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# Conflict Behavior in Maudsley Reactive and Nonreactive Rats: Effects of Noradrenergic Neuronal Destruction

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VERBANAC, J. S., H. J. ALTMAN, P. DHINGRA, G. M. HARRINGTON AND R. L. COMMISSARIS. *Conflict behavior in Maudsley reactive and nonreactive rats: Effects of noradrenergic neuronal destruction.* PHARMACOL BIOCHEM BEHAV 45(2) 429–438, 1993.—The present studies were designed to examine the effects of treatment with the noradrenergic neurotoxin *N*-(2-chloroethyl)-*n*-ethyl-2-bromobenzylamine HCl (DSP4; 65 mg/kg, IP) on conflict behavior in the Maudsley reactive (MR) and nonreactive (MNRA) rat strains. In daily 10-min sessions, water-restricted rats were trained to drink water from a tube that was occasionally electrified; electrification was signaled by the presence of a tone (7-s duration; ISI = 30 s). Consistent with previous reports, the number of shocks accepted by rats of the MR and MNRA strains did not differ initially, but MNRA rats exhibited a dramatic increase in punished responding relative to their MR counterparts over the course of several weeks of conflict testing. This MR vs. MNRA strain difference in punished responding did not exhibit extinction following discontinuation of CSD conflict behavior testing for a period of 6 weeks. Whether it was administered after conflict training or before, DSP4 treatment did not reduce the MR vs. MNRA strain difference in conflict behavior; rather, DSP4 treatment tended to increase the magnitude of the MR vs. MNRA difference in conflict behavior. The effects of DSP4 on norepinephrine (NE) and 5-hydroxytrypamine (5-HT) concentrations in the pons medulla region were determined in one group of conflict-experienced MR and MNRA rats (35 weeks after administration) and in a second group of naive MR and MNRA rats (3 weeks after administration). There were no MR vs. MNRA strain differences in NE or 5-HT concentrations in vehicle-treated rats. DSP4 treatment significantly reduced NE, but not 5-HT, concentrations when compared to control values; rats that were sacrificed 3 weeks following DSP4 administration exhibited a greater NE depletion than did rats sacrificed 35 weeks after DSP4 administration. Finally, there were no significant correlations between pons medulla region NE concentrations and conflict behavior in either strain alone or when the data from the two strains were combined. The present results are not consistent with the hypothesis that the MR vs. MNRA strain difference in conflict behavior is the result of strain differences in brain NE function.

Anxiety Locus coeruleus Maudsley rats DSP4 Noradrenergic Conflict behavior

OPEN-field defecation behavior has been used as an indicator of "emotionality" and/or anxiety in rodents, with a higher number of defecations inferred to be reflective of more emotionality and/or anxiety (3–5,11,19). The Maudsley reactive (MR) and nonreactive (MNRA) rat strains were selectively bred by Broadhurst for their differences in open-field defecation rates (3–5). Two of the Maudsley rat strains were maintained by Dr. Gordon Harrington at the University of Northern Iowa from 1965 (20) until 1986 and currently are maintained at Wayne State University (Detroit, MI). MR (anxious) rats exhibit high levels of open-field defecation, whereas MNRA (nonanxious) rats exhibit low levels of open-field defecation (2,20,21).

A second animal procedure that has been used in the study of anxiety-like behavior is the conditioned suppression of drinking (CSD) conflict paradigm (6,8,9,14,23). This test is a hybrid between the Geller-Seifter conditioned conflict test (16,17) and the Vogel acute conflict test (30). Previous testing of Maudsley rats in this paradigm has shown that the number of shocks accepted initially is comparable in the two strains but, upon repeated testing, MNRA rats accept significantly more shocks than do their MR counterparts (6,7). The basis for this gradually developing MR vs. MNRA difference in anxiety-like behavior has not been determined.

Brain norepinephrine (NE) has been implicated by several investigators as a critical neurotransmitter in anxiety-like situ-

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ations. Perhaps the most widely accepted hypothesis regarding the role of norepinephrine in anxiety argues that anxiety states are the result of increased activity of noradrenergic neurons in the locus coeruleus (26). Evidence supporting this hypothesis is that stimulation of the locus coeruleus in the monkey produced behavioral and physiological changes that simulate "natural fear" (27). Consistent with the hypothesis that increases in locus coeruleus activity are associated with increased anxiety, the $\alpha_2$-adrenoceptor antagonist piperoxane produced an anxiety-like state in primates (26). Finally, benzodiazepines and other antianxiety agents reduce the activity of locus coeruleus neurons (18).

A contrasting and perhaps less widely held theory is that anxiety is not the result of increased activity of noradrenergic neurons; rather, the increased activity of noradrenergic locus coeruleus neurons observed following the presentation of anxiety-provoking stimuli serves as a rectifying mechanism designed to help the subject cope with the stress (1). Evidence supporting this theory is that stimulation of the locus coeruleus in man, via chronically implanted electrodes, failed to produce any symptoms of anxiety but, rather, produced general arousal with increased cognitive functions in subjects tested (24).

The compound *N*-(2-chloroethyl)-*n*-ethyl-2-bromobenzylamine HCl (DSP4) has been demonstrated to be a powerful research tool available for the study of noradrenergic function. DSP4 is a noradrenergic neurotoxin that can produce a selective and permanent destruction of central norepinephrine nerve terminals after systemic administration (13,22,28).

The present studies were designed to examine the effects of DSP4 treatment on conflict behavior in Maudsley reactive and nonreactive rats. The effects of DSP4 treatment were determined both in naive and in conflict-experienced Maudsley rats.

## METHOD

### *Animals*

Subjects for the present experiments were female Maudsley rats from the Lafayette Clinic Maudsley colony (Detroit, MI). MR rats were in the F77 generation; MNRA rats were in the F82 generation. All subjects were housed in a climate-controlled room with a regulated light-dark cycle (lights on 0700–1900 h). Initially, water was available in the home cage, but after conflict testing had started all rats were placed on a water-restricted schedule as described in the General Procedure section (see below). Food was continuously available in the home cages. An additional group of MR and MNRA subjects was used only for ptosis testing and neurochemical analysis and had continuous access to food and water.

### *Apparatus*

Conflict testing was conducted in an apparatus similar to that previously described by Fontana et al. (12) and McCloskey et al. (25). The testing chamber was a rectangular box with Plexiglas sides and a metal floor and top. Recessed into one wall was a metal drinking tube, from which a calibrated (0.5-ml units) length of polyethylene tubing was attached for measuring the volume of water consumed by each subject. Programming for the session was controlled by solid-state modular programming equipment (Coulbourn Instruments Co., Lehigh Valley, PA).

### *General Procedure*

Conflict testing was conducted using the procedure described by Fontana et al. (12) and Commissaris et al. (6). For the first few sessions (10 min daily), water-deprived (24 h) rats were placed in the experimental cage and were allowed to consume water freely without any shock contingency. After 1 week of daily nonshock sessions, the tone/shock contingency was initiated. The 7-s tone periods were presented at regular [30-s interstimulus intervals (ISI)] intervals to all subjects. During the latter 5 s of these tone periods, contact between the floor and the metal drinking tube completed a circuit that resulted in the delivery of an electric shock to the mouth of the subject. The shock intensity was held constant at 0.25 mA except for the period when the current intensity vs. response experiments were conducted (see specific experiments). The duration of the shock (less than 200 ms) was equal to the duration of the tube contact. Shocks were delivered by a Coulbourn Instruments Two-Pole Small-Animal Shocker (Model E13-02).

Initially, the shock inhibited fluid consumption in the test chamber. After several days of testing, however, all subjects learned to consume stable volumes of water during the silent periods and made relatively few and brief contacts with the tube during the tone. Subjects in all experiments were tested individually in 10-min sessions at the same time of day (1000–1300 h) 4 days/week (Monday through Thursday) and were allowed free access to water on nontest days (Thursday posttest until Sunday a.m.). This schedule of 4 days/week testing was maintained throughout the study.

### *Neurochemistry*

High-performance liquid chromatography (HPLC) determinations of norepinephrine and serotonin were conducted using a modification of the method described by Galloway (15). Subjects receiving DSP4 or vehicle treatment were sacrificed by decapitation. The brains were rapidly removed and the brainstem region containing cell bodies for NE neurons (the pons medulla) rapidly dissected out, frozen on dry ice, wrapped in foil, and stored at −80 degrees C until analysis. Frozen tissues were weighed in Eppendorf tubes, sonically disrupted (Sonica and Materials, Inc., Danbury, CT) for 5s in 400 μl homogenizing solution (0.1% $Na_2S_2O_5$; 0.1 M $HClO_4$; 0.1 mg/ml each of dihydroxybenzylamine and *N*-methylserotonin) and then centrifuged at 10,000 × *g* for 5 min. After centrifugation, 50 μl 1.5 M Tris buffer were placed on acid-washed alumina (Bioanalytic Systems, Inc., West Lafayette, IN) columns and the effluent was passed into 20 μl 7.9 N HCl by centrifuging for 1 min at 2,000 × *g*. An aliquot of this effluent was used for serotonin analysis. After the alumina columns were washed with 500 μl distilled water, 150 μl 0.1 M oxalic acid was placed on the columns and catechols were eluted by centrifugation. A 50-μl aliquot of the final eluate was injected into an HPLC system for electrochemical analysis of norepinephrine. The mobile phase [0.1 M $NaH_2PO_4$, 2.0 mM octylsulfonic acid, 0.1 mM EDTA, phosphoric acid (pH between 2.6 and 2.9), MeOH (2–10%)] was pumped at a flow rate of 0.8–1.4 ml/min, depending upon the tissue under analysis. The HPLC-EC system consisted of two biophase octyl 100 × 4.6-mm 5-μm columns linked in series (Bioanalytical Systems, Inc.), an HPLC pump (series 10), and an autosampler (ISS-100), both from Perkin Elmer (Norwalk, CT).

Electrochemical detection was performed using an LC 4-B amperometric detector equipped with a glassy carbon electrode (Bioanalytic Systems, Inc.) that was set at an oxidation potential of +0.75 V relative to an Ag-AgCl reference electrode. Peak heights were measured and the concentrations of monoamines calculated based upon internal standards and comparisons to external standards containing known amounts of the measured catecholamines.

*Drug*

DSP4 was purchased from Research Biochemicals, Inc. (Natick, MA); it was dissolved in 0.9% saline and administered in a volume of 1 ml/kg body weight.

*Statistical Analyses*

The two dependent variables monitored in the CSD conflict task were the individual weekly averages (defined for each subject as the average across the Monday-Thursday test sessions) for shocks received (punished responding) and water intake (unpunished responding). Strain differences in baseline (i.e., untreated) conflict performance were statistically evaluated using 2 × 8 factorial analyses of variance (ANOVAs) with repeated measures. The effects of DSP4 or saline treatment on shocks received and water intake were compared using 2 × 2 × 9 factorial ANOVAs with repeated measures (main effects: DSP4/vehicle treatment, rat strains, test weeks). The results of the current intensity vs. response determinations were evaluated using a 2 × 2 × 3 factorial ANOVA with repeated measures (main effects: DSP4/vehicle treatment, rat strains, current intensities). Posthoc comparisons were made using the least significant differences (LSD) test. Data from the experiment examining the effects of a 2-week interruption of conflict testing on the conflict behavior were analyzed with 2 × 2 factorial ANOVA with repeated measures (main effects: rat strains, test-retest). The frequency of occurrence of ptosis in MR vs. MNRA rats at various intervals following DSP4 administration was evaluated using a $\chi^2$ for proportions. The effects of DSP4 treatment on the concentrations of NE or 5-hydroxytrypamine (5-HT) in the pons medulla region were statistically analyzed by a 2 × 2 × 2 factorial ANOVA (main effects: rat strains, DSP4/vehicle treatment, treatment-sacrifice interval). The effects of DSP4 treatment on NE and 5-HT concentrations were analyzed separately. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance (29).

SPECIFIC EXPERIMENTS CONDUCTED

*Experiment 1: Effects of DSP4 Treatment on Conflict Behavior of Trained MR and MNRA Rats*

*A: Baseline Determinations—test weeks 1-8.* The first part of Experiment 1 was designed to demonstrate that the behavioral difference previously reported between the Maudsley rat strains during the course of CSD testing was reproducible. For the first 8 weeks of the experiment, subjects were tested in the CSD conflict task for the purpose of obtaining baseline (pre-DSP4) values for punished and unpunished responding. Subjects were tested as described above in the General Procedure section. The number of shocks received and the volume of water consumed were recorded daily and averaged weekly for each subject.

*B: DSP4 or vehicle treatment Effects on CSD Behavior—test weeks 9-16.* Prior to DSP4 or vehicle treatment, subjects from each strain were assigned into two treatment groups with comparable baselines for shocks received. This assignment was based upon the average number of shocks accepted per session by each subject for the last 2 weeks prior to DSP4 or vehicle treatment. DSP4 or its vehicle was administered to subjects of each strain in a single IP injection (65 mg/kg), 1 day prior to the beginning of the ninth week of testing. CSD conflict testing was reinstated 24 h after DSP4 or vehicle treatment and was continued as described above for an additional 8 weeks following DSP4 or vehicle treatment.

*Part C: Current intensity vs. Response Determinations—test weeks 17-22.* The objective of this portion of the experiment was to determine if the differences in punished responding observed between strains was dependent upon the intensity of the shock used in the conflict task. Beginning at test week 17, current intensity vs. response functions were determined in DSP4- or vehicle-treated rats of both strains. In these studies, the shock intensity was held constant for the Monday-Thursday test sessions within a given test week and varied from week to week in a counterbalanced "ABCCBA" design using the current intensities 0.25 (A), 0.125 (B), and 0.5 mA (C), respectively. Thus, each subject was tested for 2 weeks at each of the three shock intensities.

*Experiment 2: Effects of DSP4 Treatment on Conflict Behavior in Untrained MR and MNRA Maudsley Rats*

The objective of this study was to determine whether DSP4 administration prior to behavioral testing would prevent the occurrence of the MR vs. MNRA strain difference in punished responding observed in the CSD conflict task. Untrained, naive Maudsley rats of both strains were accommodated in the CSD apparatus for a period of 1 week with no tone and no shock. At the end of this week, each subject was administered a single IP injection of DSP4 or vehicle (saline). DSP4 or vehicle treatments were randomly assigned to equal numbers of MR and MNRA subjects. CSD testing, with the tone and shock contingencies, was initiated the following week as described above in Experiment 1 (shock intensity = 0.25 mA) and was continued for a period of 9 weeks.

Conflict testing was then suspended for a period of 6 weeks. For 2 weeks, testing was reinitiated in vehicle-treated MR and MNRA subjects. The purpose of this interruption of conflict testing was to examine whether the MR vs. MNRA difference that had developed over the course of several weeks of conflict testing would persist in the absence of conflict testing. DSP4-treated subjects were not tested during this phase of the experiment.

Thirty-five weeks after DSP4 or vehicle administration, all subjects in Experiment 2 were sacrificed by decapitation; their pons medulla regions were dissected out and frozen. Concentrations of NE and 5-HT were later determined using HPLC procedures described above.

*Experiment 3: DSP4 Effects on Neurochemistry 3 Weeks After Administration*

In Experiment 2, subjects were sacrificed 35 weeks after DSP4 or vehicle treatment. It is possible that some recovery of brain NE might have occurred during this prolonged period. Therefore, the objective of the present experiment was to determine the effects of DSP4 treatment at a pretreatment interval that more closely paralleled the interval between DSP4 treatment and behavioral testing used in Experiments 1 and 2. To this end, naive subjects from the MR and MNRA strains were treated with DSP4 (65 mg/kg, IP) or vehicle 3 weeks prior to sacrifice. These subjects were not tested in the conflict procedure.

DSP4-treated subjects in Experiment 3 were scored for the presence or absence of ptosis on three occasions following treatment. At 6, 24, and 48 h after DSP4 injection, both MR and MNRA subjects were rated for the presence or absence of ptosis (droopy eyelids) by a trained observer (J.S.V.) who was blinded regarding rat strain.

Three weeks after DSP4 or vehicle administration, all subjects in Experiment 3 were sacrificed and their pons medulla

regions were dissected out and frozen until HPLC determinations of NE and 5-HT concentrations were conducted.

## RESULTS

### *Experiment 1: DSP4 Treatment Effects on Conflict Behavior in Trained MR and MNRA Maudsley Rats*

*A: Baseline Determinations.* The upper panel of Fig. 1 illustrates the time course for baseline CSD conflict behavior in MR and MNRA rats for 9 weeks of control (i.e., no treatment) CSD conflict sessions. Consistent with previous reports, there was no difference in punished responding between MR and MNRA rats in the first week of CSD conflict testing [MNRA: 21 ± 2 (mean ± SEM shocks/session); MR: 24 ± 3, n.s.]. Punished responding in rats of the MR strain did not change appreciably across test weeks 1-8. In contrast, punished responding in rats of the MNRA strain increased gradually over the course of the first 5 test weeks and then appeared to achieve an asymptote around test week 5. Statistically, there was a significant main effect for rat strains, $F(1, 29) = 8.26$, $p < 0.05$, and test weeks, $F(7, 203) = 6.09$, $p < 0.05$. Most importantly, there was a significant rat strain × test week interaction, $F(7, 206) = 4.02$, $p < 0.05$. Posthoc LSD comparisons revealed that MNRA rats accepted significantly more shocks than did MR rats at test weeks 2-8.

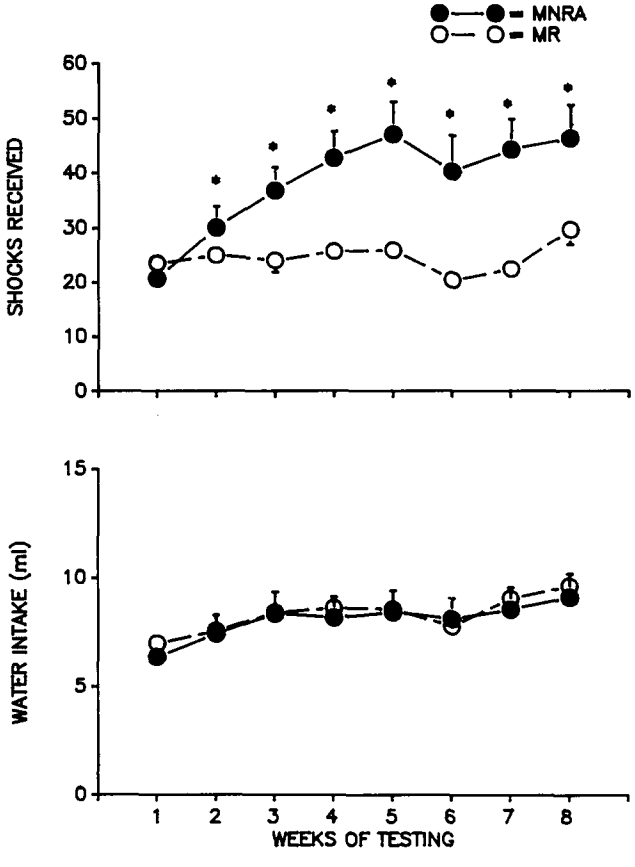


FIG. 1. Time course for 8 weeks of control conditioned suppression of drinking (CSD) conflict behavior in Maudsley reactive (MR) and Maudsley nonreactive (MNRA) rats. Plotted are the mean ± SEM shocks received (top panel) and water intake (bottom panel) per conflict session. Each symbol and vertical line represents data obtained from 16 subjects.

*MNRA rats are significantly different ($p < 0.05$) from MR rats at the indicated test week, least significant differences posthoc test following factorial analysis of variance.

The lower panel of Fig. 1 illustrates the time course for baseline water intake in control CSD conflict sessions in MR and MNRA rats. Statistically, there was no significant main effect for rat strains, $F(1, 29) = 1.13$, $p = 0.29$, n.s. There was, however, a significant main effect for test weeks, $F(7, 203) = 26.42$, $p < 0.05$, as rats from both strains increased their water intake at relatively similar rates. Finally, the rat strains × test weeks interaction was not significant, $F(7, 203) = 1.09$, n.s. It should be noted that in both MR and MNRA rats the number of punished licks (20-50 per session) was insignificant when compared to the number of unpunished licks (2,500-3,000 per session); thus, water intake is an accurate reflection of unpunished responding in both strains.

*B: DSP4 Treatment Effects.* Baseline (i.e., pre-DSP4/vehicle) values for punished responding were comparable in both groups of MR rats prior to treatment (MR/vehicle: 25 ± 4; MR/DSP4: 27 ± 2; values represent the mean ± SEM derived from the last 2 weeks of CSD conflict sessions prior to DSP4 or vehicle treatment). Similarly, baseline values for punished responding were comparable in both groups of MNRA rats (MNRA/vehicle: 46 ± 7; MNRA/DSP4: 44 ± 8).

The upper panels of Fig. 2 illustrate the effects of vehicle or DSP4 treatment (left and right panels, respectively) on punished responding in MR and MNRA rats. Both vehicle- and DSP4-treated rats of the MNRA strain exhibited a decrease in punished responding relative to baseline (i.e., pretreatment) levels. Rats of the MR strain receiving DSP4 treatment also exhibited a depression of punished responding, whereas MR rats receiving vehicle treatment did not. The magnitude of the MR vs. MNRA difference in punished responding was comparable in vehicle- and DSP4-treated subjects. Statistically, the main effect for rat strains was significant, $F(1, 27) = 7.21$, $p < 0.05$, but the main effect for DSP4/vehicle treatment, $F(1, 27) = 1.26$, n.s., and the interaction of rat strains × DSP4/vehicle treatment, $F(1, 27) < 1$, n.s., were not. The main effect for test weeks, $F(8, 216) = 12.78$, $p < 0.05$, and the interaction of rat strains × test weeks, $F(8, 216) = 2.04$, $p < 0.05$, were statistically significant. The interactions of DSP4/vehicle treatment × test weeks, $F(8, 216) = 1.38$, n.s., and rat strains × DSP4/vehicle treatment × test weeks, $F(8, 216) = < 1$, n.s., were not significant. Posthoc LSD tests revealed that vehicle-treated MNRA rats accepted significantly more shocks than did vehicle-treated MR rats at the baseline week and at test weeks 2, 3, 5, 6, 7, and 8 posttreatment and that DSP4-treated MNRA rats accepted more shocks than did DSP4-treated MR rats at the baseline week and at test weeks 2-8 post-DSP4. Thus, DSP4 treatment did not significantly alter the MR vs. MNRA strain differences in punished responding.

Baseline (i.e., pre-DSP4/vehicle) values for unpunished responding (water intake) were comparable in both groups of MR rats prior to treatment [MR/vehicle: 9.7 ± 0.5; MR/DSP4: 9.4 ± 0.2; values represent the mean ± SEM (ml) derived from the last 2 weeks of CSD conflict sessions prior to DSP4 or vehicle treatment]. Similarly, baseline values for unpunished responding were comparable in both groups of MNRA rats (MNRA/vehicle: 9.1 ± 0.4; MNRA/DSP4: 9.4 ± 0.5).

The lower panels of Fig. 2 illustrate the effects of vehicle or DSP4 treatment on water intake in rats of the MR and MNRA strains (lower left and right panels, respectively). As

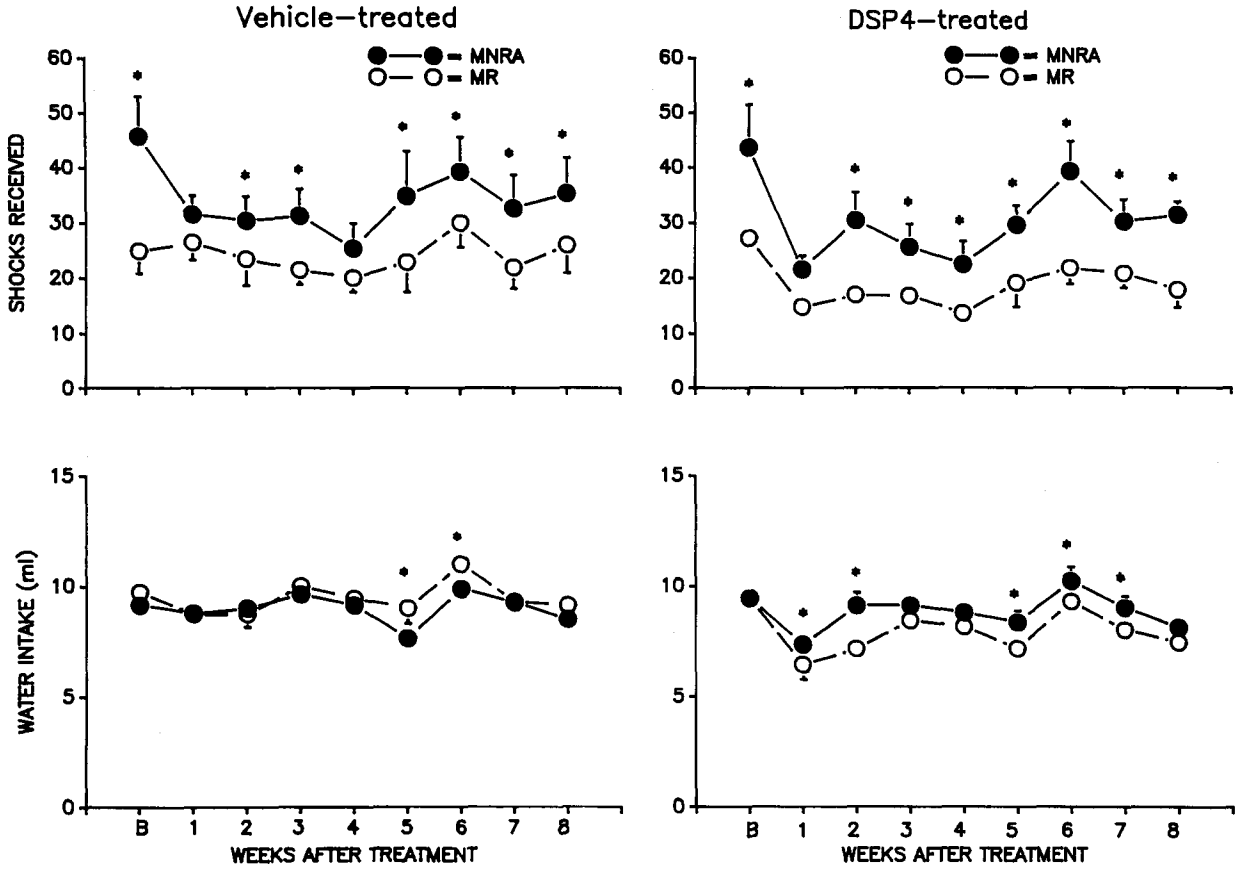


FIG. 2. Effects of DSP4 treatment on conditioned suppression of drinking (CSD) conflict behavior in Maudsley reactive (MR) and Maudsley nonreactive (MNRA) rats. Plotted are the mean ± SEM ($n$ = 8) shocks received (top panels) and water intake (bottom panels) in vehicle-treated (saline; left panels) and DSP4-treated (65 mg/kg; right panels) rats of the MR (open symbols) and MNRA (filled symbols) strains during the 2-week period prior to DSP4 or vehicle administration (baseline; B) and for each of the 8 weeks following DSP4 or vehicle treatment (test weeks 1–8).

*MNRA rats are significantly different ($p < 0.05$) from MR rats at the indicated test week and treatment condition (DSP4/vehicle), least significant differences posthoc test following factorial analysis of variance.

can be seen, DSP4 treatment reduced water intake in both MR and MNRA rats during the first week after treatment. The effect of DSP4 to reduce water intake in MNRA rats did not persist beyond the first week posttreatment; in contrast, the effect of DSP4 to decrease water intake in MR rats persisted for several weeks. Statistically, the main effect for rat strains was not significant, $F(1, 27) < 1.0$, n.s.; the main effect for DSP4/vehicle treatment, $F(1, 27) = 4.34$, $p < 0.05$, and test weeks, $F(8, 216) = 28.36$, $p < 0.05$, were significant. The DSP4/vehicle treatment × rat strain interaction also was significant, $F(1, 27) = 2.84$, $p < 0.05$, one-tailed. The test weeks × rat strains, $F(8, 216) = 2.41$, $p < 0.05$, and test weeks × DSP4/Vehicle, $F(8, 216) = 3.25$, $p < 0.05$, interaction effects were statistically significant. Finally, the test weeks × DSP4/vehicle treatment × rat strain interaction was not significant, $F(8, 216) = 1.34$, n.s. Posthoc LSD tests revealed that DSP4 treatment in MNRA rats significantly depressed water intake relative to vehicle-treated MNRA rats at test week 1, whereas DSP4 treatment in MR rats significantly decreased water intake relative to vehicle-treated MR rats at all test weeks (test weeks 1–8). Posthoc LSD tests comparing water intake in MR and MNRA rats revealed that vehicle-treated MR rats consumed significantly greater volumes of water than did vehicle-treated MNRA rats at test weeks 5 and 6, whereas DSP4-treated MR rats consumed significantly smaller volumes of water than did DSP4-treated rats at test weeks 1, 2, 5, 6 and 7 (see Fig. 2).

*C: Current Intensity vs. Response Determinations.* The upper panels of Fig. 3 illustrate the current intensity vs. response functions for punished responding in MNRA and MR rats that had received vehicle or DSP4 treatment (upper left and right panels, respectively). Rats from both strains exhibited dramatic current intensity vs. response functions, with the number of shocks received being inversely related to the shock intensity. In the MR strain, DSP4-treated rats accepted fewer shocks relative to vehicle-treated controls at all shock intensities examined. DSP4 treatment did not reliably reduce punished responding in MNRA rats, although there was a tendency for DSP4-treated rats to accept fewer shocks at the lowest intensity examined. Statistically, there were significant main effects for shock intensity, $F(2, 52) = 46.1$, $p < 0.05$, and rat strains, $F(1, 26) = 7.12$, $p < 0.05$. The shock intensity × DSP4/vehicle treatment, $F(2, 52) = 3.15$, $p < 0.05$, interaction was also significant. The main effect for DSP4/

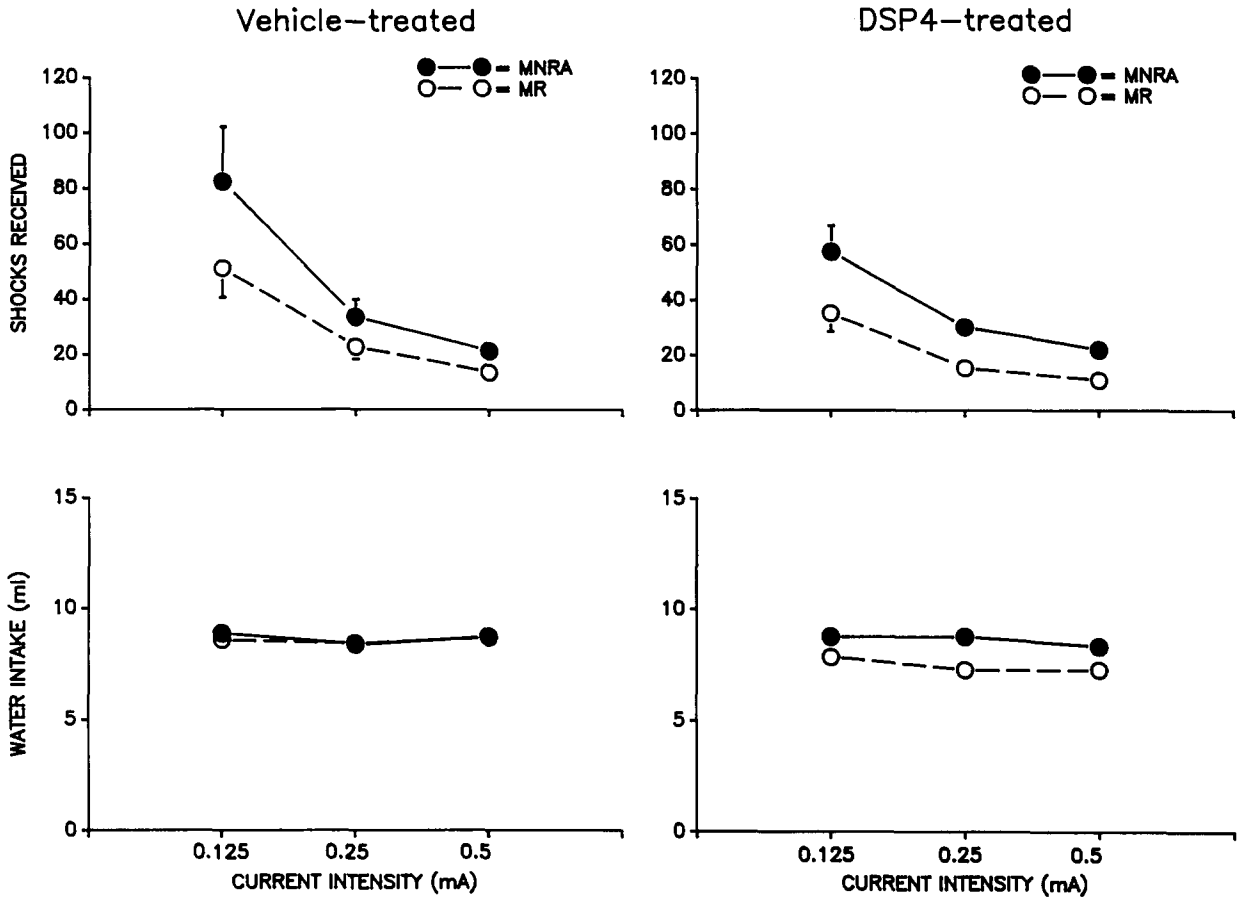


FIG. 3. Effects of varying shock intensity on conditioned suppression of drinking (CSD) conflict behavior in DSP4-treated or vehicle-treated rats of the Maudsley reactive (MR) and Maudsley nonreactive (MNRA) strains. Plotted are the mean ± SEM ($n$ = 8) shocks received (top panels) and water intake (bottom panels) in vehicle-treated (left panels) and DSP4-treated (right panels) MR (open symbols) and MNRA (filled symbols) rats when conflict testing was conducted at different shock intensities. Factorial analysis of variance revealed significant main effects for shock intensity and rat strains (see text).

vehicle treatment, $F(1, 26) = 2.37$, n.s., was not significant. The rat strains × DSP4/vehicle treatment, $F(1, 26) < 1$, n.s., and the rat strains × shock intensity, $F(2, 52) = 2.55$, n.s., interactions were not statistically significant. Finally, the rat strains × DSP4/vehicle treatment × current intensity interaction was not significant, $F(2, 52) < 1$, n.s.

The lower panels of Fig. 3 illustrate the current intensity vs. response functions for unpunished responding in MR and MNRA rats that had received vehicle or DSP4 treatment (lower left and right panels, respectively). The main effects for rat strains, $F(1, 27) = 2.48$, n.s., DSP4/vehicle treatment, $F(1, 27) = 2.04$, n.s., and current intensity, $F(2, 54) = 2.10$, n.s., were not significant. DSP4 treatment tended to decrease water intake in rats of the MR strain; however, the DSP4/vehicle treatment × rat strains interaction was not significant, $F(1, 27) = 1.78$, n.s. Neither the rat strains × current intensity, $F(2, 54) < 1.0$, n.s., nor the DSP4/vehicle treatment × current intensity, $F(2, 54) = 1.92$, n.s., interactions were significant; finally, the rat strains × DSP4/vehicle treatment × current intensity interaction was not significant, $F(2, 54) = 1.09$, n.s. Thus, alterations in current intensity produced little effect on unpunished responding in these subjects.

*Experiment 2: DSP4 Treatment Effects on CSD Conflict Behavior in Untrained MR and MNRA Rats*

The upper panels of Fig. 4 illustrate the effects of vehicle or DSP4 treatment on punished responding in previously untrained MR and MNRA rats. There was no significant difference between MR and MNRA rat strains at the onset of training (test week 1; MR/vehicle: 41 ± 5; MR/DSP4: 33 ± 7; MNRA/vehicle: 35 ± 6; MNRA/DSP4: 39 ± 7; values represent the mean ± SEM number of shocks accepted during the first week after DSP4 or vehicle treatment).

Over the course of 9 weeks of training, vehicle-treated rats of the MNRA strain exhibited a dramatic increase in punished responding relative to their MR counterparts (Fig. 4, left top panel). The MR strain, interestingly, tended to decrease in punished responding over this period. DSP4 treatment increased the magnitude of the gradually occurring MR vs. MNRA difference in punished responding, with DSP4-treated MNRA rats accepting more shocks than vehicle-treated MNRA rats and DSP4-treated MR rats accepting fewer shocks than vehicle-treated MR rats. Statistically, the main effect for rat strains, $F(1, 28) = 25.95$, $p < 0.05$, and test weeks, $F(8, 224) = 13.30$, $p < 0.05$, were significant. The main effect for

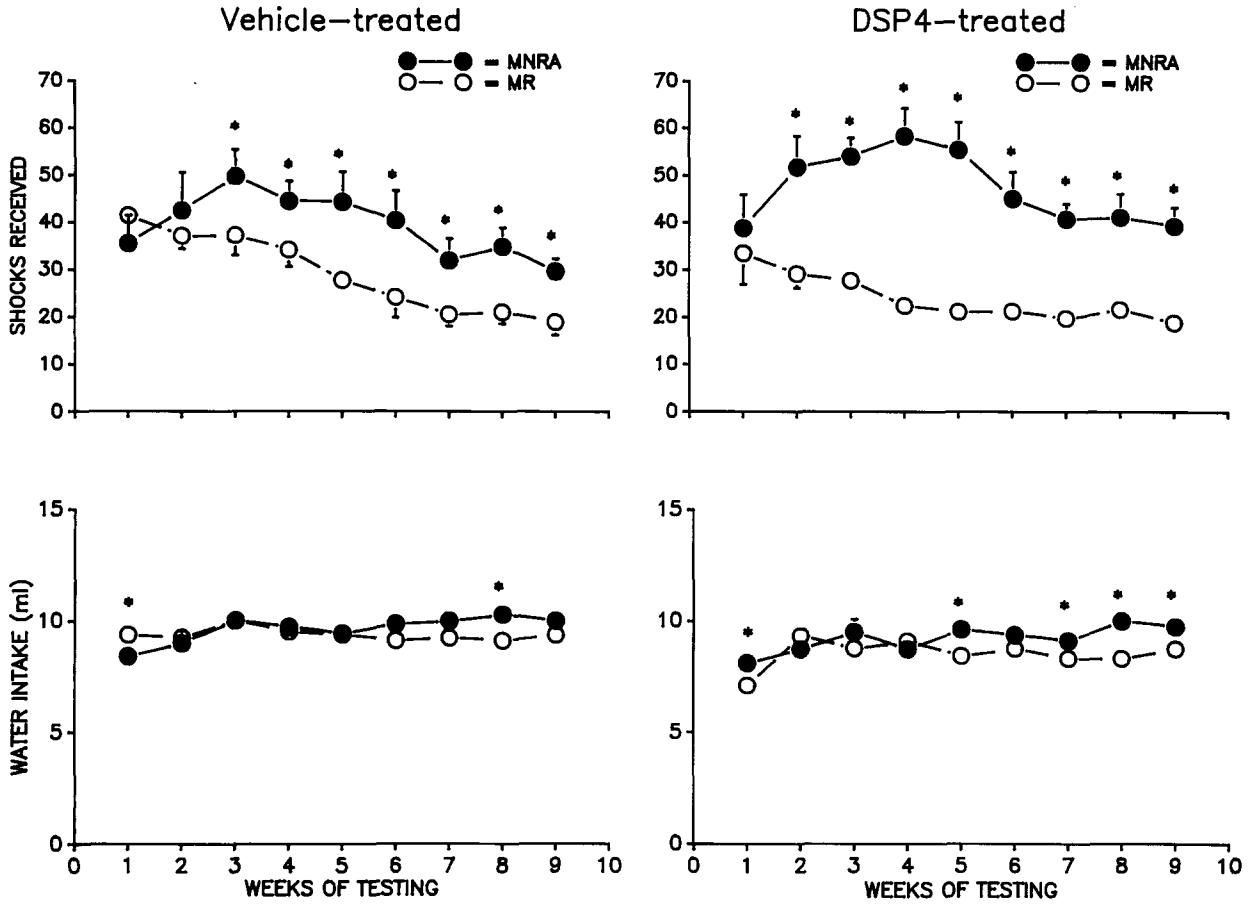


FIG. 4. Effects of DSP4 treatment on conditioned suppression of drinking (CSD) conflict behavior in previously untrained Maudsley reactive (MR) and Maudlsey nonreactive (MNRA) rats. Plotted are the mean ± SEM shocks received (top panels) and water intake (ml; bottom panels) in MR (open symbols) and MNRA (filled symbols) rats for 9 weeks following treatment with vehicle (saline; open symbols) or DSP4 (65 mg/kg, IP; filled symbols). DSP4 or vehicle were administered prior to initiation of CSD conflict testing.

*MNRA rats are significantly different ($p < 0.05$) from MR rats at the indicated test week and treatment condition (DSP4/vehicle), least significant differences posthoc test following factorial analysis of variance.

DSP4/vehicle treatment was not significant, $F(1, 28) < 1.0$, n.s. The DSP4/vehicle treatment × rat strains interaction, $F(1, 28) = 4.03$, $p < 0.05$, and the rat strains × test weeks interaction also were significant, $F(8, 224) = 5.52$, $p < 0.05$. Finally, the DSP4/vehicle treatment × test weeks, $F(8, 224) < 1.0$, n.s., and the rat strains × DSP4/vehicle treatment × test weeks, $F(8, 224) < 1.0$, n.s., interactions were not statistically significant. Posthoc LSD tests revealed that vehicle-treated MNRA accepted significantly more shocks than did vehicle-treated MR rats at test weeks 3-9, whereas DSP4-treated MNRA rats accepted significantly more shocks than DSP4-treated MR rats at test weeks 2-9. Thus, administration of DSP4 before CSD conflict training did not prevent the occurrence of gradually developing MNRA vs. MR difference in shocks received during conflict behavior testing; rather, DSP4 treatment increased slightly the magnitude of the MR vs. MNRA strain difference in punished responding in the CSD conflict paradigm.

The lower panels of Fig. 4 illustrate the effects of vehicle (lower left panel) or DSP4 treatment (lower right panel) on water intake in previously untrained MR and MNRA rats. As can be seen, for the first test week following treatment, DSP4 reduced water intake in both strains; this effect was more dramatic in MR rats. Water intake in both MR and MNRA rats treated with DSP4 returned to vehicle treatment levels by test week 2. Statistically, the main effects for rat strains, $F(1, 28) = 4.49$, $p < 0.05$, DSP4/vehicle treatment, $F(1, 28) = 7.95$, $p < 0.05$, and test weeks, $F(8, 224) = 7.33$, $p < 0.05$, were significant. The rat strains × test weeks interaction, $F(8, 224) = 3.78$, $p < 0.05$, and the rat strains × DSP4/vehicle treatment × test weeks interaction, $F(8, 224) = 1.94$, $p < 0.05$, also were significant. Finally, the rat strains × DSP4/vehicle treatment interaction, $F(1, 28) = < 1.0$, n.s., and the DSP4/vehicle treatment × test weeks interaction, $F(8, 224) = 1.58$, n.s., were not significant. Posthoc LSD tests revealed that vehicle-treated MR and MNRA rats differed significantly in their water intake at test weeks 1 (MR > MNRA) and 8 (MR < MNRA) and that DSP4-treated MR rats consumed significantly less water than did DSP4-treated MNRA rats at test weeks 1, 5, 7, 8, and 9.

Table 1 summarizes CSD conflict behavior of vehicle-treated MR and MNRA rats when conflict testing was reinitiated for 2 weeks after being suspended for 6 weeks. As can be seen, discontinuation of conflict testing for this period

TABLE 1

PERSISTENCE OF THE MR vs. MNRA STRAIN DIFFERENCE IN CSD CONFLICT BEHAVIOR

| Strain | Preinterruption | After 6 Weeks Without Conflict Testing |
|---|---|---|
| Punished responding (shocks received/session) | | |
| MR | 20 ± 3* | 16 ± 3 |
| MNRA | 32 ± 4† | 30 ± 5† |
| Unpunished responding [water intake (ml)/session] | | |
| MR | 9.2 ± 0.3 | 9.3 ± 0.2 |
| MNRA | 10.2 ± 0.4* | 10.1 ± 0.5* |

*Values represent the mean ± SEM (averaged across 2 weeks of conflict testing) obtained from 8 (MR) or 7 (MNRA) subjects.

†Values obtained from MNRA rats are significantly different from MR rats for the indicated test period, $p < 0.05$, LSD test following 2 × 2 factorial ANOVA with repeated measures.

slightly reduced punished responding in both groups; water intake was not appreciably affected. More important, however, this interruption of conflict testing did not result in a lessening of the MR vs. MNRA strain difference in punished responding. With respect to punished responding, there was a significant main effect for rat strains, $F(1, 13) = 10.20$, $p < 0.05$. There was no significant main effect for test-retest, $F(1, 13) = 3.47$, n.s., nor was the rat strains × test-retest interaction significant, $F(1, 13) = < 1$, n.s. With respect to water intake, there was a statistically significant main effect for rat strains, $F(1, 13) = 6.08$, $p < 0.05$, the main effect for test-retest, $F(1, 13) < 1.0$, n.s., and the rat strains × test-retest interaction were not significant, $F(1, 13) = < 1$, n.s.

The lower half of Table 2 illustrates pons medulla concentrations of NE and 5-HT in MR and MNRA rats sacrificed 35 weeks after DSP4 or vehicle treatment. As can be seen, there were no MR vs. MNRA differences in either NE or 5-HT concentrations in vehicle-treated rats. DSP4 treatment did not significantly affect 5-HT concentrations in either strain and significantly decreased NE concentrations to a comparable extent (approximately 20%) in both strains.

Scattergrams were constructed examining the possible relationship between conflict behavior (defined as the average number of shocks accepted per session for the last 2 weeks of conflict testing) and pons medulla concentrations of NE in MR and MNRA rats. There were no significant correlations between conflict behavior and NE concentration either when the data from each strain were analyzed separately (MR: $r = -0.19$; MNRA: $r = -0.40$) or when the data from the two strains were pooled ($r = -0.27$; data not shown).

*Experiment 3: DSP4 Effects on Neurochemistry 3 Weeks After Administration*

The upper half of Table 2 depicts the pons medulla concentrations of NE and 5-HT in MR and MNRA rats that were sacrificed 3 weeks after DSP4 or vehicle treatment. As can be seen, there were no MR vs. MNRA differences in either NE or 5-HT concentrations in vehicle-treated rats. DSP4 treatment did not significantly affect 5-HT concentrations in either strain and significantly decreased NE concentrations to a comparable extent (approximately 40%) in both strains. DSP4 produced a significantly greater depletion of NE when subjects were sacrificed 3 weeks after administration when compared to 35 weeks after administration (Experiment 3 vs. Experiment 2), as evidenced by the significant main effect for injection-sacrifice interval, $F(3, 42) = 4.34$, $p < 0.05$.

With respect to ptosis testing in DSP4-treated rats, none of the subjects in either strain exhibited a ptosis response at 6 h postadministration. Twenty-four hours after DSP4 administration, 100% of MR rats exhibited ptosis, whereas only 17% of MNRA rats were ptosis positive. At 48 h post-DSP4, 29% of MR subjects exhibited ptosis and none of the MNRA rats were ptosis positive. Statistically, the frequency of occurrence of ptosis was significantly greater in the MR strain (7/7) as compared to the MNRA strain (1/6) only at the 24-h treatment-examination interval, $\chi^2(1) = 6.285$, $p < 0.05$.

## DISCUSSION

In the CSD conflict paradigm, there was a dramatic difference in baseline punished responding between the Maudsley

TABLE 2

DSP4 TREATMENT EFFECTS ON PONS MEDULLA NE AND 5-HT CONCENTRATIONS IN MR AND MNRA RATS

| | Norepinephrine | | 5-Hydroxytryptamine | |
|---|---|---|---|---|
| | VEH | DSP4 | VEH | DSP4 |
| Sacrificed 3 weeks after DSP4/VEH treatment | | | | |
| MR | 308 ± 23* | 199 ± 65 (65)† | 460 ± 64 | 516 ± 98 (112) |
| MNRA | 390 ± 38 | 230 ± 38 (59)† | 488 ± 38 | 531 ± 103 (109) |
| Sacrificed 35 weeks after DSP4/VEH treatment | | | | |
| MR | 392 ± 36 | 307 ± 53 (78)† | 550 ± 102 | 690 ± 122 (125) |
| MNRA | 361 ± 20 | 277 ± 20 (77)† | 488 ± 39 | 427 ± 37 (88) |

Numbers in parentheses represent DSP4 treatment effects expressed as percent of VEH-treated control subjects.

*Values represent the mean ± SEM (ng amine/g wet weight tissue) obtained from six to eight subjects following 65 mg/kg DSP4 or saline (VEH) treatment.

†Values from DSP4-treated subjects are significantly different from those of VEH-treated subjects of the same strain and pretreatment interval, posthoc LSD test following 2 × 2 × 2 factorial ANOVA.

rat strains, with MNRA rats accepting significantly more shocks than their MR counterparts. As had been reported previously (6,7), this difference in baseline conflict behavior was not apparent in the early weeks of testing but developed gradually over the course of 4–8 weeks of CSD conflict testing. This MR vs. MNRA difference in conflict behavior was found to be resistant to extinction because discontinuation of conflict testing for 6 weeks did not reverse the MNRA vs. MR strain difference in punished responding that had developed over the course of several weeks of testing. Administration of the NE neurotoxin DSP4 24 h prior to initiation of CSD conflict testing did not prevent the occurrence of this time-dependent difference in conflict behavior in these Maudsley rats. DSP4 treatment in conflict-trained subjects also failed to reverse this MR vs. MNRA difference in conflict behavior. In addition, although changing the current intensity produced robust changes in punished responding in both MR and MNRA rat strains, it did not alter the effects of DSP4 on CSD conflict behavior in Maudsley rats.

The findings reported above do not support the hypothesis that anxiety-like states are the result of increased activity of noradrenergic neurons in the locus coeruleus [(26,27); see the introductory section]. This theory would predict that MR rats are more "anxious" than are rats of the MNRA strain due to increased activity of NE-containing neurons in the locus coeruleus in MR rats; thus, one would expect to see an increase in punished responding in MR rats following DSP4 administration because the noradrenergic neurons in these subjects would no longer be able to maintain the anxiety. The experimental results, however, are the opposite, with DSP4-treated rats of the MR strain accepting fewer shocks than saline-treated MR controls.

The experimental results also do not support the theory that postulates that the increased activity of NE-containing locus coeruleus neurons seen in response to anxiety-provoking stimuli represents the activity of a rectifying mechanism that helps the subject cope with the stress (1). This latter theory would predict that rats of the MNRA strain are less "anxious" than their MR counterparts in part because of the increased activity of locus coeruleus neurons in MNRA rats; thus, one would expect to see a decrease in punished responding in MNRA rats following DSP4 treatment because the anxiety-damping activity of the locus coeruleus presumably would be functioning less effectively. However, whether it was administered before or after CSD training DSP4 treatment did not decrease punished responding in rats of the MNRA strain.

In the CSD conflict paradigm, DSP4 treatment affected MR rats more than it affected MNRA rats, particularly with respect to the effects of DSP4 treatment on unpunished responding. DSP4 also produced a greater incidence of ptosis in MR rats when compared to MNRA rats. However, whether examined at 3 or 35 weeks posttreatment there was no MR vs. MNRA difference in the extent of NE depletion produced by DSP4. One might speculate from these data that MR rats are behaviorally more dependent upon normal NE tone than are MNRA rats.

In addition to the observation that treatment with DSP4 did not reduce the magnitude of the MR vs. MNRA difference in shocks received in the CSD conflict paradigm, correlation analyses comparing pons medulla concentrations of NE with conflict behavior (i.e., shocks received per session) revealed no statistical correlations between these two measures. This was true whether the data from MR and MNRA rats were evaluated separately or when the data from the two strains were pooled. Thus, the data from the present studies appear not to be consistent with either of the above-stated hypotheses regarding the role of NE in anxiety-like behavior. This conclusion must be tempered, however, given the relatively modest degree of noradrenergic depletion observed, at best 40% when subjects were sacrificed 3 weeks after treatment. Indeed, it is possible that a greater depletion of NE (e.g., using repeated administration of DSP4 or intraventricular administration of 6-hydroxydopamine) would produce different results.

The extent of NE depletion was dramatically less when subjects were sacrificed 35 weeks after administration relative to 3 weeks after DSP4 treatment. This finding is consistent with an earlier report by Dudley et al. (10), who reported that NE neurons exhibited at least partial recovery over long time periods following DSP4 administration. Nonetheless, even the 40% depletion at 3 weeks post-DSP4 is somewhat less than might be anticipated with this agent. In female Sprague-Dawley rats, pons medulla depletions have been approximately 60–70% when subjects are sacrificed 3 weeks post-DSP4 (D.J. Fontana, personal communication). Whether this apparent Maudsley vs. Sprague-Dawley strain difference in DSP4 sensitivity generalizes to other Wistar rats (the ancestors of the Maudsley lines) remains to be determined.

In summary, administration of DSP4 to MR and MNRA rats neither prevented nor reversed the time-dependent difference in baseline CSD conflict behavior observed in these Maudsley rat strains. Rather, DSP4 treatment tended to enhance the difference between the two rat strains on conflict behavior, with DSP4-treated MNRA rats accepting more shocks than vehicle-treated MNRA controls and DSP4-treated MR rats accepting fewer shocks than vehicle-treated MR controls. Thus, to the extent that the MR vs. MNRA difference in conflict behavior represents an animal model for genetically based differences in anxiety and/or fear behavior, the present findings do not support either of the two opposing theories regarding the role of NE in anxiety-like behavior. The mechanism for the gradual change in conflict behavior in MNRA rats compared to MR rats remains undetermined.

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## REFERENCES

1. Amaral, D. G.; Sinnamon, H. M. The locus coeruleus: Neurobiology of a central noradrenergic nucleus. Prog. Neurobiol. 9:147–196; 1977.
2. Blizard, D. A. The Maudsley reactive and nonreactive strains: A North American perspective. Behav. Genet. 11:469–489; 1981.
3. Broadhurst, P. L. Experiments in psychogenetics. In: Eysenck, H. J., ed. Experiments in personality, psychogenetics and psychopharmacology; London: Routledge and Kegan Paul; 1960: 44–70.
4. Broadhurst, P. L. A note on further progress in a psychogenetic experiment. Psychol. Rep. 1065–66; 1962.
5. Broadhurst, P. L. The Maudsley reactive and nonreactive strains of rats: A survey. Behav. Genet. 5:299–319; 1975.

6. Commissaris, R. L.; Harrington, G. M.; Ortiz, A. M.; Altman, H. J. Maudsley reactive and nonreactive rats: Differential performance in a conflict task. Physiol. Behav. 38:291–294; 1986.
7. Commissaris, R. L.; McCloskey, T. C.; Harrington, G. M.; Altman, H. J. MR and MNRA Maudsley rat strains: Differential response to chlordiazepoxide in a conflict task. Pharmacol. Biochem. Behav. 32:801–805; 1989.
8. Commissaris, R. L.; Moore, K. E.; Rech, R. H. The effects of *d*-lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM), pentobarbital and methaqualone on punished behavior in control and 5,7-dihydroxytrypamine-treated rats. Pharmacol. Biochem. Behav. 14:617–623; 1981.
9. Commissaris, R. L.; Rech, R. H. Interactions of metergoline with diazepam, quipazine and hallucinogenic drugs on a conflict behavior in the rat. Psychopharmacology (Berl.) 76:282–285; 1982.
10. Dudley, M. W.; Howard, B. D.; Cho, A. The interaction of the beta-haloethyl benzylamines, xylamine, and DSP-4 with catecholaminergic neurons. Annu. Rev. Pharmacol. Toxicol. 30:387–403; 1990.
11. Eysenck, H. J.; Broadhurst, P. L. Experiments with animals: Introduction. In: Eysenck, H. J., ed. Experiments in motivation. New York: Pergamon Press; 1964:285–291.
12. Fontana, D. J.; Carbary, T. J.; Commissaris, R. L. Effects of acute and chronic antipanic drug administration on conflict behavior in the rat. Psychopharmacology (Berl.) 98:157–162; 1989.
13. Fontana, D. J.; Commissaris, R. L. Anxiolytic-like effects of alpha-2-adrenoceptor agonists on conflict behavior in the rat: Pre- vs. postsynaptic receptor mechanisms. Pharmacol. Biochem. Behav. 43:697–703; 1992.
14. Ford, R. D.; Rech, R. H.; Commissaris, R. L.; Mayer, L. Effects of acute and chronic interactions of diazepam and *d*-amphetamine on punished and unpunished behavior of rats. Psychopharmacology (Berl.) 65:197–204; 1979.
15. Galloway, M. P. Neuropharmacology of cocaine: Effects on dopamine and serotonin systems. In: Lakoski, J. M.; Galloway, M. P.; White, F. J., eds. Cocaine: Pharmacology, Physiology, and Clinical Strategies. Boca Raton, FL: CRC Press; 1991:163–189.
16. Geller, I.; Kulak, J. T.; Seifter, J. Attenuation of chlordiazepoxide and chlorpromazine on a punished discrimination. Psychopharmacologia 3:374–385; 1962.
17. Geller, I.; Seifter, J. The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492; 1960.
18. Grant, S. J.; Huang, H. Y.; Redmond, D. E. Benzodiazepines attenuate single unit activity in the locus coeruleus. Life Sci. 27: 2231; 1980.
19. Hall, C. Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. J. Comp. Physiol. Psychol. 18:385–403; 1934.
20. Harrington, G. M. The Har strains of rats: Origin and characteristics. Behav. Genet. 11:445–468; 1981.
21. Harrington, G. M.; Blizard, D. A. Open-field behavior in the Maudsley reactive and nonreactive strains: Procedural variations. Behav. Genet. 13:91–94; 1983.
22. Jonsson, G.; Hallman, H.; Ponzio, F.; Ross, S. DSP4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzamine)—a useful denervation tool for central and peripheral noradrenaline neurons. Eur. J. Pharmacol. 72:173–188; 1981.
23. Kilts, C. D.; Commissaris, R. L.; Rech, R. H. Comparison of anticonflict drug effects in three experimental models of anxiety. Psychopharmacology (Berl.) 74:290–296; 1981.
24. Libet, B.; Feinstein, B.; Gleason, C. A. Stimulation of locus coeruleus in man, via chronically implanted electrodes. Soc. Neurosci. Abstr. 12:1162; 1986.
25. McCloskey, T. C.; Paul, B. K.; Commissaris, R. L. Buspirone effects in an animal conflict procedure: Comparison with diazepam and phenobarbital. Pharmacol. Biochem. Behav. 27:171–175; 1987.
26. Redmond, D. E. New and old evidence for the involvement of a brain norepinephrine system in anxiety. In: Fann, W. E., ed. The Phenomenology and Treatment of Anxiety. New York: Spectrum; 1979:153–203.
27. Redmond, D. E.; Huang, Y. H.; Snyder, D. R.; Maas, J. W. Behavioral effects of stimulation of the locus coeruleus in the stumptail monkey. Brain Res. 116:502–510; 1976.
28. Ross, S. B. Long term effects of *N*-2-chloroethyl-*N*-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurones in the rat brain and heart. Br. J. Pharmacol. 58:521–527; 1976.
29. Steele, R. G.; Torrie, J. H. Principles and procedures of statistics. New York: McGraw-Hill; 1985.
30. Vogel, J. R.; Beer, B.; Clody, D. E. A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacologia 21:1–7; 1971.