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ORIGINAL INVESTIGATION

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Effects of central noradrenaline depletion by the selective neurotoxin DSP-4 on the behaviour of the isolated rat in the elevated plus maze and water maze

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Abstract *Rationale:* Social isolation of the rat from weaning influences behaviour following central noradrenaline (NA) depletion by the selective neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4). *Objectives:* The study characterised the effects of DSP-4 on the behaviour of isolates in the elevated plus maze and water maze. *Methods:* Male Lister hooded rats were reared singly or in groups after weaning. Two weeks postweaning, the rats were injected with DSP-4 (25 mg/kg, i.p.) or saline. From week 4, rats were tested in the plus maze and in the water maze. *Results:* DSP-4 significantly reduced cortical and hippocampal NA but had no effect on hypothalamic NA. Isolation rearing alone had no significant effects on behaviour in the elevated plus maze but enhanced retention of platform placement in the water maze as measured by increased entries to the platform annulus during the probe test. DSP-4 in group-reared rats increased activity in the open arms and increased general activity in the elevated plus maze with no effect on water maze performance. DSP-4-treated isolates spent less time in the open arms and were hypoactive in the plus maze compared to group-reared DSP-4-treated rats, and had impaired retention of spatial memory in the water maze compared to isolate controls. *Conclusions:* DSP-4 treatment had an 'anxiolytic' effect in group-reared rats in the elevated plus maze. In the water maze, isolation rearing enhanced retention of spatial information, an effect normalised by NA depletion. The results demonstrate the importance of noradrenergic function in the regulation of responsiveness to environmental cues.

Keywords Social isolation · *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine · DSP-4 · Noradrenaline · Elevated plus maze · Water maze · Rat

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Introduction

Social isolation of rats after weaning produces behavioural and neurochemical changes in the adult. The most consistent behavioural effect is locomotor hyperactivity in a novel environment as tested in open field or activity cages paradigms (for example, Syme 1973; Jones et al. 1989, 1992; Fulford et al. 1994; Hall et al. 1997; Lapiz et al. 1999b). Other behavioural effects include behavioural rigidity and learning impairments (Juraska et al. 1984; Wade and Maier 1986; Jones et al. 1991) and anxiogenic behaviour profile in the elevated plus maze (Wright et al. 1991; Bickerdicke et al. 1993). Contrary findings have also been reported, such as enhanced water maze performance (Wongwitdecha and Marsden 1996) and non-anxiogenic profile in the elevated plus maze (Fone et al. 1996). Nevertheless, isolation rearing does cause behavioural changes in the adult and some have been attributed to alterations of central dopaminergic and serotonergic neurotransmitter functioning (see Wright et al. 1991; Jones et al. 1992; Bickerdicke et al. 1993; Hall et al. 1999). There is increasing evidence that the central noradrenergic system is also involved. In vivo and in vitro studies showed that isolation-reared rats have enhanced presynaptic terminal α_2-autoreceptor function in the dorsal hippocampus (Fulford et al. 1994; Fulford and Marsden 1997a, b). More recently, we have reported that isolation rearing influences the exploratory behaviour of the rat following central noradrenaline (NA) depletion by the selective neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4; Lapiz et al. 2000a, b).

DSP-4 is an alkylating agent that causes selective destruction of noradrenergic projections (distal axons and

terminals) from the locus coeruleus (Hallman and Jonsson 1984; Dudley et al. 1990). Systemic administration of DSP-4 produces a long-term depletion of brain NA in the mouse and rat without affecting dopamine and adrenaline neurones although a small loss of serotonin has been reported (Ross 1976; Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1981). The central effects of DSP-4 are long lasting while the peripheral NA depletion is transitory with recovery within days (Jaim-Etcheverry and Zieher 1980). These characteristics make DSP-4 a valuable tool for investigating the role of the central noradrenergic system in behaviour although recovery of sympathetic function is recommended prior to behavioural testing (Ross 1985).

The noradrenergic neurones ascending from the locus coeruleus innervate forebrain structures such as the cortex, hippocampus and thalamus (Fillenz 1990). The ascending noradrenergic system has been implicated in attention and arousal although there are conflicting reports on the effects of central NA depletion on aversive behaviours (Mason and Fibiger 1979; Archer et al. 1983, 1984; Dooley et al. 1983a, b; Delini-Stula et al. 1984; Harro et al. 1995). This study investigates the effects of central NA depletion by DSP-4 on the behaviour of group-reared and isolation-reared rats in the elevated plus maze and water maze.

The elevated plus maze is widely used to detect anxiolytic and anxiogenic efficacy of drugs (Pellow et al. 1985) based on the aversive nature of novelty in the rat. 'Anxiogenic' behaviour is considered to result in a reduction of entries and time spent in the open arms while 'anxiolytic' behaviour increases entries and time in the open arms. The water maze is used to test for spatial learning and memory (see Morris 1981, 1984) and can be used to dissociate learning, memory and performance deficits of behaviour (McNamara and Skelton 1993). There is conflicting evidence for the behavioural profile of the isolated rat in the elevated plus maze and water maze. Hence, this study aims to provide further evidence of the profile of these rats in these behavioural paradigms and the effect of DSP-4 treatment on these behaviours.

Materials and methods

The experiments were carried out in accordance with UK Home Office regulations governing experiments on living animals.

Animals and housing conditions

Male Lister hooded rats (Nottingham University Medical School, UK) were obtained after weaning (≥21 days postnatal). The rats (n=24) were randomly divided into two groups and were reared either singly (isolation-reared) or in groups of six (group-reared) in plastic cages. Opaque plastic cages were lined with sawdust and had metal grid tops. Isolation-reared rats were housed in cages 38×22×20 cm high, whereas group-reared rats were housed in cages 48×30×20 cm high. Food and water were available ad libitum. All rats were housed in the same room so visual, auditory and olfactory cues from other rats are maintained in the isolated rats. The room had a constant dark-light cycle (on 0800 hours, off 2000 hours, light 100 lux) and the temperature was controlled at 21±1°C.

Rats were reared in the housing conditions outlined for 2 weeks. After the 2nd week, the rats were tested in the activity photocell cages. The isolates were observed to be hyperactive (see Lapiz et al. 2000a), confirming that the isolation protocol was comparable with previous studies. The rats were then injected with DSP-4 or saline and were returned to their respective cages as above. Two weeks postinjection, the rats were tested for their behavioural profile in the elevated plus maze and cognitive performance in the water maze. All behavioural tests were conducted between 0900 and 1700 hours.

Drug treatment and NA depletion in brain regions

Rats were injected intraperitoneally (i.p.) with either 25 mg/kg DSP-4 (Sigma, UK) or 0.9% saline at 1 ml/kg volume. DSP-4 was dissolved in saline immediately before injection. All rats were given wet-mash and 10% glucose supplement to their diet for 5 days postinjection.

The dose was chosen on the basis of the work of Cheetham et al. (1996) in which DSP-4 (10, 20, 50 and 100 mg/kg, i.p.) dose-dependently depleted cortical NA by 51%, 73%, 100% and 100%, respectively, with no significant effects on dopamine and 5-HT levels. An initial study using male Lister Hooded rats (n=12 weighing 315–380 g) injected with DSP-4 (25 mg/kg, i.p.) or saline was conducted to confirm that DSP-4 at this dose produces adequate cortical NA depletion. Four days after injection, the rats were decapitated and the brain rapidly removed. The cortices were dissected out on ice (Heffner et al. 1980) and snap frozen. NA was measured using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) optimised for the detection of NA. Briefly, tissues were sonicated in 1 ml 0.1 M perchloric acid containing 0.02% 0.15 M $Na_2S_2O_5$ and centrifuged at 14,000 g for 30 min at 4°C. The resulting supernatant was centrifuged in a microcentrifuge at 30,000 g for 4 min. Samples (1:5 dilution; 100 µl sample plus 400 µl H_2O) were injected onto a column (100×2 mm i.d. Phenomenex) packed with 3 µm ODS_{c18} material (Hypersil) via a Rheodyne 7125 sample injector. The mobile phase (pH 4.0) consisted of 0.15 M Na_2HPO_4, 1.0 mM EDTA, 2.8 mM 1-octane sulphonic acid sodium salt and 5% methanol. NA was measured electrochemically using a dual glassy carbon working electrode set at +0.75 V maintained by a Ag/AgCl reference electrode (BAS LC4B cell). Changes in current were detected by a BAS LC4C amperometric detector linked to an integrator (Spectra-Physics Chromjet) for the display of chromatograms.

The same method for measuring brain NA was used for the dissected cortex, hippocampus and hypothalamus of each rat used in this behavioural study 28 days after DSP-4 treatment.

Elevated plus maze

Behaviour was tested on an elevated plus maze made of matt black plastic comprising four arms (45×15 cm) extending perpendicularly from a central square (15×15 cm). Two opposite arms were enclosed (closed arms) at the far end and along the sides (10 cm high wall) while the other opposing arms are open (open arms). The maze was raised 65 cm with 200 lux at the central square. At the start of a trial, the rat was placed in the centre of the maze facing the closed arm and was allowed to explore the arena for 5 min. Activity was captured by a video camera and a computerised tracking system (VideoTrack CPL; Beckett and Marsden 1995). Activity measured included number of open and closed arm entries (all four feet off the central square) and locomotor speed. Risk assessment activities including stretch attend posture (investigating the open arm from a protected compartment in a stretched position) and head dipping (investigating the area beneath the platform) were manually recorded. The apparatus was cleaned after each animal trial with 70% (v/v) ethanol. An alternating schedule

of rearing (group-/isolation-reared) and treatment (DSP-4/saline) testing schedule was observed to maintain consistency of circadian rhythm variation.

Water maze test: place navigation and probe test

The behavioural test used was a modification of the protocol used by Morris (1984). A circular pool (200 cm in diameter with 60 cm wall height) with a featureless white inner surface was filled to depth of 40 cm with 21±1°C water which was opacified with Opacifier E-308. The pool was divided into four equal quadrants: namely, Northwest, Northeast, Southeast and Southwest. The hidden escape platform, consisting of a clear 10×10 cm Plexiglas stand, was submerged in the middle of the Northwest quadrant 1.5 cm below the water surface. The location of the platform was not altered throughout the training sessions. Visual cues consisting of 78×52 cm unique black and white patterns were attached to the centre of two opposing walls of the room. Other visual cues consisted of standard room objects and a curtain. The cues were not changed throughout the experimental period. An alternating testing schedule was observed. All experiments were conducted between 0900 and 1700 hours.

The acquisition phase consisted of 4 consecutive trial days (four trials/day). On the first trial of day 1, the rat was allowed to swim in the water for 90 s. At the end of the first trial, none of the rats found the escape platform so each rat was guided manually to the platform and was allowed to stand on it for 20 s. For the succeeding sessions, the rat was placed in the water facing the pool wall at one of the three randomly determined starting locations. As previously, each rat was given 90 s to find the hidden platform. If the platform was not found within this time period, the rat was guided manually to it and allowed to stand on it for 20 s. The trial was stopped when the rat found the platform before the predetermined time. After each trial, all rats were removed and brought behind the curtain, dried, and given a 30-s rest before the start of the next trial. Latency to find the platform (escape latency) was recorded for each trial.

The transfer or probe test was conducted on the 5th day using the same set-up but with the escape platform removed. Each rat was placed in the pool and was allowed to swim freely for 60 s. The activity of each rat was recorded by a video camera and tracked by a computerised system as above. Activity measured included swimming distance and speed, zone transitions, entries to and time spent in quadrants. To measure memory of exact location of platform, entries made to the platform annulus were also measured.

Statistical analyses

All data are presented as mean (± SEM). Behavioural data from the elevated plus maze were analysed using two-way analysis of variance (ANOVA) with two between-subject factors: rearing condition and drug treatment. To further compare differences between groups, one-way ANOVA was performed with Bonferroni's test for selected pairs as post hoc. Escape latency in the water maze during the 4-day acquisition phase was analysed using two-way ANOVA with two between factors: rearing condition and drug treatment, and one within factor: time. Comparisons of escape latency for each trial day and data from the probe test were analysed using ANOVA with Bonferroni's test as post hoc.

HPLC-ECD data from the initial group of DSP-4 and saline-treated group-reared rats were analysed using unpaired *t*-test. Data from the rats used in this behavioural study was analysed using ANOVA with Bonferroni's test as post hoc. In all statistical tests, a value of $P<0.05$ was considered significant.

Results

Activity in the elevated plus maze 2 weeks after DSP-4

All groups significantly ($P<0.001$) preferred the closed compared to the open arms. Times spent in the closed arms were comparable for all groups but this is not so in the open arms. DSP-4 treatment of grouped rats significantly increased time spent in the open arms compared to group controls ($P<0.05$) and DSP-4 treated isolates ($P<0.01$). This was reflected in the significantly higher percentage time spent in the open arms of DSP-4-treated grouped rats compared to group controls ($P<0.05$) and DSP-4-treated isolates ($P<0.01$; Fig. 1A). Percentage

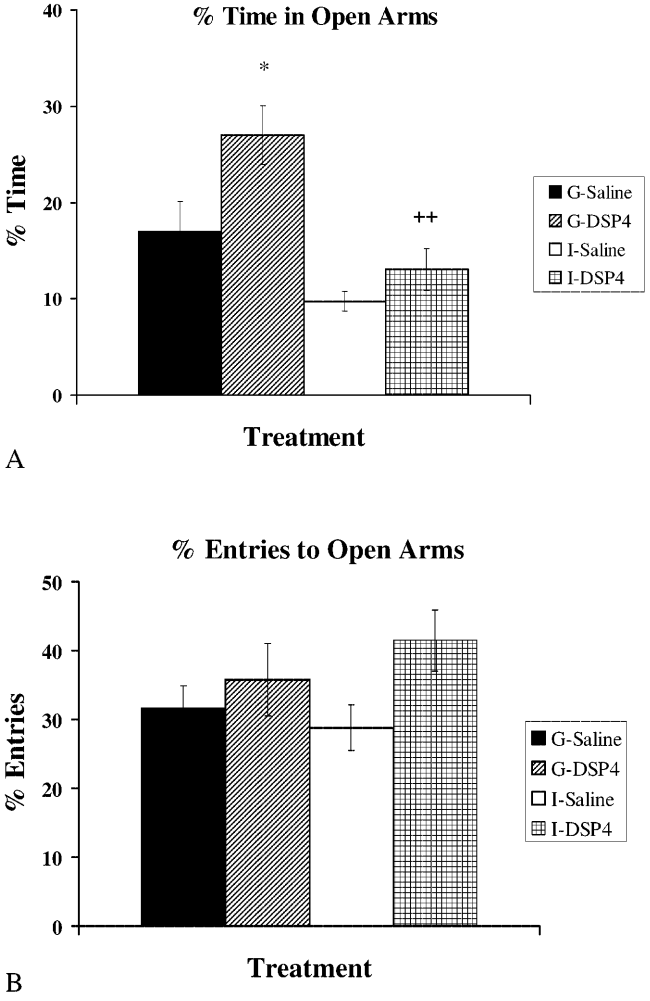
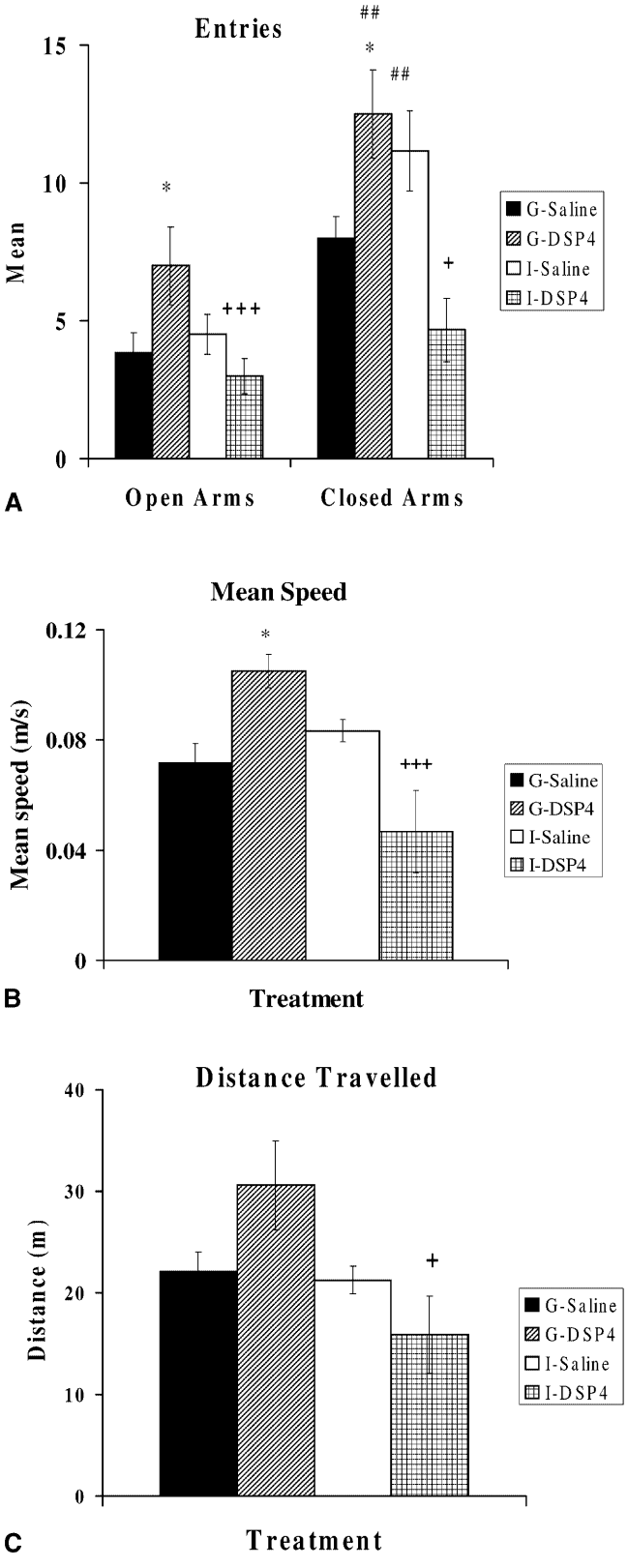


Fig. 1 Mean (± SEM) percentage time (**A**) and entries (**B**) in the open arms of the elevated plus maze of DSP-4 (25 mg/kg, i.p.)- or saline-treated group (*G*)- and isolation (*I*)-reared rats. **A** DSP-4 treatment of grouped rats significantly increased percentage time spent in the open arms compared to group controls [$F(3,20)=9.08$; $P<0.05$] and DSP-4-treated isolates [$F(3,20)=9.08$; $P<0.01$]. **B** Percentage entries into the open arms were comparable for all groups. *$P<0.05$ vs saline controls; ++ $P<0.01$ vs group-reared DSP-4 using ANOVA with Bonferroni's test as post hoc

entries to open and closed arms (Fig. 1B) were comparable for all groups although group-reared DSP-4 rats made more (P<0.05) entries into the open and closed arms compared to saline controls (Fig. 2A). Grouped DSP-4 rats and saline-treated isolates significantly preferred (P<0.01) to enter into the closed arms than into the open arms (Fig. 2A). DSP-4-treated grouped rats had faster (P<0.05) speed compared to group controls (Fig. 2B). Isolation rearing had no significant effects on these measures. DSP-4-treated isolated rats had reduced activity in the elevated plus maze. They have less closed arms entries (P<0.05) compared to both isolate controls and grouped DSP-4-treated rats (Fig. 2A). DSP-4-treated isolation-reared rats also had less entries (P<0.001) into the open arms and less distance travelled (P<0.05; Fig. 2C) and speed (P<0.001; Fig. 2B) compared to DSP-4-treated grouped rats. The stretch attend posture and head dipping activities were comparable for all groups.



Behaviour in the water maze

All treatment groups learned the placement of the platform after 4 days as shown by the significant reduction (P<0.001) in latency to find the escape platform (Fig. 3). There was no significant difference in the performance of treatment groups for all days except for day 2 where DSP-4-treated group-reared rats had significantly (P<0.01) shorter latency in finding the escape platform compared to group controls (Fig. 3).

Data from the probe test show that all rats significantly preferred to swim into the Northwest quadrant, where the platform was previously located, compared to all other quadrants (Fig. 4A). Neither DSP-4 treatment nor isolation rearing had any significant effects on the number of entries made to the Northwest quadrant. Figure 4B shows the number of entries to the platform annulus (see Fig. 4B diagram), a more precise measure of the memory of the platform location. Isolation-reared rats made significantly (P<0.05) more entries to the platform annulus compared to group controls and DSP-4-treated isolates (Fig. 4B), indicating that the isolate controls had a more precise memory of the platform location.

Fig. 2 Mean (± SEM) number of entries into the open and closed arms (**A**), speed (**B**) and distance travelled (**C**) in the elevated plus maze of DSP-4 (25 mg/kg, i.p.)- or saline-treated group (*G*)- and isolation (*I*)-reared rats. **A** Grouped DSP-4 [F(7,40)=9.91; P<0.01] and isolation-reared control rats [F(7,40)=9.91; P<0.01] made significantly more entries to the closed arms than the open arms. DSP-4 treatment of grouped rats significantly increased entries to the open [F(7,40)=9.91; P<0.05] and closed [F(7,40)= 9.91; P<0.05] arms compared to group controls while DSP-4 treatment of isolates significantly decreased these activities compared to grouped rats given DSP-4 [F(7,40)=9.91; P<0.05]. **B** DSP-4 treatment in grouped rats significantly increased speed compared to group controls [F(3,20)=7.02; P<0.05] and DSP-4-treated isolation-reared rats [F(3,20)=7.02; P<0.001]. **C** DSP-4-treated isolates travelled shorter distances compared to group-reared DSP-4-treated rats [F(3,20)=3.76; P<0.05]. *P<0.05 vs saline controls; + P<0.05, +++ P<0.001 vs group-reared DSP-4; ## P<0.01 open vs closed arms using ANOVA with Bonferroni's test as post hoc

Table 1 Noradrenaline levels (ng/g wet weight of tissue, mean ± SEM) in brain regions of group-reared (*G*) and isolation-reared (*I*) rats 28 days after DSP-4 (25 mg/kg) treatment

Brain regions	G-saline	G-DSP-4	Percentage decrease from control	I-saline	I-DSP-4	Percentage decrease from control
Cortex	219.3±26.46	101.1±23.28	53.90*	205.6±33.14	102.9±19.06	49.95*
Hippocampus	319.7±47.82	154.9±41.83	51.44*	327.4±27.09	153.5±35.75	53.06*
Hypothalamus	1,838±191.6	1,641±139.5	10.72	1,994±245.5	1,765±295.6	11.38

*P<0.05 vs saline control using ANOVA with Bonferroni's test as post hoc

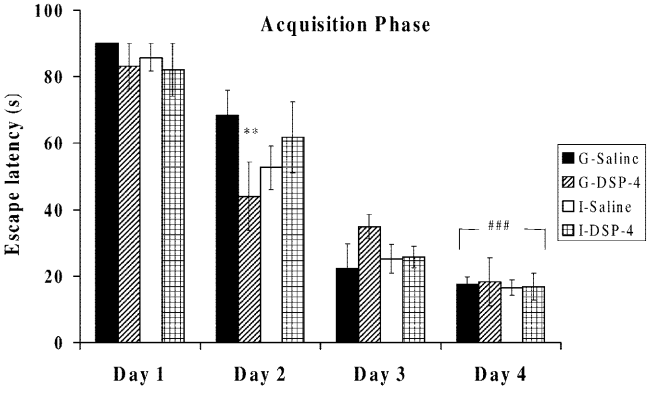


Fig. 3 Mean (± SEM) activity during the acquisition phase in the water maze of DSP-4 (25 mg/kg, i.p.)- or saline-treated group (*G*)- and isolation (*I*)-reared rats. The downward trend of the escape latencies indicates that the learning of the location of the platform improved as a function of time [F(3,12)=14.14; P<0.001]. Escape latencies at day 4 were significantly lower than in day 1 [F(3,12)=110.00; P<0.001]. DSP-4 treatment in grouped rats decreased latency at day 2 compared to saline controls [F(3,12)=32.32; P<0.01] but this effect was abolished on days 3 and 4. **P<0.01 vs saline controls; ### P<0.001 vs day 1 using ANOVA with Bonferroni's test as post hoc

Isolation-reared controls had fewer zone transitions [F(3,20)=3.45; P<0.05] and had reduced swimming distance [F(3,20)=7.63; P<0.05] at a lower speed [F(3,20)= 5.50; P<0.01] than group controls during the probe test. DSP-4-treated isolates swam a significantly longer distance [F(3,20)=7.63; P<0.05] at a faster speed [F(3,20)= 5.50; P<0.05] compared to isolate controls (data not shown).

Regional NA levels

Systemic administration of DSP-4 (25 mg/kg, i.p.) achieved a significant 75% reduction (mean ± SEM ng/g net weight of tissue; control: 248.9±31 and DSP-4: 62.4±33, P<0.01) in cortical NA 4 days after injection. Twenty-eight days after DSP-4 administration, cortical and hippocampal levels of NA were significantly decreased (P<0.05) in group-reared and isolation-reared rats compared to their saline controls (Table 1). There was no significant difference between the two rearing groups. Hypothalamic NA levels were unaffected.

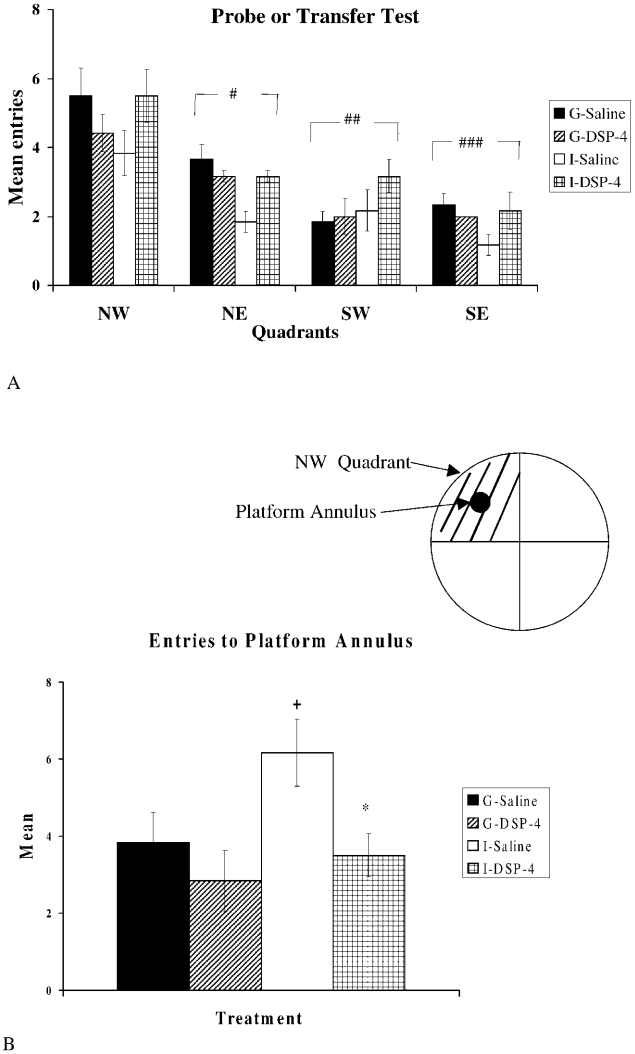


Fig. 4A, B Mean (± SEM) activity during the probe test in the water maze of DSP-4 (25 mg/kg, i.p.)- or saline-treated group (*G*)- and isolation (*I*)-reared rats. **A** All rats significantly preferred the Northwest (*NW*) quadrant where the platform had been placed compared to the Northeast [*NE*; F(3,12)=7.47; P<0.05], Southwest (*SW*; F(3,12)=7.47; P<0.01] and Southeast (*SE*; F(3,12)=7.47; P<0.001] quadrants. **B** *Inset diagram* shows the location of the platform annulus (the exact previous location of the platform) in the Northwest quadrant (*hatched area*). The number of entries or crossings to the platform location was significantly higher in isolate controls compared to group-reared counterparts [F(3,20)= 4.04; P<0.05] and isolation-reared DSP-4-treated rats [F(3,20)= 4.04; P<0.05]. * P<0.05 vs I-saline; + P<0.05 vs G-saline; # P<0.05, ## P<0.01, ### P<0.001 vs NW quadrant using ANOVA with Bonferroni's test as post hoc

Discussion

DSP-4 (25 mg/kg, i.p.) treatment of the rats caused a substantial depletion of cortical NA levels 4 days post-treatment and a persistent decrease in cortical and hippocampal NA 28 days after DSP-4 administration while hypothalamic NA was unaffected. These results support findings that DSP-4 selectively destroys noradrenergic projections from the locus coeruleus, results confirmed by immunocytochemical studies (Fritschy and Grzanna 1989, 1992; Schuerger and Balaban 1999). The HPLC-ECD method used in the present study was optimised to measure NA so data on dopamine and serotonin are not available. However, several previous studies have shown that DSP-4 spares dopamine, adrenaline and serotonin neurotransmission even at higher doses (50 mg/kg or higher) than that used in the present study (Jonsson et al. 1981; Dooley et al. 1983a, b; Ho et al. 1995; Cheetham et al. 1996; Al-Zahrani et al. 1997). Higher doses of DSP-4 are associated with gross toxicity (Ross 1985), and can result in mortality (Schuerger and Balaban 1999) probably as a consequence of acute cerebellar and cerebral cortical oedema (Tengvar et al. 1989). With the 25 mg/kg dose used in this study, we achieved a decrease in NA levels comparable to that reported by Cheetham et al. (1996). The use of the lower dose of DSP-4 has been shown to minimise effects on the serotonergic system so avoiding the need to pretreat with a serotonin uptake inhibitor (Ross 1985). Hence, this study achieved comparable NA depletion with less deleterious effects on the rats.

The present behavioural results may be a consequence of partial rather than total NA depletion or perhaps reflect compensatory changes in the noradrenergic system. Evidence shows that monoamine neurones respond to lesions with a wide range of compensatory adaptations aimed at preserving their functional integrity. These may include increased synthesis and release of neurotransmitter from residual monoamine fibres as shown for serotonergic neurones (Hall et al. 1999), axonal sprouting (Fritschy and Grzanna 1992), and changes in receptor functions (Dooley et al. 1983a, b; Heal et al. 1993). Although DSP-4 decreases α_2-adrenoceptors and functional presynaptic terminals, its overall effect on the noradrenergic system maybe masked by a proliferation of post-synaptic receptors in response to noradrenergic denervation (Heal et al. 1993). Other compensatory changes may include delayed appearance of hyperresponsivity within systems regulating stimulus-directed behaviour involving NA release and/or receptor function or increased activity in other systems influenced by NA (Berridge and Dunn 1990). However, despite evidence of plasticity and recuperative responses of noradrenergic neurones to 6-hydroxydopamine (6-OHDA)-induced lesions, 90% or greater depletion of telencephalic NA produced robust and long-lasting behavioural deficits (Abercombie et al. 1988; also see Everitt et al. 1990). It can not be determined whether the behavioural changes reported in the present study reflect an overall reduction or increase in noradrenergic function despite a loss of central NA of more than 50%.

Effects of postweaning social isolation

Isolation rearing had no significant effects on the behaviour in the elevated plus maze, supporting previous studies (Fone et al. 1996) and contradicting reports of isolation-induced ‘anxiogenic’ profile in the elevated plus maze (Wright et al. 1991; Maisonnette et al. 1993). Several factors could account for the discrepancy. Fone et al. (1996) suggested that the difference in behavioural profile could be due to the higher light intensity used compared to the previous study in the same laboratory (Wright et al. 1991). Light intensity, strain and housing conditions have been shown to affect the behaviour of isolation- and group-reared rats in the elevated plus maze and other behavioural paradigms (Hall et al. 1997, 1998). Pellow et al. (1985) failed to observe effects of varying lighting conditions on the behaviour of rats on the elevated plus maze. This could be due to factors such as differences in strain, sex and housing conditions of rats as Hall et al. (1998) observed behavioural differences in Fawn Hooded but not Wistar rats tested on the elevated plus maze under low and bright light conditions.

In the water maze, isolation rearing had no significant effect during the acquisition phase. However, during the probe test, isolation-reared rats made more entries to the platform annulus, indicating that these rats retained a more accurate memory of the platform location. Isolation-induced enhancement of place and reversal learning in the water maze has been reported (Wongwitdecha and Marsden 1996). These findings are in contrast with reports of isolation-induced impairment in the water maze (Wade and Maier 1986). The differing results may again reflect the differences in housing conditions and rat strain. Alternatively, it could be due to other factors associated with the test environment. Wade and Maier (1986) reported a very rapid restoration or normalisation of function of individually housed rats in the water maze following exposure to loud noise for half an hour before testing. The isolated rats may have a lowered stress response, causing an abnormal activation of processes that support learning. Noise exposure before maze testing may have returned this threshold to normal sensitivity thus restoring learning performance (Wade and Maier 1986).

Isolation rearing significantly decreased speed, distance swam and zone transitions of rats in the water maze during the probe test. Considering that isolates are hyperactive in the open field and activity cages (Lapiz et al. 1999b, 2000a), these behavioural changes do not represent a generalised reduction in motor activity. Swimming speed in the water maze can be used to assess motor and motivational deficits (McNamara and Skelton 1993) so the behaviours observed may indicate changes in the motivational status of the isolates.

Effects of DSP-4 treatment

On the elevated plus maze, DSP-4-treated group-reared rats were more active and spent more time in the open arms compared to controls, suggestive of an 'anxiolytic' effect of DSP-4. In contrast, DSP-4-treated isolated rats spent less time in the open arms and were hypoactive compared to group-reared DSP-4-treated rats. Since there was no significant difference between saline and DSP-4 isolates, the difference between the behaviour of group and isolated DSP-4-treated rats in the elevated plus maze may indicate changes in the isolated rat resulting in a differential effect of NA depletion.

An anti-exploratory effect of DSP-4 in group-reared rats on the elevated plus maze and other behavioural paradigms has been reported (Delini-Stula et al. 1984; Harro et al. 1995; Skrebuhhova et al. 1999). DSP-4 treatment prevented virtually all activities of rats in the open field arena during the initial sessions indicating an increase in neophobia (Harro et al. 1995). Perhaps these results were analogous to the behaviour seen in the DSP-4-treated isolation-reared rats in this study with isolation rearing making these rats more vulnerable to the effects of central NA depletion. Pisa and Fibiger (1983) reported that 6-OHDA lesions to the dorsal noradrenergic bundle impaired learning in a Y-maze. The lesioned rats persistently avoided the illuminated goal arm. Interestingly, all the subjects (male Wistar rats) used in their experiments were individually housed although housing condition was not a factor taken into consideration.

Selden et al. (1990a, b) reported that lesions to the locus coeruleus alter perception of danger through alteration of attention focus, resulting in loss of ability to ignore irrelevant stimuli (Mason and Iversen 1978) thus increasing susceptibility to danger (Mason and Iversen 1975; Everitt et al. 1990). The hyperactivity and 'anxiolytic' profile of DSP-4-treated group-reared rats in this study may indicate failure to attend to the aversiveness of the test condition causing them to be more active and less fearful of the open arms. In contrast, the hypoactivity and 'anxiogenic' profile of DSP-4-treated isolates probably indicates increased neophobia. Harro et al. (1995) suggested that denervation of the locus coeruleus increased neophobia, lengthening the avoidance phase of exploration. However, this would not explain the difference in response to DSP-4 treatment between group- and isolation-reared rats.

DSP-4 treatment of group- and isolation-reared rats had no significant effects on the performance in the water maze during acquisition (Lapiz et al. 1999a). Previous studies have also shown that DSP-4 lesioning does not affect acquisition and retention of platform location in the water maze (Björklund et al. 1999). 6-OHDA lesions to the dorsal noradrenergic bundle from the locus coeruleus may or may not affect place learning depending on the water temperature of the maze. No effect was seen when water temperature was between 22–26°C (Valjakka et al. 1990) while with colder water (11–12°C), improvement (Selden et al. 1990a) or lack of effect have been reported (Valjakka et al. 1990). Pharmacological manipulations (Decker et al. 1990; Sirviö et al. 1991, 1992) tend to support the lesion data to suggest that the noradrenergic systems may not have a major role in place learning.

DSP-4-treated isolation-reared rats learned equally well the placement of the escape platform. However, during the probe test, they made fewer entries to the platform annulus compared to the isolate controls, indicating impairment of spatial memory. Depletion of forebrain NA induces persistent attention to motivationally irrelevant stimuli (Mason and Iversen 1978). The DSP-4-treated isolated rats may have been attending to other irrelevant stimuli as indicated by the greater swimming distance and speed of these rats. These findings support our previous findings that DSP-4-treated isolated rats attend more to the general environment rather than to specific items within the environment, indicating changes in attention (Lapiz et al. 2000a).

Overall, the results indicate that the behavioural response of grouped and isolation-reared rats with depleted brain NA depends on the nature of the test environment. In the water maze and open field where a sizeable part of the arena is equally exposed and aversive, DSP-4 treatment of isolates increased attention to irrelevant stimuli, i.e. attention to the general environment rather than specific items (Lapiz et al. 2000a). In the elevated plus maze where the rat has a choice between the aversive open arms and the secure enclosed arms, the DSP-4-treated isolated rat spent less time in the open arms compared to DSP-4-treated group-reared rats.

In summary, DSP-4 treatment of group-reared rats produced an 'anxiolytic' response on the elevated plus maze while in isolates, it normalised memory in the water maze. The results demonstrate the importance of noradrenergic function in attention and the regulation of responsiveness to environmental cues. Modification of this system by isolation rearing results in differential effects in response to disruption of brain noradrenergic function.

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