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Behavioral and neurochemical changes following predatory stress in mice

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Abstract

This article had several objectives. First it aimed at investigating the anxiogenic-like behaviors elicited by unavoidable cat exposure and/or cat odor across nine strains of mice (BALB/c, C57BL/6, C3H, CBA, DBA/2, NMRI, NZB, SJL, Swiss) in a modified version of the free-exploration test. The second objective was to investigate possible neurochemical changes following cat exposure in Swiss mice by measuring the turnover of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in several brain regions known to be involved in the modulation of emotional processes (hippocampus, hypothalamus and striatum). Finally, the third objective was to examine the effects of anxiolytic drug treatments on the anxiogenic responses elicited by a cat odor (i.e. a feces) in Swiss mice previously exposed to a cat using the free-exploration test. Results from the strain comparison showed that mice could be divided into three distinct groups: two non-reactive strains (NZB and SJL) which were relatively insensitive to predatory exposure and/or odor; five intermediate-reactive strains (Swiss, NMRI, CBA, C3H and BALB/c) which displayed clear anxiogenic-like responses only when exposed to both cat and, subsequently, to feces; and two high reactive strains (C57BL/6 and DBA/2) which showed anxiogenic-like reactions following cat exposure, regardless of the stimulus (clay or feces) present in the free-exploration cage. Neurochemical data revealed that, while brain levels of NA, DA, 5-HT in cat exposed Swiss mice were not significantly different from those of control animals, turnover rates of these monoamines were increased in the hippocampus (NA and 5-HT), hypothalamus and striatum (DA) after cat exposure. Results from pharmacological experiments indicated that repeated administration of the 5-HT reuptake inhibitor fluoxetine (5–20 mg/kg, twice a day, for 5 days) completely abolished avoidance of the cat feces in Swiss mice previously exposed to the predator. Neither acute nor repeated administration of the classical anxiolytic diazepam was able to reduce avoidance behavior of the anxiogenic stimulus in the free-exploration test. Taken together, these findings indicate that the exposure of mice to unavoidable predatory stimuli is associated with behavioral and neurochemical changes consistent with increased anxiety.

Keywords: Anxiety; Cat exposure; Diazepam; Fluoxetine; Free-exploration test; Mice; Monoamine turnover; Predator

1. Introduction

It is widely acknowledged that the exposure of rodents to natural predators or to their odors may induce anxiety-like states (e.g. Adamec and Shallow, 1993; Berton et al., 1998; Blanchard et al., 1990; Dielenberg et al., 1999; Hogg and File, 1994a; Kavaliers et al., 1994; Zangrossi and File, 1992a). For example, Blanchard and colleagues (1990) showed that in a straight alley containing a cat odor stimulus, rats show high rates of risk assessment, including flat back approach and stretch attend behaviors oriented toward the threat stimulus and contact with the stimulus. Further, it was shown that rats exposed to a cloth impregnated with cat odor showed a decreased number of contacts with the cloth and time in contact with it and increased time sheltering from it. More recently, Dielenberg et al. (1999) demonstrated that rats confronted with a cat-odor impregnated collar displayed robust avoidance responses towards this stimulus. Such exposure also resulted in anxiogenic responses in the social interaction and elevated plus-maze tests

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(Zangrossi and File, 1992b), that is, when animals are no longer in the presence of the stressful predator odor.

The possibility that such procedures may model specific anxiety states in humans and thus may serve as screening tests of novel anxiolytic drugs has prompted several research groups to study the effects of clinically effective anxiolytics on the behavior of rodents exposed to predatory stimuli. Most of these studies showed that the anxiogenic response to predator exposure or to their odors is relatively insensitive to the effects of such compounds. As an illustration, Blanchard et al. (1990) showed that the benzodiazepine (BZ) diazepam failed to reduce in a specific manner (i.e. at non sedative doses) risk assessment behavior of rats exposed to a cat-impregnated cloth. In another study, the BZ chlordiazepoxide was found to increase time spent by rats in contact with a cat odor-saturated cloth, but failed to affect sheltering response (Zangrossi and File, 1992a). The weak positive action of BZs in these studies suggests that it is unlikely that the behaviors elicited by predatory cues model certain aspects of generalized anxiety disorder (GAD) that is successfully treated with these drugs. A recent study with rats previously exposed to a cat reported that subsequent avoidance responses towards a cat odor-saturated cue in the staircase test were modified by the mixed noradrenaline/serotonin reuptake inhibitor imipramine, but not by diazepam (Griebel et al., 1999). Moreover, chronic treatment with imipramine and the selective serotonin reuptake inhibitor (SSRI) fluoxetine reduced defensive responses of mice exposed to a predator in the mouse defense test battery (Griebel et al., 1995). SSRIs have been successfully used in the clinical management of several anxiety disorders, including social phobia, panic, obsessive-compulsive and post-traumatic stress (PTSD) disorders (Den Boer and Westenberg, 1995; Fichtner et al., 1997; Nagy et al., 1993; Van Ameringen et al., 1993). This raises the possibility that predatory exposure may serve as an ethologically-relevant model of some anxiety disorders in rodents.

In this context, one objective of the present experiments was to characterize the anxiogenic-like behaviors elicited by cat exposure and/or a cat odor in nine strains of mice (BALB/c, C57BL/6, C3H, CBA, DBA/2, NMRI, NZB, SJL, Swiss) in the free-exploration test. These mice were chosen on the basis of differences in behavioral profile as revealed in several models of anxiety (Beuzen and Belzung, 1995; Griebel et al. 1997, 2000; Trullas and Skolnick, 1993). A second objective was to investigate possible neurochemical changes seen in Swiss mice following cat exposure by measuring the turnover of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in several brain regions known to be involved in the modulation of emotional processes (hippocampus, hypothalamus and striatum). This strain was chosen because it was the sole one that displayed anxiogenic behavior following exposure to the cat associated with the presence of cat odor in the novel environment without modification of locomotion and rearing and also because it is widely used in pharmacological and neurochemical studies. Finally, a third objective was to examine further the effects of anxiolytic drug treatments on the anxiogenic-like responses elicited by a cat odor (i.e. a feces) in mice previously exposed to a cat, using a modified version of the free-exploration test (Griebel et al., 1993). The compounds used were diazepam and the SSRI, fluoxetine.

2. Animals, material and methods

2.1. Ethics

All procedures described here fully comply with French legislation on research involving animal subjects.

2.2. Animals

Subjects were naive male mice from seven inbred strains (BALB/cByJIco, C57BL/6JIco, C3H/HeOuJIco, CBA/JIco, DBA/2JIco, NZB/Ola/Hsd, SJL/J) and from two outbred lines (NMRI, Swiss) aged 9 weeks at the time of testing. They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental testing, they were housed in groups of six in standard-sized cages (30×20×14 cm) containing a constant supply of food pellets and water. All animals were maintained under standard laboratory conditions (21–23EC) and kept on a 12 h light/dark cycle with light onset at 06:00 h. The number of animals per group ranged from 5 to 15, depending on the experiment.

2.3. Drugs

Diazepam and fluoxetine hydrochloride (purchased from Sigma-Aldrich, Saint Quentin Fallavier, France) were prepared as suspensions in physiological saline containing Tween 80 (0.1%). They were injected intraperitoneally in a volume of 20 ml/kg. All doses are expressed as the bases.

2.4. Experiment 1: Effects of cat exposure and/or cat feces on the behavior of different strains of mice in the free-exploration test

A modified version of the free-exploration test (Griebel et al., 1993) was used. The apparatus consisted of a PVC box (30×20×20 cm) covered with Plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small entries. It could be divided in half lengthwise by closing three temporary partitions. Approximately 20 h before cat exposure and/or free-exploration testing, each subject was placed

in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half was covered with fresh sawdust and the animal was given unlimited access to food and water. On the test day, mice of each strain were randomly allocated to the following four groups.

(a) Naive+clay: animals were exposed to both familiar and novel compartments by removal of the temporary partitions. The novel compartment contained three modeling odor-free clay pellets.
(b) Naive+feces: animals were exposed to both familiar and novel compartments. The novel compartment contained three cat feces pellets.
(c) Exposed+clay: subjects were removed from the free-exploration box and confronted individually with a cat during a 5-min session. The cat cage consisted of a PVC box (82×56×62 cm) subdivided into two compartments, one containing the cat, the other the mouse. Separation consisted of a transparent PVC wall with holes allowing the cat to reach the other side with its paws. The mouse was then put back in the free-exploration apparatus and was exposed 1 h later to both familiar and novel compartments. The novel compartment contained three modeling odor-free clay pellets.
(d) Exposed+feces: same as previous group, but the novel compartment contained three pellets of feces from the cat used during exposure.

The behavior of the mouse was observed under red light for 5 min via a closed circuit TV camera by an observer located in an adjacent room. The following parameters were recorded: (a) time spent in the novel compartment; (b) total unit entries and (c) total number of rearings. The results were expressed as mean percentage of time spent in the novel compartment, mean total number of novel unit changes, and mean total number of rearings.

2.5. Experiment 2: Effects of cat exposure on central DA, 5-HT and NA turnover in Swiss mice

Mice were familiarized with the free-exploration cage as described in experiment 1. On the test day, they were randomly allocated to the following two groups: (a) naive: animals were removed from the free-exploration box and sacrified; (b) cat exposed: subjects were removed from the free-exploration box and confronted individually with a cat during a 5-min session. Immediately after exposure, they were sacrified. Animals were killed by decapitation and the following brain structures were dissected out on ice: striatum, hippocampus, hypothalamus. NA, DA, 5-HT and their metabolites, normetanephrine (NMN), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAAY), respectively, were measured by high pressure liquid chromatography (HPLC) with electrochemical detection. Frozen tissues were sonicated in 800 μl of 0.05 M $HClO_4$ containing 0.5 mM of EDTA, 2 mM sodium metabisulfite and 3,4-DHBA (final concentration 1 ng/50 μl) as the internal standard. After centrifugation, 50 μl of the supernatant were injected onto the liquid chromatographic column using a refrigerated (4°C) autoinjector Wisp 512 (Waters, Milford, MA, USA). Separation was achieved at room temperature. The HPLC system consisted of a pump and a stainless steel separation column (0.46×7 cm) packed with an Ultrasphere XL ODS C18, 3 μm particle size (Beckman, Fullertone, CA, USA). The mobile phase contained 0.1 M NaH_2PO_4, 1 mM EDTA, 2.5 mM octane sulfonic acid, 7% CH_3CN, pH 3.4. The flow rate was 0.9 ml/min. Electrochemical detection was carried out by means of an amperometric detector (Waters 460, Milford, USA) with a glassy carbon working electrode and an Ag/AgCl reference electrode. The detector potential was set at +0.8 V versus the reference electrode. Concentrations of each compound were calculated using a computing integrator (Maxima, Waters, Milford, USA) with reference calibration curves obtained after injection of standards.

2.6. Experiment 3: Effects of diazepam and fluoxetine in Swiss mice in the free-exploration test following cat exposure

The apparatus, familiarization period and cat exposure procedure were the same as described in condition (d) in experiment 1 (i.e. cat exposure+cat feces). The effects of diazepam and fluoxetine were tested in Swiss mice only because this strain is among the most commonly lines used in psychopharmacological studies, and it has been shown to be more sensitive than the other strains to the administration of anxiolytics (Griebel et al., 2000). Diazepam and fluoxetine were administered twice a day for four consecutive days. The last administration was performed on the fifth day 30 min before testing in the free-exploration test (30 min after cat exposure). Diazepam was also given acutely 30 min before free-exploration testing. The latter regimen was not used with fluoxetine because the drug has been shown to display positive action mostly after repeated treatment (for review, see Griebel, 1995).

2.7. Statistical analysis

Due to the rather large number of experimental groups, it was not possible to undertake a parallel group design for the whole experiment. A parallel group design (all types of pre-exposure and exposure conditions within a given strain) was run in a single session. Therefore, no strain comparison could be undertaken. Behavioral data of experiment 1 were analyzed by two factor analysis of variance (ANOVA) with naive versus

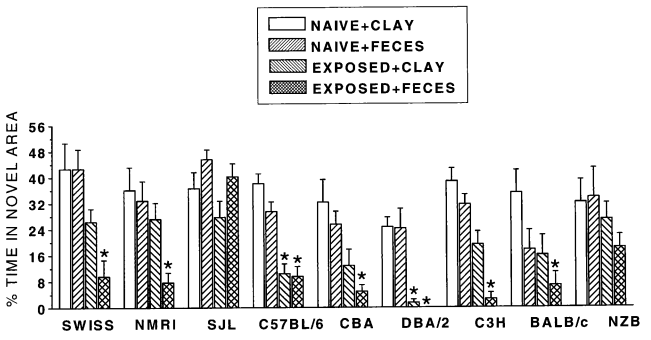

Fig. 1. Percentage of time spent by different strains of mice in areas containing modeling clay pellets or cat feces pellets in the free-exploration test in naive animals or in animals exposed to a cat. Data represent mean±SEM. *$P<0.05$ (vs naive+clay condition).

cat pre-exposure and clay versus feces exposure condition as dependent factors. Subsequent comparisons between treatment groups and control were carried out using Newman–Keuls tests. Data from experiment 2 data were analyzed by Student's *t*-test. Data from experiment 3 were analyzed with a one way ANOVA followed by Newman–Keuls a posteriori comparisons.

3. Results

3.1. Experiment 1: Effects of cat exposure and/or cat feces on the behavior of different strains of mice in the free-exploration test

Data are presented in Fig. 1 and Table 1. A significant naive versus cat pre-exposure *x* clay/feces exposure was never observed for time spent in the novel area (*p* always >0.13). For locomotion, such an interaction was seen only in the NMRI strain [$F(1,56)=11.39$, <0.001; $p>0.18$ in all other strains] while for rearing, significant or marginally significant interaction could be seen in the NMRI and the C3H strain [respectively, $F(1,56)=8.4$, $p<0.O05$; $F(1,56)=3.51$, $p<0.06$] but not in any other strain (*p* always >0.15). For time spent in the novel compartment, further analyses revealed a significant effect of pre-exposure in SWISS [$F(1,44)=10.31$, $p=0.002$], in DBA [$F(1,44)=51.77$, $p<0.0001$], in C3H [$F(l,56)=51.51$, $p<0.001$], in CBA [$F(1,56)=18.11$, $p<0.001$], in C57 [$F(1,56)=43.41$, $p<0.001$] and in SWISS mice [$F(l,35)=14.82$, $p<0.0001$], but not in BALB, NZB and SJL mice. Furthermore, for this same parameter, a difference could be detected between clay and feces exposure in NMRI mice [$F(1,56)=4.58$, $p=0.03$], in C3H mice [$F(1,56)=12.36$, $p=0.001$] and in SJL mice [$F(1,56)=6.2$, $p=0.01$] but not in the other strains. Further analyses indicated that for C57BL/6, CBA, C3H, BALB/c and DBA/2 mice, cat exposure was sufficient to decrease significantly time spent by subjects in the novel compartment, regardless of its content (clay or feces). Both exposure plus feces were necessary to reach a significant decrease in this parameter with Swiss and NMRI mice.

For locomotion and rears, a significant effect of pre-exposure could be detected in NMRI mice [$F(1,56)=19.04$, $p<0.001$ for locomotion and $F(1,56)=13.93$, $p<0.001$ for rears], in DBA mice [$F(1,44)=56.76$, $p<0.001$ for locomotion, $F(1,44)=61.26$ for rears], in C3H [respectively, $F(1,56)=51.77$, $p<0.001$ and $F(1,56)=56.65$, $p<0.001$], in CBA [$F(1,56)=25.64$, $p<0.001$ for locomotion and $F(1,56)=29.11$, $p<0.001$ for rears], in C57 [$F(1,56)=42.36$, $p<0.001$ for locomotion and $F(1,56)=24.89$, $p<0.001$ for rears] and in NZB mice [$F(1,40)=3.93$, $p=0.05$ for locomotion and $F(1,40)=9.37$, $p=0.004$ for rears]. In SWISS mice, pre-exposure had an effect on rears and a marginal significant effect on locomotion [$F(1,35)=3.39$, $p=0.07$ for locomotion and $F(1,35)=8.25$, $p<0.007$ for rears] while the opposite was seen in BALB/c mice [$F(1,56)=5.08$, $p=0.02$ for locomotion and $F(1,56)=3.57$, $p=0.06$ for rears]. No effect of pre-exposure could be seen in SJL mice. A difference between exposure to clay and feces could be observed for locomotion in SJL mice [$F(1,56)=8.33$, $p=0.006$] and for rears in NMRI mice [$F(1,56)=6.58$, $p=0.013$], in C3H mice [$F(1,56)=3.73$, $p=0.05$], in CBA mice [$F(1,56)=9.69$, $p=0.003$]. In the other strains, no effects of exposure could be seen in any of these two parameters. Confrontation with the cat was sufficient to induce a decrease of activity and rears in DBA/2, CBA, C57BI/6 and C3H mice, even in the absence of any predator odor in the novel environment. Both exposure to the cat and presence of cat odor in the novel compartment were necessary to induce a modification of rears and locomotion in the NMRI strains.

3.2. Experiment 2: Effects of cat exposure on DA, 5-HT and NA turnover in Swiss mice

Data are presented in Fig. 2 and Table 2. Student's *t*-test revealed no significant differences in NA, DA or 5-HT levels in the hippocampus, hypothalamus and/or striatum between naive animals and subjects exposed to a cat. However, NMN, the metabolite of NA, was significantly increased in the hippocampus ($t=2.9$, $P<0.05$) of exposed mice compared with naive animals, whereas DOPAC, the metabolite of DA, was significantly increased in the hypothalamus ($t=2.4$, $P<0.05$) and in the striatum ($t=3$, $P<0.05$) following cat exposure. Moreover, Fig. 2 shows that the ratios NMN/NA and 5-HIAA/5-HT were significantly increased in the hippocampus ($t=2.6$, $P<0.05$ and $t=4.1$, $P<0.05$, respectively) in exposed animals. Similarly, the ratio DOPAC/DA was significantly increased in the hypothalamus ($t=2.6$,

Table 1
Measures of activity in the free-exploration test in different strains of mice under different conditions[a]

		Total unit changes	Rearings
Swiss			
	Naive+clay	41.5±8.3	38.0±7.5
	Naive+feces	53.4±5.2	46.9±2.6
	Exposed+clay	34.2±5.6	32.0±3.7
	Exposed+feces	33.6±24.3	28.7±4.1
NMRI			
	Naive+clay	40.5±3.9	36.6±2.8
	Naive+feces	38.1±5.3	37.7±3.3
	Exposed+clay	35.9±5.9	33.9±3.9
	Exposed+feces	12.1±2.8*	16.5±2.6*
SJL			
	Naive+clay	48.3±5.1	38.5±3.8
	Naive+feces	62.4±6.9	37.7±4.6
	Exposed+clay	36.6±4.7	27.5±2.5
	Exposed+feces	59.9±8.6	34.5±4.6
C57BL/6			
	Naive+clay	53.2±4.4	33.2±3.0
	Naive+feces	55.5±4.1	34.7±3.1
	Exposed+clay	24.1±3.7*	16.2±2.2*
	Exposed+feces	24.8±4/5*	18.7±3.3*
CBA			
	Naive+clay	53.6±6.5	39.2±3.6
	Naive+feces	55.9±5.4	28.7±3.8*
	Exposed+clay	34.7±4.5*	21.8±2.4*
	Exposed+feces	24.1±2.8*	13.6±1.8*
DBA/2			
	Naive+clay	37.6±3.8	33.0±3.2
	Naive+feces	40.1±4.5	28.4±2.6
	Exposed+clay	15.5±3.5*	12.9±1.9*
	Exposed+feces	8.3±2.2*	7.4±2.6*
C3H			
	Naive+clay	50.6±2.9	42.9±3.1
	Naive+feces	49.1±39	42.7±39
	Exposed+clay	26.1±4.5*	23.7±4.3*
	Exposed+feces	10.9±2.1*	10.7±1.7*
BALB/c			
	Naive+clay	44.7±8.1	28.1±3.2
	Naive+feces	39.0±4.1	26.5±3.0
	Exposed+clay	28.9±5.3	23.5±2.8
	Exposed+feces	28.8±4.7	19.9±2.8
NZB			
	Naive+clay	37.3±3.8	28.0±2.1
	Naive+feces	41.0±7.7	30.6±4.4
	Exposed+clay	25.9±4.8	21.5±2.7
	Exposed+feces	29.3±5.6	17.5±2.8*

[a] The conditions were: (1) naive+clay (naive mice were confronted with modeling clay pellets); (2) naive+feces (naive mice were exposed to cat feces pellets); (3) mice were exposed to a cat, then confronted with modeling clay pellets); (4) exposed+feces (mice were exposed to a cat, then confronted with cat feces pellets). Data represent mean±SEM. *$P<0.05$: different from mice from the same strain, for different conditions.

$P<0.05$) and in the striatum ($t=4.9$, $P<0.05$) after cat exposure.

3.3. Experiment 3: Effects of diazepam and fluoxetine in Swiss mice in the free-exploration test following cat exposure

Fig. 3 shows that none of the treatments with diazepam significantly modified the percentage of time spent in the novel compartment and total unit changes. However, the drug significantly reduced rearings following a single injection of 3 mg/kg (Table 3). After repeated administration, fluoxetine significantly changed the percentage of time spent in the novel area [$F(3,19)=4.1$, $P<0.05$], but did not modify the other two measures (Table 3). Post-hoc analysis showed that the drug significantly increased time spent in the compartment containing cat feces at all doses.

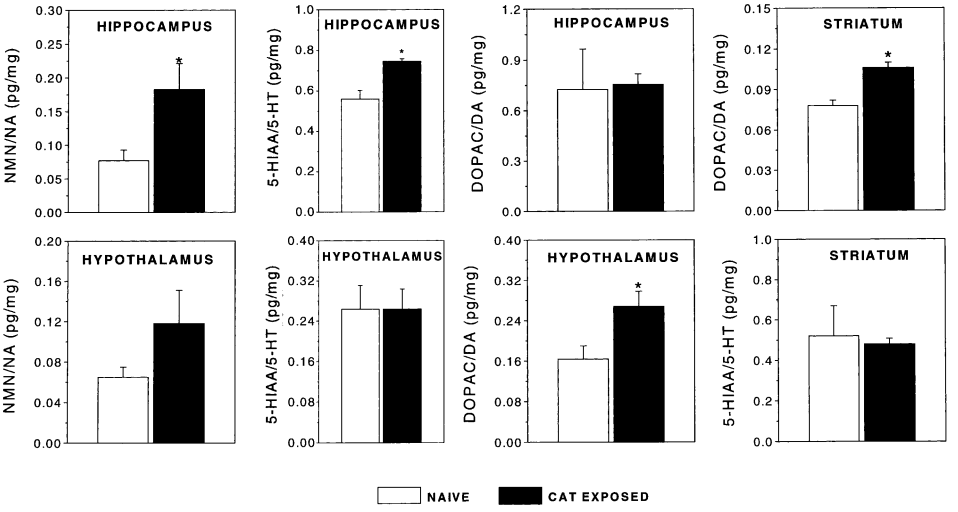

Fig. 2. Effects of unavoidable cat exposure on the turnover of noradrenaline, 5-HT or dopamine in several brain regions of Swiss mice. Animals were killed 5 min after confrontation with the cat. Data represent mean±SEM. *$P<0.05$.

Table 2
Effects of unavoidable cat exposure on the levels of noradrenaline, 5-HT, dopamine and their metabolites (pg/mg) in several brain regions of Swiss mice[a]

		Naive	Exposed
Hippocampus	NA	338±18	286±21
	NMN	25±4	49±7*
	DA	29±6	27±4
	DOPAC	20±9	20±1
	5-HT	490±30	442±15
	5-HIAA	277±33	330±13
Hypothalamus	NA	1649±127	1442±93
	NMN	108±17	161±43
	DA	603±45	519±44
	DOPAC	96±14	134±9*
	5-HT	1967±768	2365±1101
	5-HIAA	352±40	412±53
Striatum	DA	10351±703	9282±566
	DOPAC	799±43	982±42*
	5-HT	1233±230	1092±98
	5-HIAA	502±34	513±30

[a] Animals were killed 5 min after confrontation with the cat. Data represent mean±SEM. *$P<0.05$.

4. Discussion

One objective of the present study was to investigate the anxiogenic-like responses of mice exposed to predatory stimuli using the free-exploration test in different strains of mice. Our results showed that while SJL and NZB mice were relatively insensitive to predatory exposure and/or odor, all other strains displayed clear anxiogenic-like behaviors when confronted with these stimuli. The latter could be divided into three distinct groups based on their sensitivity to both predatory stimuli: two high reactive strains (C57BL/6 and DBA/2) which showed anxiogenic-like reactions in the free-exploration following cat exposure regardless of the stimulus (clay or feces) in the novel compartment, five intermediate-reactive strains (Swiss, NMRI, CBA, C3H and BALB/c), which displayed clear anxiogenic-like responses only when exposed to both cat and feces and two non reactive strains which did not display any reaction, regardless of the stimulus (NZB and SJL). These results are in part consistent with previous studies with these strains that have reported differences on variables designed to measure fear-related behaviors in the light/dark and the elevated plus-maze tests (Beuzen and Belzung, 1995; Griebel et al., 2000; Trullas and Skolnick, 1993). SJL and, to a lesser extent, NZB mice were reported in these studies to have low emotionality and DBA/2 to exhibit high reactivity — a result confirmed

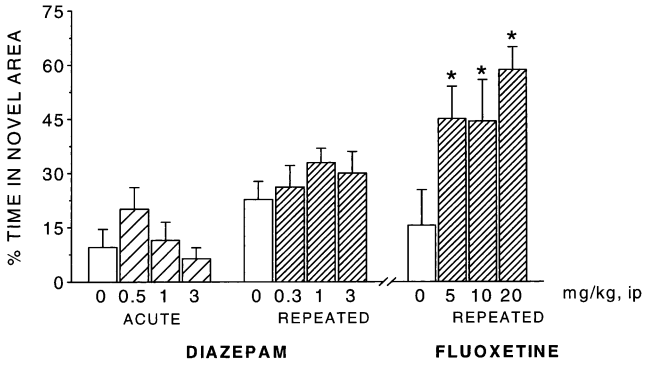

Fig. 3. Effects of diazepam and fluoxetine on the percentage of time spent by Swiss mice in areas containing cat feces pellets 1 h after unavoidable cat exposure. The drugs were administered repeatedly twice a day for 5 consecutive days, and diazepam was also given acutely 30 min before testing in the free-exploration apparatus. Data represent mean±SEM. *$P<0.05$.

here. However, C57BL/6 are generally described as non reactive, or as displaying an intermediate level of reactivity, while we report the contrary here. A possible explanation is that the behavioral response measured here might not be related to the same psychological processes as the one measured in classical animal models of state anxiety, such as the elevated plus maze or the light/dark tests.

The present findings fully agree with previous studies in rodents showing that cat exposure produces behavioral changes consistent with increased anxiety (Adamec and Shallow, 1993; Blanchard et al., 1995; Blanchard and Blanchard, 1989; Kavaliers and Colwell, 1991; Lester and Fanselow, 1985). However, unlike previous reports (Blanchard et al., 1990; Hogg and File, 1994a,b; Kemble and Gibson, 1992; McGregor and Dielenberg, 1999; Zangrossi and File, 1992a,b), our results revealed that the cat odor was not really repellent to naive animals since their performance did not significantly differ from those of non exposed subjects confronted with modeling clay. It is possible that the nature of the olfactory stimulus (cat feces vs cat odor-saturated cloth, litter or collar) may at least in part explain these differences. However, we demonstrated recently that the feces of cats produced clear anxiogenic-like behaviors in naive B6D2Fl mice which had free access from their nest to an unfamiliar straight runway that contained either modeling clay or cat feces (Berton et al., 1998). Interestingly, when the feces came from cats that were subjected to a vegetarian diet, anxiogenic-like responses were weaker than those of mice confronted with feces resulting from a carnivorous diet. Although cats used in the present study were subjected to a mixed vegetarian/carnivorous diet, it is possible that the predator's diet was of crucial importance in the degree of aversiveness of its feces. While exposure to predator feces failed to induce behavioral changes in any of the strains tested, pre-exposure to the cat was sufficient to produce anxiogenic-like responses in two strains, even when the mice were no longer in the presence of the cat. This suggests that confrontation with the cat induced changes that lasted at least 1 h after the stressful event. This is further strengthened by the finding that exposure to both the cat and the feces induces behavioral modifications in most of the strains tested. Complementary experiments may be necessary to verify whether these alterations persist over a longer period.

Although it was reported previously that exposure of rodents to aversive situations (e.g. elevated plus-maze, social interaction test, forced-swimming test, visible burrow system) generally increases activity of brain monoamines (e.g. Bickerdike et al., 1993; Blanchard et al., 1991; File et al., 1993; Jones et al., 1996; Lorens et al., 1990; Miura et al., 1993; Richardson, 1984; Shekhar

Table 3
Effects of diazepam and fluoxetine on measures of motor activity in Swiss mice confronted with cat feces pellets 1 h after unavoidable cat exposure[a]

Treatment (mg/kg, ip)		Total unit changes	Rearings
Diazepam (acute)	0	33.6±7.7	28.7±4.1
	0.5	40.7±8.6	30.4±6.0
	1	30.4±5.8	21.5±3.8
	3	18.3±5.9	9.4±2.5*
Diazepam (repeated)	0	50.3±5.7	40.0±3.7
	0.3	56.4±7.4	42.8±4.4
	1	62.2±5.0	40.4±3.1
	3	56.1±9.1	31.8±5.8
Fluoxetine (repeated)	0	51.2±11.7	50.8±12.5
	5	82.8±11.1	61.7±7.4
	10	60.8±14.9	46.6±7.8
	20	58.0±8.2	45.3±3.2

[a] The drugs were administered repeatedly twice a day for 5 consecutive days, and diazepam was also given acutely 30 min before testing in the free-exploration apparatus. Data represent mean±SEM. *$P<0.05$.

et al., 1994) there is as yet no evidence that exposure to a cat may produce similar effects. Our results showed that, while brain levels of NA, DA, and 5-HT in cat exposed Swiss mice were not significantly different from those of control animals, NMN and DOPAC, the metabolites of NA and DA, respectively, were increased in the hippocampus (NMN), hypothalamus and striatum (DOPAC). In addition, NMN and the metabolite of 5-HT, 5-HIAA, were increased in the hypothalamus and hippocampus of exposed mice, but these effects just failed to reach statistical significance. In contrast, cat exposure did no change levels of DOPAC and 5-HIAA in the hippocampus (DOPAC), hypothalamus and striatum (5-HIAA). These findings indicate that cat exposure increases the turnover rates (metabolite/monoamine) of NA, DA and 5-HT in a region-specific manner. These results fit well with previous studies in rats showing that a variety of stressors may produce region-specific increases in the turnover of NA in the hippocampus and hypothalamus (e.g. McQuade et al., 1999; Shirao et al., 1988; Tsuda and Tanaka, 1985) of DA in the striatum and hypothalamus (e.g. Lorens et al., 1990) and of 5-HT in the hippocampus (e.g. Adell et al., 1989; Takada et al., 1996). Some of these studies also observed stress-induced increases in the turnover of these monoamines in the frontal cortex of rats; however, because of the difficulty of dissecting out this structure from the mouse brain, tissues of this structure were not removed in the present study. In conclusion, the finding that cat exposure is associated with increased monoamine turnover in brain regions known to be involved in the modulation of emotional processes, indicates that unavoidable predator exposure may represent a valid model of stress in Swiss mice. However, further studies investigating neurochemical modifications in other strains of mice following predator exposure will be necessary before any definite conclusion can be drawn on the relationship of the neurochemistry to the behavioral observations. Indeed, Swiss mice exhibited only intermediate reactivity in the strain comparison study; neurochemical modifications should also be studies in strains displaying high and low reactivity.

Results from pharmacological experiments showed that repeated administration of the 5-HT reuptake inhibitor fluoxetine completely abolished avoidance of the cat feces in Swiss mice exposed to the predator. Neither acute nor repeated administration of the classical anxiolytic diazepam was able to reduce avoidance behavior of the anxiogenic stimulus in the free-exploration test. The sole effect observed after acute diazepam challenge was a weak sedation at the highest dose. This confirms that BZs show weak or no efficacy in reversing the anxiogenic-like behavior displayed by rodents during or after exposure to predatory stimuli (Blanchard et al., 1990; Griebel et al., 1999; Zangrossi and File, 1992a). Moreover, the lack of efficacy of diazepam in this model, taken together with the positive effects of fluoxetine, raises the possibility that the responses elicited by the predator exposure procedure used in this study reflect avoidance components of an anxiety state unrelated to GAD. As mentioned above, fluoxetine has been successfully used against several anxiety disorders, including social phobia, panic, obsessive-compulsive disorder and PTSD (Den Boer and Westenberg, 1995; Fichtner et al., 1997; Nagy et al., 1993; Van Ameringen et al., 1993), suggesting that the present cat exposure procedure may model certain aspects of any of these conditions. However, it has been argued that lasting effects of cat exposure on anxiety-related behaviors in rodents may model aspects of anxiety disorder associated with PTSD (Adamec, 1997).

In conclusion, findings from the present series of experiments showed that predatory stimuli produce in mice behavioral and neurochemical changes consistent with increased anxiety.

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