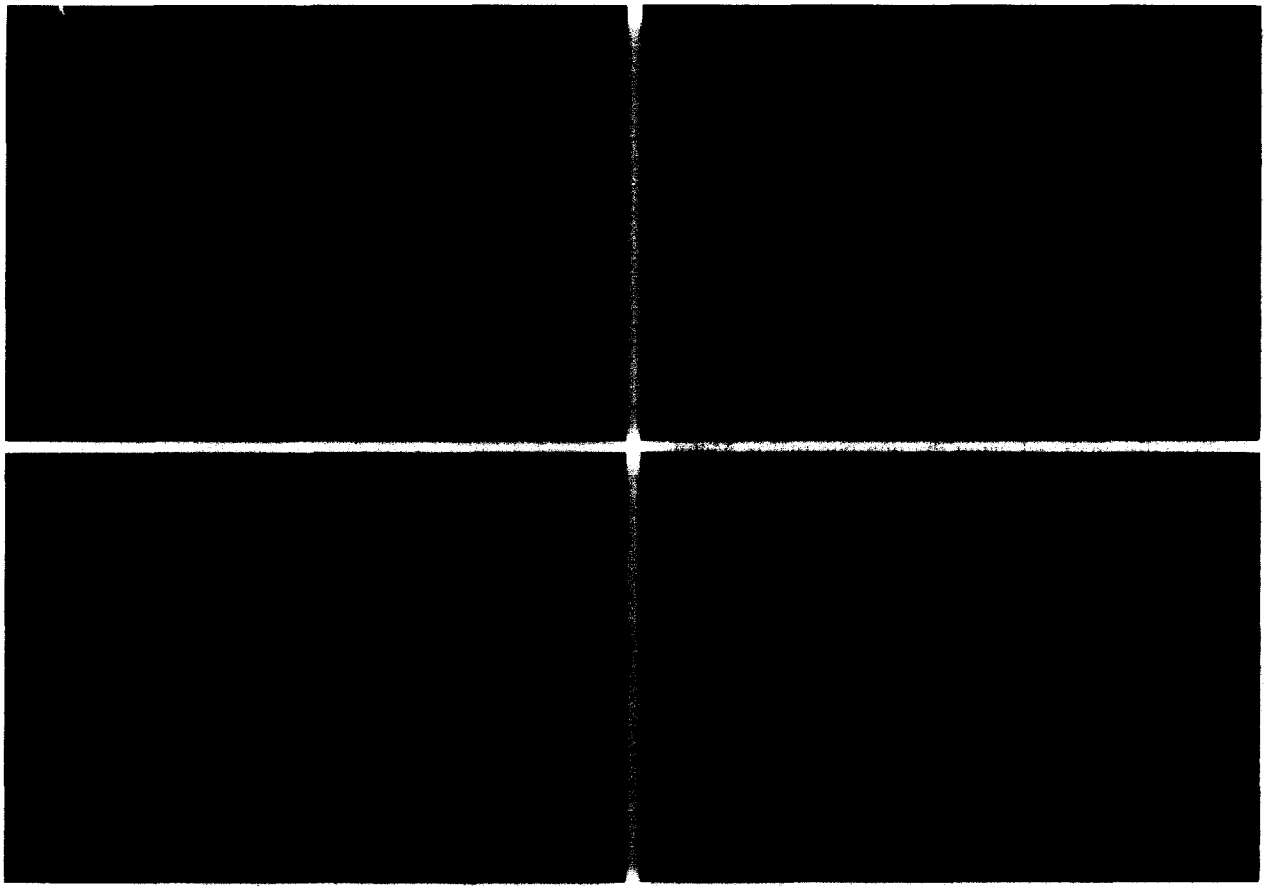

Fig. 4. Autoradiographs following in situ hybridization of mRNA encoding for c-*fos* in rat brain coronal sections taken at bregma −1.8 mm (Paxinos and Watson, 1986). Results are presented to demonstrate the lack of effect of chlordiazepoxide on gene expression after exposure to the X-maze (a) vehicle-treated, remained in home cage (b) vehicle-treated, 30 min on X-maze (c) chlordiazepoxide-treated, remained in home cage (d) chlordiazepoxide-treated, 30 min exposure to the X-maze. Cx = cortex, Hi = hippocampus, Pir = piriform cortex, Th = thalamus. Scale bar = 2 mm.

3.4. Effect of CI-988 on immediate early gene expression after exposure to the elevated X-maze

The effect of an acute dose of CI-988, 40 min prior to exposure to a 30 min exposure to the X-maze on c-*fos* expression is shown in Table 1. A potentiation in the levels of c-*fos* expression in the cingulate cortex, amygdala, CA1 and dentate gyrus were observed compared with rats exposed to the X-maze in the absence of CI-988. An increase in the levels of c-*fos* mRNA in the CA1 region of the hippocampus was also observed, despite the lack of significant upregulation in this area after exposure to the X-maze without CI-988. The piriform cortex showed no potentiation by CI-988 of c-*fos* mRNA above levels seen after exposure to the X-maze. Again a similar pattern was seen after CI-988 for NGFI-A and NGFI-B mRNAs (data not shown). CI-988 administered to control rats (not exposed to the X-maze) had no detectable effect on any of the immediate early genes studied with the methodology employed.

3.5. Effect of chlordiazepoxide on immediate early gene expression after exposure to the elevated X-maze

Chlordiazepoxide had no detectable effect on the levels of gene expression of the immediate early genes: c-*fos*, c-*jun*, NGFI-A or NGFI-B in any of the brain areas studied with the methodology employed (Fig. 4).

4. Discussion

This study demonstrates for the first time that a range of immediate early gene mRNAs are upregulated in response to exposure to the rat elevated X-maze, which is a frequently used animal model of anxiety. Exposure to the X-maze is a relatively mild stimulus when compared with that used in some of the earlier studies looking at upregulation of these genes such as convulsive agents (Morgan et al., 1987), ischaemia (Onodera et al., 1989) or peripheral application of CCK (Day et al., 1994). Although these are known to produce much larger immediate early gene responses than are seen here, this work does emphasise the sensitive nature of the immediate early gene response, implying a physiological role in novel environment exploration or adaptation. Additionally, these observations are in agreement with other studies of Fos/AP-1 induction in response to a novel environment (Handa et al., 1993; Kinney and Routtenberg, 1993). Neither of the anxiolytic agents CI-988 nor chlordiazepoxide, both of which demonstrate an anxiolytic behavioural profile in this model, exert their anxiolytic effects by reducing the levels of immediate early gene expression in the brain. Since the upregulation of gene expression is anatomically widespread, in fact sufficiently so to be readily detectable by northern blot analysis using RNA extracted from whole brain, it would seem that this response represents a more generalised upregulation necessitated by the multiple demands being placed on the animal whilst exploring the maze. Thus this study demonstrates that a reduction in a behavioural index of anxiety/stress does not coincide with a detectable reduction in immediate early gene expression. The cingulate cortex (Diorio et al., 1993), amygdala (Davis, 1992), hippocampus (Gray, 1990) and periaqueductal gray (Carrive, 1993) belong to the limbic system of the brain and increased neuronal activation in these areas may be functioning to integrate the fear and mnemotropic components of the response to the exploration of a novel environment. Increased expression of immediate early genes in the piriform cortex (an area receiving input from the olfactory lobe) is possibly of particular interest in the rodent or for any species with a highly developed sense of smell. Olfactory information probably represents a major sensory input for a rat exploring a novel environment, and since the piriform cortex also sends massive sensory input to the amygdala, this area may function to integrate incoming sensory information with limbic responses. Although in situ hybridization studies were carried out at the level of the hypothalamic paraventricular nucleus no consistent upregulation was observed in this area. This is possibly surprising in view of the previously reported increases in circulating glucocorticoids in rats after exposure to the X-maze (File, 1990), which may be produced via the neurohypophyseal system. This indicates that either there are other mechanisms operating to produce adrenal activation or that the response is below the detection limits of the present system.

There has been one previous report of an anxiolytic benzodiazepine, diazepam, dose dependently reducing Fos-like immunoreactivity after conditioned fear in response to footshock (Beck and Fibiger, 1995). As with our novel environment model c-*fos* expression was increased in numerous brain regions after conditioned fear. Possible explanations why the benzodiazepine effects on Fos appear different to those reported here may be that whilst Beck and Fibiger see great reductions in behavioural activity increasing with dose indicating sedation, the dose of CDP used in this study did not affect locomotion around the maze, only the time spent on the ends of the open arms of the maze. Thus it may be that any reduction in Fos produced by CDP is due to the sedative action of this compound. Alternatively, conditioned fear may have different neural components to those in novel environment, such as anticipation of previously experienced pain and associative learning of place and pain, which would not be involved in exploration of a new environment. Thus this discrepancy could be due to the dose of benzodiazepine chosen or that the Fos expression, although appearing similar, may actually reflect very different neural mechanisms/pathways being activated.

Much consideration was given during the course of these experiments to the nature of the control groups.

Comparisons were made between controls left in the home cage, animals handled and then replaced in the home cage and animals placed on the X-maze with the open arms blocked off. This showed that although simple handling or intraperitoneal injection, followed by return to the home cage did not produce any significant upregulation of c-*fos* expression, the animals that were placed on the closed arm maze did show a small response (results not shown). This is presumably because despite the less 'stressful' nature of the maze it still represents a novel environment to the animal. In view of this it was considered most suitable to handle the animals but return them to the home cage so as not to expose them to any novel environment.

The levels of c-*fos* gene expression seen after CI-988 treatment and exposure to the X-maze indicate that the drug has caused a small potentiation of gene expression. However, the lack of constitutive expression of c-*fos* in the brain indicates that the initial increase in response to the maze is probably of much greater functional significance to the animal as this represents an increase from virtually nothing, whereas an approximate doubling of the density levels is probably indicative of the greater exploratory behaviour displayed by the animal. This potentiation was not seen with the benzodiazepine which still displays an anxiolytic profile on the maze. This difference in effect may be a reflection of the different mechanisms by which these two compounds operate. Since there was no observed changes in general locomotor activity around the maze, as indicated by the number of total transitions into all arms of the maze, the observations reported here cannot be explained purely by changes in locomotor activity.

The only gene studied which showed changes after a 5 min exposure to the maze was c-*fos*, with the other genes requiring a longer exposure. Additionally, c-*fos* expression was higher after a longer exposure indicating a relationship between the length of the stimulus and the intensity of the response during the initial first half hour on the maze. Routine behavioural scoring only takes place for the first 5 min on the maze whilst the immediate early gene response follows on after scoring has ceased, so the upregulation of these factors represents a more long term cellular response to the increased neuronal activity, and shows a relationship to the intensity of the stimulus given. However, the failure of either anxiolytic to produce a detectable reduction in this response indicates that the increased time spent on the open arms of the maze is not linked to a detectable decrease in neuronal activation, so that any cellular basis for the changes in behavioural profile on the X-maze remains elusive.

In summary, exposure of rats to the elevated X-maze leads to neuronal activation, as visualised by immediate early gene expression, within areas of the limbic system. This upregulation is seen for c-*fos*, NGFI-A and NGFI-B but not for c-*jun* or *jun* B, indicating an immediate early gene profile for this stimulus. Pretreatment with the CCK_B receptor antagonist CI-988 leads to a small potentiation of the IEG response to the X-maze, possibly due to increased exploratory/inquisitive behaviour. Comparison with the benzodiazepine chlordiazepoxide again shows that there is no block of the response, indicating that this increase in neuronal activation remains elevated despite the changes in the behavioural index as measured by the rat elevated X-maze.

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