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Biochemical and Behavioral Correlates of Chronic Stress: Effects of Tricyclic Antidepressants

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SOBLOSKY, J. S. AND J. B. THURMOND. *Biochemical and behavioral correlates of chronic stress: Effects of tricyclic antidepressants.* PHARMACOL BIOCHEM BEHAV **24**(5) 1361–1368, 1986.—Using a chronic stress model of depression, the biochemical, hormonal, and neurochemical effects of chronic stress were determined in male CD-1 mice. The effects of chronic administration of three tricyclic antidepressants (TCA): chlorimipramine, amitriptyline and desmethylimipramine, as well as fluoxetine, a specific serotonin uptake inhibitor, were also evaluated. Exposure to acute noise/light stress dramatically increased motor activity (behavioral activation) in comparison with basal (unstressed) activity. However, animals with a history of chronic stress exhibited reduced basal activity levels as well as a decreased behavioral activation response to acute stress. There was also exaggerated corticosterone (CS) responding in both of these behavioral test situations attributable to prior chronic stress exposure. Chronic treatment with any of the TCAs significantly restored the behavioral activation response to acute stress and normalized CS responding in chronically stressed animals. Chronic fluoxetine treatment was ineffective. In chronically stressed, but behaviorally untested (quiescent) mice, there were no changes in CS levels, but norepinephrine (NE) and 5-hydroxyindoleacetic acid (5-HIAA) levels were increased. However, chronically stressed mice tested for basal motor activity showed large NE decreases, while those receiving acute stress exposure prior to testing showed large NE decreases and further 5-HIAA increases. There were no alterations on neurochemical parameters due to any drug treatment which could be correlated with a possible mechanism for their efficacy, although evidence suggested NE involvement. It was further proposed that the chronic stress paradigm induced conditioned neuroendocrine and neurochemical responses.

Locomotor activity Animal model Depression Chronic stress Antidepressant Amitryptyline Chlorimipramine Desmethylimipramine Fluoxetine Corticosterone Serotonin 5-Hydroxyindoleacetic acid Norepinephrine

ALTHOUGH clinical depression can be subdivided into several categories based on their etiologies, there is reason to believe that some endogenous depressions may be precipitated by environmental factors such as stress [4]. It is well known that the inability to successfully cope with or adapt to stress can lead to ulcers, heart disease, hormonal imbalances, neurochemical changes, and altered affective states. A review of pervasive effects of stress on physiological and psychological parameters may be found elsewhere [10].

The ability to cope with stress is a factor in determining how and if stress-induced pathologies are manifested. It has been shown that uncontrollable stress can alter neurochemical parameters and induce a behavioral state of learned helplessness in rats which can be ameliorated by tricyclic antidepressant (TCA) treatments [30,37]. The phenomena of learned helplessness has been proposed as a suitable animal model of depression [35]. Evidence has also been obtained from mice indicating that uncontrollable stress influences behavioral and neurochemical activity to varying degrees and specificity as a function of experimental, environmental, and organismic factors [4].

Another stress-dependent animal model of depression which appears to adequately fulfill the requirements of predictive, face, and construct validities [44] was developed by Katz and colleagues utilizing chronic, or more appropriately, chronic intermittent stress. Rats were subjected to a variety of unpredictable stressors on a daily basis over a three week period. Two days after the end of this period the rats were

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TABLE 1
CHRONIC STRESS REGIMEN PROTOCOL

Day	Stressor
1.	Shock (30 min)
2.	Food Deprivation (48 hr)
3.	Shaker (30 min)
4.	Tail Pinch (1 min)
5.	Cold Swim (30 min at 4°C)
6.	Water Deprivation (24 hr)
7.	Shock (30 min)
8.	Isolation (48 hr)
9.	Cold Swim (3 min at 4°C)
10.	Heat (5 min at 40°C)
11.	Tail Pinch (1 min)
12.	Cold Swim (3 min at 4°C)
13.	Shock (60 min)
14.	Shaker (60 min)
15.	—
16.	Test

exposed to a novel acute stress (noise/light) then tested for open field activity. In nonchronically stressed rats exposure to the novel acute stress resulted in an increase in open field activity, termed an activation response, and increased corticosterone (CS) output. However, chronically stressed rats exhibited a severe blunting of the behavioral activation response and exaggerated CS responding. It was subsequently shown that the activation response to the noise/light stress and exaggerated CS response could be significantly normalized in chronically stressed rats whom also received chronic concominant treatments with TCAs [19, 24, 33], monoamine oxidase inhibitors [18,20], the novel antidepressants iprindole, bupropion or mianserin [22], or electroconvulsive shock [21]. The necessity for chronic TCA treatment was determined [24], as were the ineffectiveness of antihistamines, antipsychotics, anxiolytics [23], anticholinergics [19], and amphetamines [20] in restoring normal responding.

As this animal model has been well examined using rats as subjects, it was of interest to determine if similar behavioral, hormonal, and drug effects would be produced in mice subjected to a chronic intermittent stress regimen. Furthermore, in order to get a more complete picture of this chronic stress paradigm, additional control groups were utilized and brain neurochemical analyses were performed.

METHOD

Animals

Male mice of the CD-1 strain (Carworth Farms, Wilmington, MA), 90–150 days old, were housed five to a cage with food and water available ad lib. The laboratory was maintained at a temperature of 21°C, with a light cycle of 12 hr on, 12 hr off. All procedures were performed during the animals' active period between the second and fifth hours of the dark cycle.

Apparatus and Behavioral Procedure

Locomotor activity was assessed using the motimeter described by Knoll [26]. In this device the animal moves over aluminum contact plates mounted 4 mm apart in a clear Plexiglas box (testing cage) and a count is automatically recorded for every passage between two plates during a timer operated test period (6 min).

The acute stress (noise/light) procedure and parameters were similar to those previously described by Katz *et al.* [18–24, 33]. Subjects were randomly chosen, placed into individual cages without food or water, transported to a soundproof room illuminated by three 75 W bulbs and placed one meter from a speaker emitting 95–100 dB as white noise. After one hour exposure, the animal was transported to the test room, immediately placed in the motimeter for 6 min, then sacrificed for subsequent neurochemical or CS determination within 2 min of completion of the test period. Half (n=5) the mice for each experimental group (n=10) were used for neurochemical determinations and the other half for CS assays. Animals not receiving acute noise/light stress prior to behavioral testing were instead transported to a darkened room with only background noise exposure one hour prior to behavioral testing. To assess the neurochemical and CS effects of behavioral testing alone (i.e., without prior acute stress exposure) a second control was included. This group differed in that the animals were left undisturbed on the test day and were sacrificed upon immediate removal from their home cage.

The chronic stress regimen used was a variant of Katz *et al.* [18–24, 33]. Stressors were administered one per day over a period of 14 days, between the first and eight hour of the dark cycle. This was done to maximize the unpredictability of the nature of the stressor and the time of delivery. The stressors used were: 30 minutes of scrambled unpredictable/uncontrollable footshock (three times; approximately one 0.75 mA shock/60 seconds; shocks averaged 10 seconds duration and ranged from 1–15 seconds; shock generator was a C. J. Applegate Model 230 stimulator); 48 hour food deprivation (one time); 24 hour water deprivation (one time); 30 and 60 minutes of horizontal shaker stress (two times; a standard laboratory shaker was used and run at 200 displacements/minute); tail pinch (two times; rubber dammed forceps applied one cm from the base of the tail, closed to the first notch and pressured maintained for one minute); 3 minutes cold swim (three times; water temperature was maintained at 4°C with ice added as necessary); 48 hour isolation (one time); 5 minutes, 40°C heat stress (one time; heat stress apparatus consisted of a foiled lined box in which two 150 W spotlights were enclosed and temperature controlled via a rheostat). The exact order of presentation is detailed in Table 1.

On day 16, chronically stressed animals were assigned to one of the three experimental groups: nontested, behavioral testing only (no acute stress) or exposure to acute noise/light stress prior to behavioral testing.

Drugs and Injection Protocol

The drugs used in this study consisted of three tricyclic antidepressants: amitryptyline hydrochloride (Merck, Sharpe and Dohme, Cincinnati, OH), desmethylimipramine hydrochloride (USV Pharmaceuticals, Tuckahoe, NJ) and chlorimipramine hydrochloride (Cibe-Geigy, Summit, NJ) as well as the specific serotonin (5-HT) uptake inhibitor, fluoxetine hydrochloride (Eli Lilly, Indianapolis, IA). All drugs were freshly prepared in 0.9% saline and were injected intraperitoneally in a volume of 2.5 ml/kg body weight. Control animals received 0.9% saline only. All drugs were administered in 5 mg/kg doses (as hydrochloride salts)

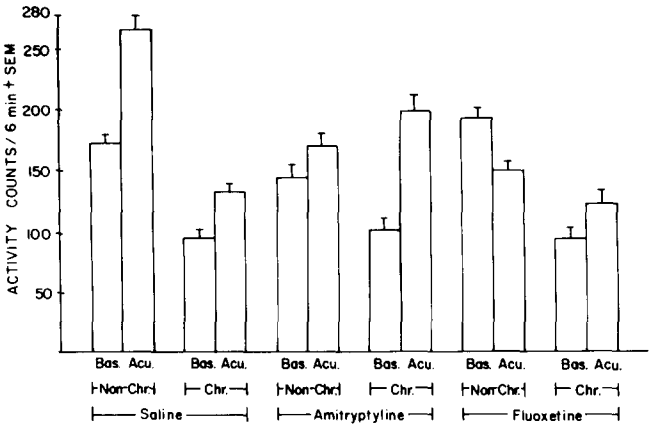

FIG. 1. Reversal of chronic stress produced activation deficit by amitryptyline. Locomotor activity expressed as number of counts (with SEM) in 6 minutes of testing in a motimeter (see test) are presented. Bas=basal (no acute stress). Acu=acute stress, consisting of 1 hour exposure to 95 dB of white noise and bright light prior to testing. Non-Chr=no chronic stress; standard laboratory housing. Chr=2 weeks of chronic intermittent stress exposure involving various stressors (see text). All animals received daily injections of saline (vehicle) or drug (5 mg/kg) for 2 weeks. Although amitryptyline had no intrinsic activating effects, it did restore normal acute noise/light stress elicited activation.

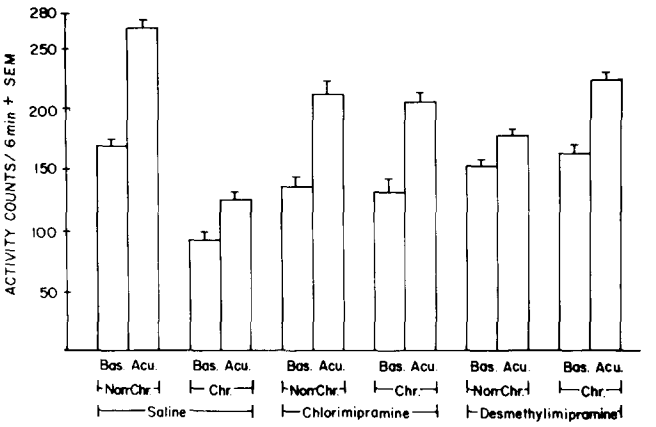

FIG. 2. Reversal of chronic stress produced activation deficit by chlorimipramine and desmethylimipramine. Locomotor activity expressed as number of counts (with SEM) in 6 minutes of testing in a motimeter (see text) are presented. Abbreviations used are identical to those in Fig. 1. All animals received daily injections of saline (vehicle) or drug (5 mg/kg) for two weeks. Although chlorimipramine and desmethylimipramine had no intrinsic activating effects, it did restore normal acute noise/light stress elicited activation. (NOTE: The saline data represents the results of separately conducted experiments from the saline data in Fig. 1.)

and were based on clinically effective doses of these compounds.

All drugs were administered once daily for two weeks to all experimental groups. Animals receiving chronic stress were injected one to three hours prior to exposure to the stressor required for that day. No drugs or stressors were administered 36–48 hours prior to testing and/or sacrifice on day 16.

Biochemical Procedures

Animals used for neurochemical assays were quickly sacrificed by cervical dislocation. Brains were then removed, split equally into two halves down the longitudinal fissue and weighed prior to being frozen for subsequent assays. One half was used to determine 5-HT and the metabolites, while the other half was used to determine the catecholamines. Procedures for quantification of the amines and their metabolites involved the use of high pressure liquid chromatography (HPLC) with electrochemical detection. The method of Perry and Fuller [29] was used for measuring 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) levels. Samples were put on a HPLC unit (Bioanalytical Systems, West Lafayette, IN) equipped with a Hexyl C6 HiChrom Reversible column (Regis Chem. Co., Morton Groves, IL). The mobile phase was 0.1 M dibasic sodium phosphate, 0.05 M citric acid, 10% methanol, pH 4.8. The mobile phase was filtered through a Millipore system equipped with a GS 0.22 μM filter and degassed prior to use. Flow rate was 0.75 ml/min for the metabolites and 1 ml/min for 5-HT. The detector was BAS model LC-2A equipped with a carbon paste electrode and run at a potential of 0.9 V versus silver-silver chloride reference electrode. Norepinephrine (NE) and dopamine (DA) were quantified using the procedures of Wagner *et al.* [41] with modifications. Samples were put on a separate HPLC unit equipped with a Bio-Sil ODS-5S (250×4 mm), Reverse Phase Column (Bio Rad Labs., Richmond, CA). The mobile phase consisted of 0.007 M dibasic sodium phosphate, 0.015 M citric acid, 2.5–5% methanol, 35–50 mg/l octyl sodium sulfate, pH 3.85 and was run at a flow rate of 1 ml/min. The detector was a BAS model LC-3 glassy carbon electrode maintained at a potential of 0.9 V versus a silver-silver chloride reference electrode. The fluorometric method described by Guillemen *et al.* [15] with the modifications by Givener and Rocheforte [14] was employed for the determination of blood plasma CS levels.

Statistics

Two overall multivariate analyses of variance (MANOVA) for independent observations were performed, one for behavioral measures (n=10 per cell) and another for biochemical data (n=5 per cell). In order to evaluate the effects of chronic stress on corticosterone and neurochemical parameters of quiescent or otherwise undisturbed mice, additional untested controls (chronically and nonchronically stressed) were included in the study. Therefore, for biochemical comparisons there are three levels of a condition factor: untested vs. behavioral testing alone (no prior acute stress) vs. acute stress prior to behavioral testing. For behavioral comparisons the condition factor is termed the acute stress factor as there are only two levels: behavioral testing alone vs. acute stress prior to behavioral testing. Since the design of the study specifies in advance which comparisons are to be made, subsequent analyses were performed as planned comparisons or Dunnetts' tests were appropriate [27].

RESULTS

Locomotor Activity

The univariate tests provided by the MANOVA analysis indicated a treatment × chronic stress interaction,

TABLE 2

EFFECTS OF ACUTE AND CHRONIC STRESS AND FOUR COMPOUNDS ON PLASMA CORTICOSTERONE LEVELS

Treatment Condition	Non-Chronic NT	Non-Chronic Bas.	Non-Chronic Acu.	Chronic NT	Chronic Bas.	Chronic Acu.
Saline	9.6 ± 1.6	16.7 ± 1.4*	27.7 ± 1.5†	11.6 ± 1.6	27.9 ± 1.6‡	37.0 ± 2.0‡
Amitriptyline	12.6 ± 3.0	22.4 ± 1.7*	33.3 ± 2.8†	7.8 ± 0.9	21.0 ± 1.1	30.1 ± 0.9
Fluoxetine	9.9 ± 0.9	16.8 ± 1.4*	26.0 ± 2.4†	9.2 ± 1.4	27.0 ± 0.7‡	34.4 ± 2.3‡
Saline	9.9 ± 1.3	20.4 ± 1.0*	27.8 ± 2.2†	11.4 ± 1.4	28.8 ± 1.9‡	38.5 ± 1.5‡
Chlorimipramine	11.4 ± 2.5	20.9 ± 2.5*	32.5 ± 1.6†	11.5 ± 1.5	19.2 ± 1.9	32.8 ± 2.4
Desmethylimipramine	10.0 ± 0.9	17.5 ± 2.6*	24.2 ± 3.9†	9.2 ± 0.6	17.8 ± 1.0	28.7 ± 2.0

Results are given as mean (μg/100 ml plasma) ± SEM (n=5). NT=non-tested; mice were sacrificed upon immediate removal from group housing. Bas.=basal (no acute stress); mice received behavioral testing only, Acu.=acute stress; mice were exposed to one hour of noise/light stress prior to behavioral testing. All animals received daily injections of saline (vehicle) or drug (5 mg/kg) for two weeks.

*=significantly increased from non-tested control.

†=significantly increased from control (Bas.).

‡=significantly increased from nonchronically stressed control.

TABLE 3

EFFECTS OF ACUTE AND CHRONIC STRESS AND FOUR COMPOUNDS ON WHOLE BRAIN SEROTONIN LEVELS

Treatment Condition	Non-Chronic NT	Non-Chronic Bas.	Non-Chronic Acu.	Chronic NT	Chronic Bas.	Chronic Acu.
Saline	519 ± 8	497 ± 10	572 ± 18*	496 ± 15	537 ± 27	628 ± 7*
Amitriptyline	502 ± 4	549 ± 7	542 ± 19	540 ± 9	590 ± 14	660 ± 18*
Fluoxetine	521 ± 16	475 ± 7	495 ± 19	481 ± 9	461 ± 14	455 ± 18
Saline	498 ± 18	484 ± 6	588 ± 6*	513 ± 6	507 ± 18	557 ± 14*
Chlorimipramine	528 ± 19	545 ± 19	570 ± 15	522 ± 16	519 ± 10	568 ± 6*
Desmethylimipramine	538 ± 4	565 ± 12	572 ± 15	521 ± 4	560 ± 13	642 ± 11*

Results are given as mean (ng/g) ± SEM (n=5). Abbreviations are identical to those in Table 2.

*=significantly increased from control (Bas.).

F(5,216)×39.03, a treatment × acute stress (noise/light) interaction, F(5,216)=11.39 and a treatment × acute stress × chronic stress interaction, F(5,216)=10.25, $p<0.001$ in all cases.

Planned comparisons indicated that in nonchronically stressed vehicle control mice there were activity increases due to acute stress exposure, F(1,108)=4.37 and 4.32, $p<0.001$, in both cases. This effect has been described as the "activiation response" to acute noise/light stress exposure. The effect of chronic stress on locomotor activity was evident, as chronically stressed vehicle control mice exhibited decreases in basal activity, F(1,108)=31.36 and 29.70, $p<0.001$ in both cases, as well as in the activation response to acute stress, F(1,108)=85.20 and 80.30, $p<0.001$ in both cases, as compared to the nonchronically stressed vehicle control mice. However, there was a significant increase, or partial restoration, of the activation response to acute stress, as compared to the chronically stressed vehicle control groups, in mice receiving chronic treatment with one of the TCAs: amitryptyline, $t(27)=4.51$, chlorimipramine, $t(27)=4.38$ and desmethylimipramine, $t(27)=6.23$, $p<0.01$ in all cases. Chronic treatment with fluoxetine was ineffective in restoring the activation response (Figs. 1 and 2).

Corticosterone

The univariate tests provided by the MANOVA analysis indicated a treatment × chronic stress interaction, F(5,144)=8.43, $p<0.001$, a condition × chronic stress interaction, F(2,144)=4.40, $p<0.014$, and a treatment × condition × chronic stress interaction, F(10,144)=2.62, $p<0.01$.

In nonchronically stressed mice planned comparisons revealed increases in CS levels of mice tested for basal activity in all treatment groups as compared to untested controls: F(1,72)=7.30, 7.09 and 8.06, $p<0.01$ for saline, fluoxetine and desmethylimipramine respectively and Fs=16.89, 17.95 and 16.00, $p<0.001$, for saline, amitryptyline and chlorimipramine respectively. These results indicate that mere exposure to the testing apparatus was stressful. There were additional increases in CS output in mice exposed to the acute noise/light stress prior to behavioral testing in all treatment groups: desmethylimipramine, F(1,72)=6.48, $p<0.05$, saline, F(1,72)=8.40, $p<0.01$, saline, fluoxetine, amitryptyline and chlorimipramine, Fs=17.30, 18.14, 14.28 and 30.58, respectively, $p<0.001$ in all cases. These results indicate that noise/light exposure prior to testing was indeed stressful (Table 2).

TABLE 4

EFFECTS OF ACUTE AND CHRONIC STRESS AND FOUR COMPOUNDS ON WHOLE BRAIN 5-HYDROXYINDOLEACETIC ACID LEVELS

Treatment Condition	Non-Chronic NT	Non-Chronic Bas.	Non-Chronic Acu.	Chronic NT	Chronic Bas.	Chronic Acu.
Saline	422 ± 10	431 ± 17	499 ± 7*	490 ± 9†	473 ± 15	560 ± 13*
Amitriptyline	386 ± 1	423 ± 25	492 ± 24*	494 ± 21*	514 ± 19	649 ± 18*
Fluoxetine	310 ± 10	311 ± 15	315 ± 13	359 ± 3†	351 ± 16	379 ± 15
Saline	415 ± 17	434 ± 6	492 ± 9*	519 ± 12*	502 ± 18	577 ± 7*
Chlorimipramine	279 ± 7	303 ± 15	301 ± 13	428 ± 16†	415 ± 7	469 ± 20*
Desmethylimipramine	418 ± 20	394 ± 15	465 ± 10*	471 ± 15†	462 ± 11	507 ± 15*

Results are given as mean (ng/g) ± SEM (n=5). Abbreviations are identical to those in Table 2.
*=significantly increased from control (Bas.).
†=significantly increased from nonchronic, nontested control.

TABLE 5

EFFECTS OF ACUTE AND CHRONIC STRESS AND FOUR COMPOUNDS ON WHOLE BRAIN NOREPINEPHRINE LEVELS

Treatment Condition	Non-Chronic NT	Non-Chronic Bas.	Non-Chronic Acu.	Chronic NT	Chronic Bas.	Chronic Acu.
Saline	461 ± 19	442 ± 6	457 ± 22	525 ± 12*	456 ± 14†	464 ± 17
Amitriptyline	468 ± 14	452 ± 6	436 ± 22	536 ± 14*	481 ± 17†	483 ± 10
Fluoxetine	465 ± 15	460 ± 18	466 ± 18	510 ± 8*	458 ± 11†	462 ± 10
Saline	469 ± 20	452 ± 11	431 ± 16	534 ± 8*	440 ± 10†	461 ± 20
Chlorimipramine	465 ± 18	464 ± 14	445 ± 9	528 ± 14*	480 ± 11†	467 ± 11
Desmethylimipramine	441 ± 11	460 ± 12	453 ± 8	513 ± 8*	472 ± 11†	463 ± 14

Results are given as mean (ng/g) ± SEM (n=5). Abbreviations are identical to those in Table 2.
*=significantly increased from nonchronic, nontested control.
†=significantly decreased from nontested control.

In chronically stressed untested mice there were no increases in the resting CS levels of any treatment group, with all probabilities >0.5, however, there were significant increases in CS levels of chronically stressed mice tested for basal activity in the saline, $F(1,72)=7.60$, $p<0.01$ and $F(1,72)=18.40$, $p<0.001$, and fluoxetine, $F(1,72)=21.25$, $p<0.001$, treatment groups as compared to the nonchronically stressed controls. Conversely, mice receiving treatments with one of the three TCAs did not exhibit significantly different levels (all probabilities >0.2). There were also significant additional CS increases due to prior acute stress exposure in chronically stressed mice receiving saline, $F(1,72)=8.30$, $p<0.01$ and $F(1,72)=12.50$, $p<0.001$, or fluoxetine, $F(1,72)=7.92$, $p<0.01$, as compared to the nonchronically stressed controls. Again, mice receiving treatments with one of the three TCAs did not show this exaggerated CS output due to chronic stress (all probabilities >0.1). In fact, the CS values of chronically stressed mice receiving one of the TCAs did not significantly differ (all probabilities >0.1) even if compared to the values of the appropriate nonchronically stressed saline controls (Table 2).

Neurochemistry

The univariate tests provided by the MANOVA analysis indicated on 5-HT due to a treatment × condition interaction, $F(10,144)=9.83$, a treatment × chronic stress interaction, $F(5,144)=11.94$, and a treatment × chronic stress × condition interaction, $F(10,144)=3.08$, $p<0.001$ in all cases.

In nonchronically stressed mice, there were increases in 5-HT due to acute noise/light stress only in the saline control groups, $F(1,72)=8.93$ and 9.24, $p<0.01$ in both cases. However, all chronically stressed mice, except those receiving fluoxetine treatment, exhibited increases in 5-HT levels in the same situation: saline, $F(1,72)=13.61$, $p<0.001$ and $F(1,72)=4.25$, $p<0.05$, chlorimipramine, $F(1,72)=4.10$, $p<0.05$, amitriplyline and desmethylimipramine, $Fs=8.05$ and 11.05 respectively, $p<0.01$ in both cases (Table 3).

Significant effects were obtained on 5-HIAA due to a treatment by condition interaction, $F(10,144)=8.18$, a treatment × chronic stress interaction, $F(5,144)=18.13$, and a treatment × condition × chronic stress interaction, $F(10,144)=3.47$, $p<0.001$ in all cases.

Planned comparisons indicated that in nonchronically stressed mice exposed to acute stress there were increases in 5-HIAA levels in those groups receiving saline, $F(1,72)=4.52$ and 11.31, $p<0.05$ and $p<0.01$ respectively, amitriptyline, $F(1,72)=11.31$, $p<0.01$, or desmethylimipramine, $F(1,72)=4.01$, $p<0.05$, treatments. In chronically stressed mice, there were increases in the 5-HIAA levels of the untested mice of all the treatment groups: saline, $F(1,72)=11.08$ and 29.91, $p<0.01$ and $p<0.001$ respectively, fluoxetine,

chlorimipramine, desmethylimipramine, Fs=7.20, 8.23 and 11.13 respectively, all probabilities <0.01, and amitryptyline, F=32.94, $p<0.001$. These results indicate that chronic stress by itself caused increased 5-HT turnover in quiescent mice. Although behavioral testing alone had no additional effect on 5-HIAA levels, there were further increases found in chronically stressed mice exposed to the acute noise/light stress in all the treatment groups except fluoxetine: chlorimipramine, F(1,72)=6.91, $p<0.05$, both saline groups, amitriptyline and desmethylimipramine, Fs=13.65, 18.14, 43.42, 11.64 respectively, all probabilities <0.001 (Table 4).

Univariate tests indicated significant effects on NE due a treatment × condition interation, F(10,144)=3.12, a treatment × chronic stress interaction , F(5,144)=10.92, $p<0.001$ in both cases, and a treatment × condition × chronic stress interaction, F(10,144)=2.26, $p<0.02$.

In nonchronically stressed mice there were no significant effects on NE levels under any condition or treatment group. However, planned comparisons indicated that there were increases in NE levels of all the chronically stressed untested mice in all the treatment groups as compared to nonchronically stressed controls: fluoxetine, desmethylimipramine, F(1,72)=5.18 and 5.72 respectively, $p<0.05$, both saline groups, amitriptyline and chlorimipramine, Fs=7.56, 7.67, 11.84 and 10.16 respectively, all probabilities <0.01. However, there were decreases in the NE levels in the behaviorally tested mice of all the treatment groups, as compared to untested controls: chlorimipramine and desmethylimipramine, F(1,72)=5.90 and 5.10 respectively, $p<0.05$, fluoxetine, F=7.10, $p<0.01$, both saline groups and amitriptyline, Fs=11.30, 12.19 and 14.40 respectively, all probabilities <0.001. Those chronically stressed mice exposed to acute stress prior to testing exhibited no further alteration in NE levels and appeared similar to those receiving only behavioral testing (Table 5).

Significant effects were obtained for DA due to a treatment × condition interaction, F(10,144)=1.87, $p<0.05$. There were no effects due to chronic stress, F(1,144)=0.79, $p<0.35$. In nonchronically stressed mice, there were increases in DA levels only in saline control groups exposed to acute noise/light stress prior to testing, F(1,72)=4.25 and 4.63, $p<0.05$ in both cases. There were no significant effects on HVA (data not shown).

DISCUSSION

The behavioral results obtained in this study using mice essentially parallel those obtained by Katz *et al.* [18–24, 33] in rats when similar stressors and testing procedures were employed. Firstly, the effectiveness of the acute noise/light stress in eliciting behavioral activation was clearly evident in nonchronically stressed saline control mice. However, nonchronically stressed drug pretreated mice failed to show a large behavioral activation to the acute stressor. This effect has also been noted in rats and it was proposed that this may represent a lack of intrinsic activating effects of the drugs [22].

It was clearly evident that after chronic stress exposure, there was a lack of basal behavioral activity as well as a severe blunting of the behavioral activation response to acute stress. However, mice which received concominant chronic pretreatment with one of the three TCAs displayed a significant restoration of the behavioral activation response, while those treated with fluoxetine exhibited no improvement (Figs. 1 and 2).

The CS results in nonchronically stressed mice under all drug treatment conditions followed expected patterns of responding. Exposure to the testing apparatus alone caused increased CS output, which is indicative of stress due to exposure to a novel situation [31]. Exposure to acute noise/light stress prior to behavioral testing produced a seemingly additive CS response.

After chronic stress the CS levels of undisturbed quiescent mice were not different from the nonchronically stressed controls. Similar results have been reported in rats which have also been exposed to a chronic stress regimen composed of various stressors [5]. However, chronically stressed saline control mice displayed significantly increased or exaggerated CS responding whether receiving only behavioral testing or acute stress prior to behavioral testing. Moreover, mice pretreated with a TCA failed to display abnormal CS responding and appeared identical to nonchronically stressed controls. Again, fluoxetine failed to restore normal responding (Table 2).

In chronic stress studies, usually one type of stressor is employed. It has been frequently shown that under these circumstances, CS levels decline to baseline levels during the course of chronic stress, which signifies adaptation or habituation to the particular stressor [25]. The habituation to stress has also been reported to be stressor specific, i.e., no cross adaptation between different types of stressors [17]. Under chronic isolation and cold stresses, adaptation does occur; however, if a novel acute stressor is presented, CS and ACTH responses are faster and markedly elevated when compared to nonchronically stressed rats [34,40]. Additionally, chronically stressed rats were shown to elicit significantly more CS in response to ACTH injections [5]. The present results also show that chronically stressed mice exhibited normal baseline levels, but reacted to novel situations with exaggerated CS responses. It was subsequently found that over a period of time in a chronic stress procedure, the animals began to exhibit exaggerated CS responding before the stressor was even presented [8,9]. The results were explained in the framework of a conditioned neuroendocrine response wherein noises and disturbances made by the experimenter were predictive of ensuing stress. This could explain why mice who were not exposed to the acute stressor prior to testing also showed exaggerated CS responding. Although the saline control mice showed exaggerated CS responding, it is evident that the response did not reach its highest level since mice pre-exposed to the acute noise/light stress exhibited additional and exaggerated CS responding also. These results support the contention that changes in corticoid levels can sensitively reflect the intensity of stimulation to which mice are exposed [16] and may represent components of physical and psychological stress.

Taken together, the behavioral and CS results obtained in the present study using mice are similar to those reported by Katz *et al.* in rats, suggesting that chronic stress has similar effects on these parameters in both species.

In nonchronically stressed untested mice there were no effects on 5-HT levels due to any drug treatment. Acute noise/light stress exposure caused increased 5-HT levels only in the saline controls. However, 5-HIAA levels are more indicative of 5-HT functioning and in this measurement there were increases due to acute stress in the saline, amitriptyline and desmethylimipramine pretreated mice. Increases were absent in the chlorimipramine and fluoxetine pretreated mice and were probably due to the potent effect of these drugs have on inhibiting 5-HT uptake [13,39]. In-

creased 5-HT activity is a common finding in studies of acute stress involving rats and mice, and an excellent review may be found elsewhere [1].

After chronic stress all treatment groups, except those receiving fluoxetine, displayed increased 5-HT and 5-HIAA levels due to acute noise/light stress exposure (Tables 3 and 4). It should also be noted that determinations of 5-HIAA in chronically stressed, but untested mice, indicated that there were increases in 5-HT activity due to chronic stress alone (Table 4). There is a sparse amount of information available involving the effects of chronic stress on 5-HT activity, especially those which use various stressors in a chronic regimen. It has been reported that exposure of mice to chronic crowding conditions resulted in chronically elevated 5-HIAA and CS levels, when measured at various intervals between 10 and 40 days [6]. Corticosterone has also been shown to induce tryptophan hydroxylase activity, as well as facilitate tryptophan uptake into 5-HT nerve terminals. It was hypothesized that this effect may be a compensatory mechanism for the increased 5-HT turnover seen during stress [28,36]. However, in this study there was no correlation between 5-HIAA and CS levels in chronically stressed untested mice, as 5-HIAA levels were increased, but CS levels were normal.

The most interesting neurochemical results after chronic stress are those in regard to NE, which was not significantly affected in any of the nonchronically stressed conditions. Chronic stress exposure resulted in increased NE levels in all the untested treatment groups (Table 5). This finding is consistent with most studies employing chronic stress and has been shown to be indicative of increased NE synthesis [32] and turnover [1,38]. Conversely, marked decreases in NE occurred in all chronically stressed animals whether behaviorally tested or exposed to acute noise/light stress prior to behavioral testing (Table 5). The decreases were detected in both situations and to approximately the same extent, suggesting that this finding may not be directly related to exposure to the acute noise/light stress. The most reasonable explanation for this occurrence is that a conditioned neurochemical change has also been produced. This possibility was recently advanced and supported by findings in mice [2] and rats [11,42]. It should be noted that NE reductions associated with acute shock exposure have been reported to be prevented by prior chronic exposure [1,43], however a similar habituation was not found in the present study. Possibly, the multiplicity of stressors precluded neurochemical adaptation.

It is likely that in the present study both neuroendocrine and neurochemical conditioning had occurred, since placement in the motimeter with or without prior acute noise/light stress exposure, resulted in: (1) gross behavior changes; (2) exaggerated CS responding; and (3) significant NE decreases. It is interesting to note that increases in 5-HIAA did not follow this pattern and were only exhibited after physical exposure to the acute noise/light stressors or chronic stress. As such, it tentatively appears that the 5-HIAA response may not be part of the conditioned emotional response.

Chronic treatments with any one of the three TCAs tested, but not fluoxetine, were able to ameliorate the behavioral and CS alterations due to chronic stress in the present study. Results obtained using inescapable shock pardigms strongly suggest that the induced behavioral deficit may be correlated with decreased NE and/or DA functions [2,13]. It was further reported that chronic, but not acute, treatment with nortriptyline (TCA) counteracted the escape deficits due to prior inescapable stress exposure [35]. It is possible that the behavioral activation deficit seen in the present study may have also been due NE depletions but whole brain monoamine analyses precludes any further speculation.

In the present study fluoxetine was ineffective in ameliorating the behavioral and CS effects of chronic stress and it may be concluded that fluoxetine is not an antidepressant. Fluoxetine shares a common property with two of the TCAs tested, chlorimipramine and amitriptyline, which is the inhibition of 5-HT reuptake. The present results suggest that the ameliorative effects of these TCAs are at least not solely due to 5-HT reuptake inhibition. Recent clinical trials have indicated that fluoxetine may be an antidepressant [7,12]. Since clinical depression is believed to be composed of various subtypes of different ethiologies and subsequent treatments, it is also probable that animal models may also vary in the types of depressions they are purported to mimic and the types of drugs which may prove efficacious for each.

In summary, chronic stress alone was found to increase both 5-HIAA and NE levels, but have no effects on the resting CS levels in untested mice. In a behavioral testing situation chronically stressed mice were found to exhibit decreased levels of locomotor activity as well as decreases in NE levels and abnormal CS output. Chronically stressed mice exposed to a novel acute stress (noise/light) prior to testing exhibited a severe blunting of the activation response, decreases in NE levels, further increases in 5-HIAA levels and abnormal CS responding. The behavioral and hormonal, but not the neurochemical, effects of chronic stress were normalized or partially restored in animals which received chronic treatment with one of the TCAs but not fluoxetine. Examination of the overall biochemical and neurochemical results of the chronic stress paradigm indicated that conditional neuroendocrine and neurochemical responses may have been induced.

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