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ORIGINAL INVESTIGATION

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Differential modulation of antipredator defensive behavior in Swiss-Webster mice following acute or chronic administration of imipramine and fluoxetine

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Abstract The Mouse Defense Test Battery (MDTB) has been designed to assess defensive reactions in Swiss–Webster mice to situations associated with a natural predator, the rat. Primary measures taken before, during and after predator confrontation comprise escape attempts, predator assessment, defensive attack and flight. Previous reports from this laboratory have shown that the panic-promoting drug yohimbine potentiated flight behavior, while long-term treatment with the panicolytic agent alprazolam reduced this response. In order to evaluate further the possibility that the MDTB may represent an effective animal model of panic attacks, the present study investigated the behavioral effect of imipramine and fluoxetine, two serotonin reuptake inhibitors (SRIs) known to alleviate panic symptoms when given on a repeated basis. Both drugs were administered acutely and chronically (one daily IP injection for 21 days) at 5, 10 and 15 mg/kg. Our results showed that a single dose of imipramine or fluoxetine strongly potentiated flight reactions in response to an approaching predator and increased defensive attack toward the rat. This was in contrast to chronic treatment with each drug which dramatically decreased flight responses and defensive attack behaviors. In addition, long-term administration with both SRIs produced a reliable attenuation of predator assessment activities. Taken together, these findings suggest an acute anxiogenic-like effect of imipramine and fluoxetine followed by a fear/anxiety reducing effect after repeated administrations. These results support clinical observations revealing an acute anxiogenic effect of SRIs followed by an anxiolytic and/or panicolytic effect after chronic use, and support previous results suggesting that the MDTB may be useful for the investigation of panic-modulating agents.

Key words Imipramine · Fluoxetine · 5-HT reuptake inhibitors · Flight · Antipredator defense · Fear · Anxiety · Panic · Predator assessment · Acute and chronic treatments · Swiss-Webster mouse

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Introduction

A range of pharmacological, neuroanatomical and clinical studies have suggested a role for 5-hydroxytryptamine (5-HT, serotonin) in modulating some dimensions of personality and behavior as well as the symptomatology of a range of related psychiatric disorders. Thus, there is a substantial literature implicating the 5-HT system as a mediator of emotional responses in animal and man (Kahn et al. 1988; Griebel 1995). As an example, the therapeutic mechanism of action of clinically effective antipanic medications may be mediated by the 5-HT system. Indeed, the antipanic efficacy of chronic treatment with selective 5-HT reuptake inhibitors (SRIs) such as clomipramine (e.g. Kahn et al. 1987), fluvoxamine (e.g. Den Boer and Westenberg 1990), fluoxetine (e.g. Gorman et al. 1987; Schneier et al. 1990) or the non-selective SRI imipramine (e.g. Garakani et al. 1984) is well documented. In addition, a transient increase in anxiety level is often reported at the beginning of treatment with SRIs in panic disorder (PD) patients (e.g. Westenberg and Den Boer 1993). This intriguing phenomenon suggests an acute anxiogenic

and/or panicogenic effect of SRIs followed by an anxiolytic and/or panicolytic effect after chronic use. It as been hypothesized that such behavioural effects might be due in part to adaptive changes of the 5-HT receptors (Westenberg and Den Boer 1988): enhancement of 5-HT availability, after a single injection of SRIs (Fuller 1993, 1994; Rutter and Auerbach 1993), would lead to stimulation of the central 5-HT system, and hence increase anxiety level; conversely, repeated treatment with these drugs would decrease the responsiveness of 5-HT receptors (Maj and Moryl 1992; Burnet et al. 1994) and thus improve anxiety states (Van Praag 1988).

Preclinical investigations with SRIs in animal models of anxiety disorders reveal highly variable effects of these drugs (for review, see Griebel 1995). For example, several studies reported no effect after a single administration of imipramine in the Geller–Seifter and Vogel conflict tests (Kilts et al. 1981), the elevated plus-maze situation in rats (Pellow et al. 1985), the light/dark choice task in mice (Onaivi and Martin 1989) or in the fear-potentiated startle reflex paradigm in rats (Cassella and Davis 1985), while other data in rats revealed that the drug potentiated anxious responses in the Geller–Seifter and Vogel conflict tests (Fontana and Commissaris 1988; Sanger 1992), the conditioned emotional response paradigm (Sanger 1990) and the open-field test (Dwyer and Roy 1993). Further, some studies in rats and mice revealed an anxiolytic-like profile of this compound in the light/dark choice task (Young and Johnson 1991), the ultrasonic "distress" vocalization test (Molewijk et al. 1993) or the shock-probe- and marble-burying paradigms (Meert and Colpaert 1986; Craft et al. 1988). Chronic administration of imipramine in animals has also been reported to produce anxiolytic (Bodnoff et al. 1988, 1989; Fontana and Commissaris 1988; Blanchard et al. 1993a), anxiogenic (Dwyer and Roy 1993) or even no effect at all (Cassella and Davis 1985). Acute as well as chronic administration studies with fluoxetine in animal models of anxiety have provided a similar profile of inconsistency, including anxiolysis, no effect and anxiogenesis (Griebel 1995). The reason for this variability is in great part unknown. One possibility may be that different animal models represent qualitatively different types of "anxiety" or "fear", only some of which are reliably modulated by SRIs (Griebel et al. 1994b). The relatively weak efficacy of SRIs in other anxiety pathologies like generalized anxiety disorder (GAD) and social phobia (Nutt and Glue 1991; Murphy et al. 1993) seems to support this view.

Thus far, there is no single animal model of anxiety that may be said to strictly correspond to one type of anxiety disorder (Lister 1990; Treit 1991). However, several authors have recently developed experimental procedures which claim to have validity as an animal model of panic attack. For instance, Graeff (1991) described a procedure in which electrical or chemical stimulation of the dorsal periaqueductal grey (DPAG) of the midbrain leads to explosive motor behavior, including vigorous flight and aimless vertical jumps, which is identified as panic-like. Recently, Martin (1993) proposed a model in which rats are treated with panicogenic drugs, then exposed briefly to an uncontrollable and aversive situation and finally, are subjected to an avoidance task in a shuttle box. The behavioral deficits induced in these rats are described as homologous to those observed in panic attack, especially in patients who are inhibited in cognitive and behavioral processes. A more ethological model of panic has also been described by Hendrie and Neill (1992), who identified the behavioral responses of mice following exposure to cries of raptors as "panic-like". However, discrepancies have been obtained between the clinical effect of some panic-modulating drugs and their behavioral outcome in these models. For example, in the DPAG stimulation paradigm, an acute dose of clomipramine elicited an increase in the threshold of aversive DPAG stimulation (Kiser et al. 1978), while in a clinical trial patients reported increased severity and frequency of panic attacks during the initial days of treatment (Kahn et al. 1987). Furthermore, these tests are only useful in the detection of panicolytic effects, as they were unable to detect an anxiogenic- or panicogenic-like action of acute SRIs (Schütz et al. 1985; Graeff et al. 1986; Audi et al. 1988; Hendrie and Neill 1991; Martin 1993) or of mCPP (Jenck et al. 1989), a nonselective 5-HT direct agonist, known for its strong panic-inducing action in humans (e.g. Mueller et al. 1985).

Based on the assumption that the spontaneous activation of neuronal systems mediating the flight component of defense reactions may underlie human PD (Graeff 1990; Deakin and Graeff 1991; Deakin et al. 1991), we recently developed an experimental paradigm (the Mouse Defense Test Battery (MDTB)) in which Swiss–Webster mice are confronted with immediate, discrete or potential threat stimuli (e.g. rat). The primary measures, taken before, during and after rat presentation, include escape attempts, predator assessment, defensive attack and flight. In this test, the panic-promoting drug yohimbine potentiated flight behavior in response to an approaching human (Blanchard et al. 1993b), while long-term treatment with the panicolytic agent alprazolam reduced the prey-predator distance at which flight occurred (Griebel et al. 1994a). In addition, neither the traditional benzodiazepine (BZP) chlordiazepoxide nor the novel serotonergic anxiolytic gepirone affected this particular defensive response (Griebel et al. 1994a, 1995a). We concluded that panic-modulating drugs potentiate or inhibit neural mechanisms mediating flight, and further suggested that the MDTB may have some utility for the investigation of panicolytic as well as panicogenic compounds. Finally, our previous findings showing that the anti-GAD agents chlordiazepoxide and gepirone reduced contextual escape attempts to the situation associated with the predator, while neither acute or chronic alprazolam altered these responses, suggested that flight

responses, but not contextual defense behaviors, respond to panicolytic treatments.

The purpose of the present study was to investigate further the validity of the MDTB as an animal model of PD by assessing the behavioral effects of acute as well as chronic imipramine and fluoxetine treatment in this experimental paradigm. First, in the light of previous findings with panic-modulating drugs, we predict that administration of both SRIs on a chronic basis will produce a reduction in flight measures, while a single acute dosing will potentiate these responses; second, based on our recent data with the panicolytic agent alprazolam, we can tentatively predict that imipramine and fluoxetine will fail to counteract post-predator escape attempts.

Materials and methods

Animals

Subjects were 240 naive male Swiss-Webster mice obtained from Simonsen Laboratories (CA), 60–75 days old at the beginning of the experiment. They were housed singly in polycarbonate cages in a room maintained under a 12-h light/dark cycle.

Drug and treatment groups

Imipramine (RBI, Natick, Mass.) and fluoxetine (Eli Lilly and Company, Indianapolis, Ind.) were dissolved in an isotonic saline vehicle to various concentrations such that injections were always at a constant volume of 10.0 ml/kg. Mice were randomly assigned to following four conditions: a) acute imipramine: control group ($n = 15$) and drug treatment groups (5, 10 and 15 mg/kg; $n = 15$); b) chronic imipramine: control group ($n = 15$) and drug treatment groups (5, 10 and 15 mg/kg; $n = 15$); c) acute fluoxetine: control group ($n = 15$) and drug treatment drugs (5, 10 and 15 mg/kg; $n = 15$); d) chronic fluoxetine: control group ($n = 15$) and drug treatment groups (5, 10 and 15 mg/kg; $n = 15$). In conditions b) and d), mice received 21 daily intraperitoneal injections of saline, imipramine or fluoxetine. The last injection for each subject was given 30 min before the experiment was carried out.

Apparatus

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 6.0 m in total length, consisting of two 2-m straight segments joined by two 0.4-m curved segments and separated by a median wall ($2.0 \times 0.30 \times 0.06$). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with videocameras mounted above the apparatus. Experiments were performed under red light between 1 p.m. and 5 p.m.

"Predator"

Preliminary behavioral investigations have clearly demonstrated the importance of a rat to elicit the full range of specific fearful/defensive behaviors in the mouse. Looming stimuli or visual cues (e.g. approaching hand or leather glove) produced reliably less severe defensive responses (Griebel et al. 1995b). In addition, because of the great importance of achieving a high degree of control over the eliciting stimulus and its movements and actions, it was decided to use anaesthetized rats.

Procedure

Contextual fear defense

Evaluation of the impact of predator exposure on locomotor responses. Subjects were placed into the runway for a 3-min familiarization period, in which line crossings, wall rears and escape attempts (wall climbs and jump escapes) were recorded (min 1–3). The same behavioral parameters were also recorded during an equivalent period following tests involving exposure to a predator (post-test) (min 12–14). Changes in the latter measure during the post-predator period provides an index of contextual defense.

Reactions to the predator

Predator avoidance test (min. 4–6). Immediately after the 3-min familiarization period, a deeply anesthetized hand-held rat (Long-Evans male) was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) and escape distance were recorded. This was repeated five times.

Chase/flight (min 7–8). The hand-held rat is brought up to the subject at a speed of approximately 2.0 m/s. The time it took to chase the subject a distance of 15 m was recorded. Overall flight speed (m/s) and maximum flight speed (an average of three measures of uninterrupted straight flight, over 1-m linear segment of the runway) were subsequently calculated from these measures. Previous studies (Griebel et al. 1994a, 1995b) have shown that this latter measure, taken together with line crossing and wall rearing, both recorded in the contextual defense test, enables evaluation of the potential motor-impairing effect of drugs. By contrast, overall flight speed measure seems more specifically altered by potential fear/anxiety-modulating drug treatments (Griebel et al. 1995b). In addition, the number of stops (pause in movement) and orientations (subject stops, then orients the head toward the rat) were recorded.

Straight alley (min 9–11). The runway was then converted to a straight alley by closing two doors at both ends. The hand-held rat was moved to a distance of 1.20, 0.80 and 0.40 m from the subject and held at each location for 15 s. Measures taken included immobility time, closest distance between the subject and the rat and number of approaches/withdrawals (subject must move more than 0.2 m forward from the closed door, then return to it). Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites, vocalizations, upright postures and jump attacks by the subjects were noted.

Statistics

Data were analysed by a one-way analysis of variance (ANOVA) or the nonparametric Kruskal–Wallis ANOVA for some non-parametrically distributed infrequently occuring or highly variable behaviors. Subsequent comparisons between treatment groups and control were carried out using Newman–Keuls procedures or the

nonparametric Mann–Whitney *U*-test. In the contextual defense test, differences were evaluated by a combined repeated measures ANOVA followed by a Newman–Keuls post-hoc comparison or by the Mann–Whitney *U*-test and Wilcoxon matched pair test if the behavior occurred infrequently.

Results

Contextual fear/defense: locomotor activity before and after exposure to the predator (Fig. 1)

Imipramine (acute). ANOVA 4 × 2 (dose × pre/post-test) failed to indicate a reliable interaction for line crossings and wall rearings, but revealed a significant effect on escape attempts (Friedman ANOVA: $N_{1,60} = 58$, $P < 0.00001$) and subsequent analyses (Wilcoxon pair test) showed a post-test increase in this measure for all doses (0, 5, 10 and 15 mg/kg).

Imipramine (chronic). ANOVA did not reveal a reliable interaction for line crossing and wall rearing, but showed a significant overall effect with respect to escape attempts ($N_{1,60} = 44$, $P < 0.0001$). Subsequent Mann–Whitney analyses indicated that chronic imipramine significantly decreased the latter at all doses tested ($P < 0.003$ versus control). Post-hoc analyses also revealed that predator exposure reliably increased post-test escape attempts in both saline- and drug-treated groups.

Fluoxetine (acute). ANOVA 4 × 2 failed to indicate a reliable interaction effect for line crossing and wall rearing, but this interaction was reliable for escape attempts ($N_{1,60} = 56$, $P < 0.00001$). Post-hoc analyses indicated reliably fewer escape attempts ($P < 0.02$ versus control) at 10 and 15 mg/kg as well as a post-test increase in this measure for saline and drug-treated groups (5, 10 and 15 mg/kg).

Fluoxetine (chronic). ANOVA 4 × 2 did not reveal a reliable main effect with respect to the number of line crossings and wall rearings, but indicated a significant overall effect on the escape attempt responses ($N_{1,60} = 45$, $P < 0.00001$). Subsequent analysis indicated that fluoxetine treatment decreased the occurence of escape attempts at all doses tested (5, 10 and 15 mg/kg). In addition, a significant increase in this latter measure was seen during the post-rat period in the saline-treated mice as well as in the drug-treated groups (5, 10 and 15 mg/kg).

Reactions to the predator

Predator avoidance test (Fig. 2)

Imipramine (acute). ANOVA indicated a reliable main effect for the avoidance ($F_{3,53} = 4.4$, $P < 0.008$) as well as the escape distances ($F_{3,56} = 3.24$, $P < 0.03$), but failed to show any significant action of the drug for the number of avoidances and escapes. Post-hoc analysis with the Newman–Keuls test revealed that a single treatment with imipramine significantly increased avoidance distance at 10 and 15 mg/kg and escape distance at 5 mg/kg.

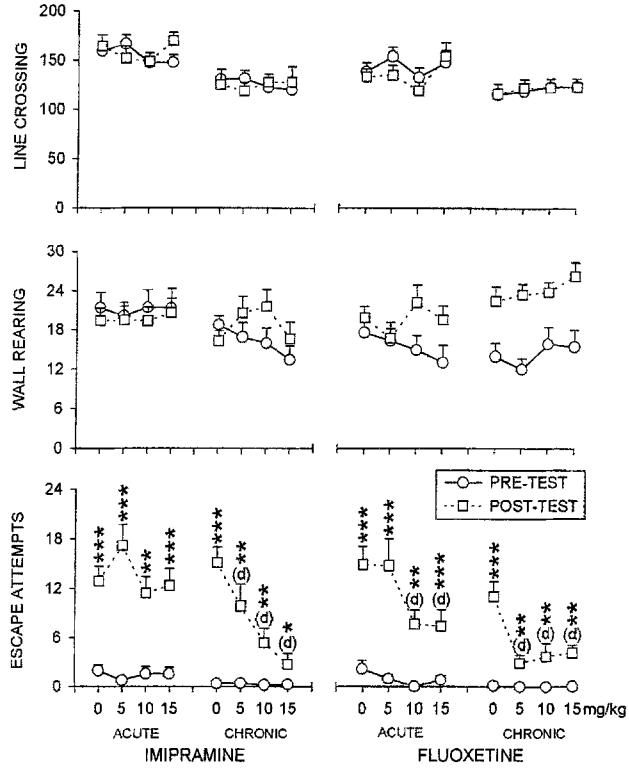

Fig. 1 Effects of an acute and chronic (21 days, once a day) treatment of imipramine and fluoxetine on the frequency of three response measures before (*pre-test*) and after (*post-test*) the exposure to the predator. *Data points* and *vertical bars* represent means and SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.0001$ (vs. pre-test); (d) (vs. vehicle)

Imipramine (chronic). ANOVA revealed that the drug treatment significantly affected avoidance distance ($F_{3,56} = 27.69$, $P < 0.0001$) and frequency of escapes ($H_{3,60} = 9.28$, $P < 0.03$), but did not modify number of avoidances and escape distance. Subsequent post-hoc analysis indicated that long-term imipramine administration induced a dramatic reduction in the avoidance distance at all doses and also decreased the number of escapes at 5 and 10 mg/kg.

Fluoxetine (acute). ANOVA indicated a reliable drug effect on the predator-subject distance at which avoidance occurred ($F_{3,49} = 3.21$, $P < 0.03$), but failed to show any marked action of the drug on avoidance as well as escape frequencies and escape distance. The former measure was reliably increased at 5 and 10 mg/kg.

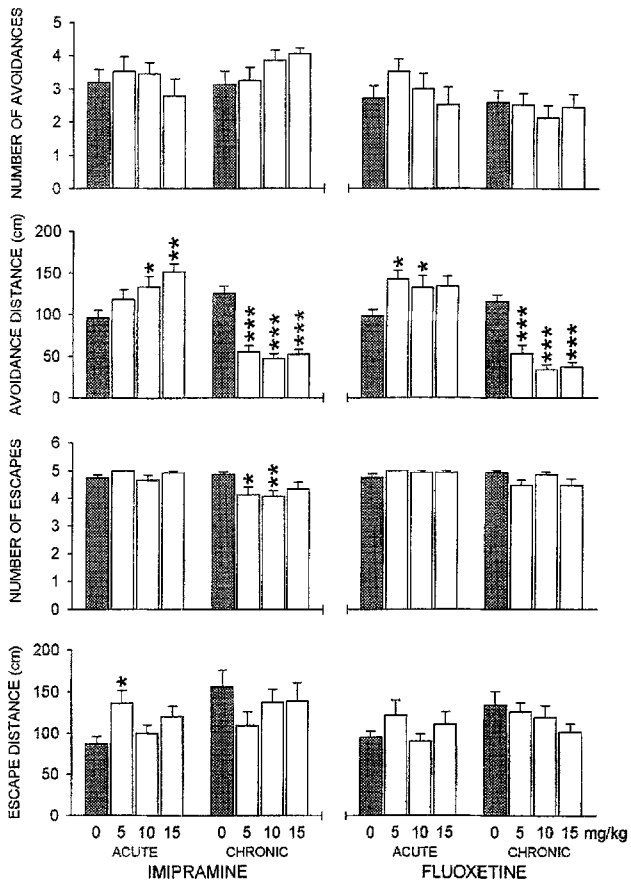

Fig. 2 Runway measures of avoidance to an approaching predator for mice administered imipramine and fluoxetine. *Columns* and *vertical bars* represent means and SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.0001$

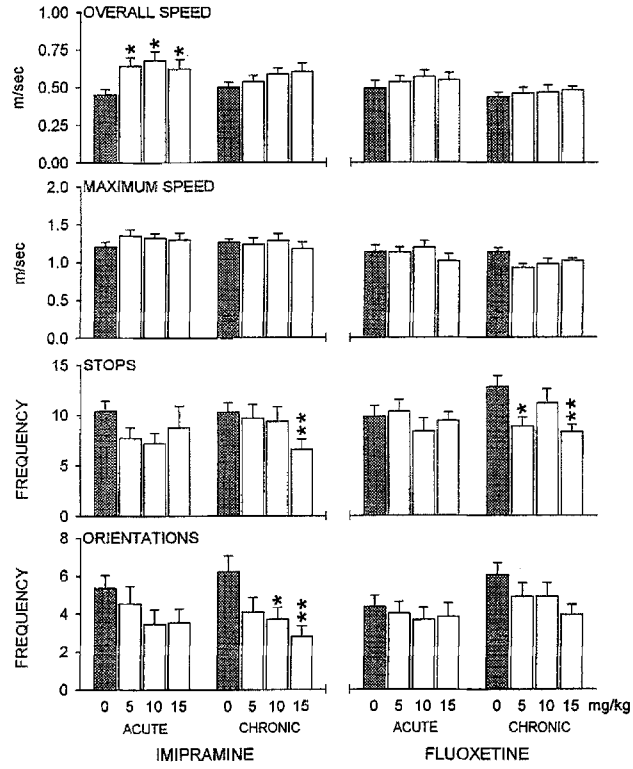

Fig. 3 Effects of imipramine and fluoxetine on behavioral responses of mice chased by a predator. *Columns* and *vertical bars* represent means and SEM. $*P < 0.05$, $**P < 0.01$

Fluoxetine (chronic). ANOVA revealed an overall main effect on the avoidance distance ($F_{3,50} = 24.44$, $P < 0.0001$) but not on the three other measures. Subsequent post-hoc analysis indicated that the treatment reliably reduced the distance at which flight occurred at all doses tested (5, 10 and 15 mg/kg).

Flight/predator orientation test (Fig. 3)

Imipramine (acute). ANOVA failed to reveal a reliable drug effect on maximum flight speed, arrests in movement and orientation to the predator, but indicated a main effect on overall flight speed ($F_{3,56} = 3.44$, $P < 0.02$). Newman–Keuls post-hoc comparison showed that a single injection of imipramine induced a reliable increase in the overall flight speed when the subject is chased by the predator. This effect occurred at all doses tested (5, 10 and 15 mg/kg).

Imipramine (chronic). ANOVA revealed that neither speed measure was affected by this treatment. However, the number of stops ($H_{3,59} = 8.09$, $P < 0.04$) as well as orientation movements ($H_{3,59} = 9.28$, $P < 0.02$) were significantly altered by the drug. Analysis with the Mann–Whitney test showed that chronic treatment with imipramine reduced the two latter measures at 15 mg/kg and also decreased orientation at 10 mg/kg.

Fluoxetine (acute). No significant effect of drug condition was found for any of the behavioral measures taken.

Fluoxetine (chronic). ANOVA indicated a reliable main effect on the number of stops ($H_{3,58} = 10.71$, $P < 0.01$), but not on the three other measures. Subsequent Mann–Whitney tests revealed that long-term fluoxetine treatment produced a significant decline in the occurence of arrests at 5 and 15 mg/kg.

Predator approach: straight alley (Fig. 4)

Imipramine (acute). ANOVA failed to reveal a reliable main effect for any of the behavioral measures taken.

Imipramine (chronic). None of the behavioral responses was significantly affected by the drug treatment.

Fluoxetine (acute). ANOVA failed to indicate any reliable effects of acute fluoxetine treatment for the frequency of approaches/withdrawals and the immobility

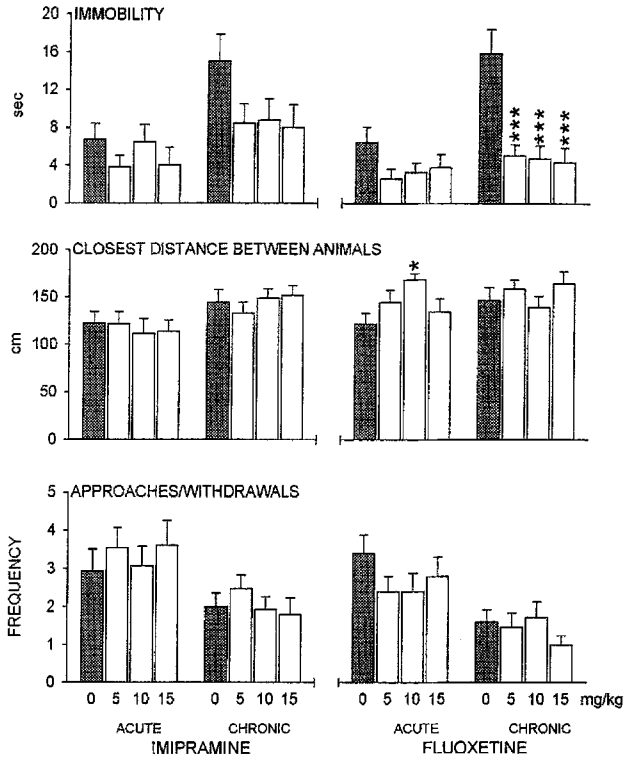

Fig. 4 Effects of imipramine and fluoxetine in the straight alley on behavioral reactions to a predator which remains at constant distance from the subject. *Columns* and *vertical bars* represent means and SEM. $*P < 0.05$ and $***P < 0.0001$

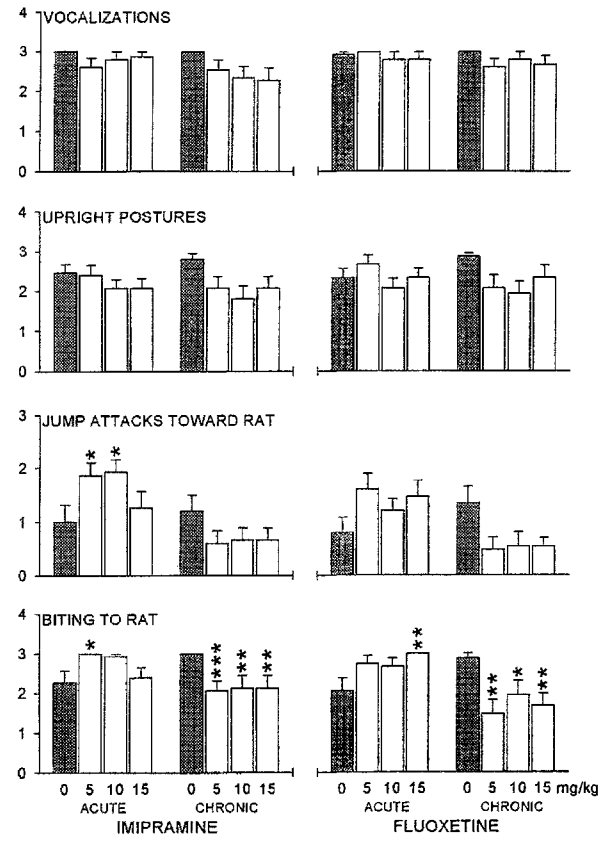

Fig. 5 Frequency of biting, defensive threat vocalization, upright posture and jump attacks to forced contact with a deeply anesthetized rat for subjects under varying doses of imipramine and fluoxetine. *Columns* and *vertical bars* represent means and SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.0001$

time, but revealed a significant main effect of drug treatment for the closest distance between animals measure ($F_{3,56} = 2.89$, $P < 0.04$), which subsequent Newman–Keuls post-hoc analysis showed to be increased at 10 mg/kg.

Fluoxetine (chronic). ANOVA revealed a reliable overall effect of the treatment for immobility time ($F_{3,56} = 10$, $P < 0.0001$), but not on the two other measures. Post-hoc comparison showed that repeated fluoxetine administrations markedly reduced immobility time in mice facing a predator.

Forced contact with the predator (Fig. 5)

Imipramine (acute). ANOVA indicated a reliable effect for frequency of biting to the rat ($H_{3,60} = 9.4$, $P < 0.02$), and jump attacks toward the predator ($H_{3,60} = 7.99$, $P < 0.05$), but not for the frequencies of vocalization and upright postures. Subsequent Mann–Whitney *U*-tests revealed significant increases in biting at 5 mg/kg and in jump attacks at 5 and 10 mg/kg.

Imipramine (chronic). ANOVA indicated a reliable effect for frequency of biting ($H_{3,60} = 10.77$, $P < 0.01$) which subsequent Mann–Whitney tests showed to be due to a marked reduction of this response at all doses (5–15 mg/kg). ANOVA failed to reveal a reliable main effect for any of the other behavioral measures.

Fluoxetine (acute). ANOVA indicated a reliable main effect for the frequency of biting ($H_{3,60} = 10.22$, $P < 0.01$), but not on the three other measures. Mann–Whitney *U*-tests indicated a reliable increase in biting at the highest dose of fluoxetine (15 mg/kg).

Fluoxetine (chronic). ANOVA indicated a reliable main effect for frequency of biting to the rat ($H_{3,60} = 11.54$, $P < 0.009$), but not on the three other measures. Subsequent Mann–Whitney *U*-tests revealed that long-term fluoxetine treatment significantly decreased biting to rat at all doses.

Discussion

MDTB: behavioral analysis of drug-free controls

Present data provide a series of behavioral profiles in response to predatory or contextual threat stimuli which are generally consonant with previous studies in this laboratory using the MDTB (Griebel et al. 1994a,

1995a). Thus, in the contextual defense situation, escape attempts were strongly potentiated during the post-rat period, in comparison to an equivalent period preceding the confrontation to the predator. Furthermore, in reaction to an approaching predator, control mice often showed active flight responses, with a consistent prey-predator distance of about 1.0 m in the acute experiments and nearly 1.20 m in both chronic saline groups. Similarly, in these latter groups, the escape distance (1.40 m) was increased compared to the acute saline-treated mice (0.90 m). When the control mice ran to escape the chasing predator, they frequently displayed predator assessment, consisting of an abrupt movement arrest often followed by orientation to the approaching rat. In this particular situation, acute and chronic saline-treated animals showed similar performances. This was in contrast to the inescapable straight alley test, where both chronic control groups showed a much higher level of freezing (immobility) and less active predator assessment responses (approaches/withdrawals) in comparison to the acute groups. Finally, defensive threat and attack to the predator almost invariably occurred upon forced contact in all saline-treated groups.

These results suggest that 3 weeks of daily handling and drug injection potentiated some aspects of the defensive repertoire for mice in the chronic administration groups. Interestingly, this difference was much more apparent in less threatening situations, when the predator remained at a constant distance and did not have direct contact with the subject.

Drug effects

Present data show that systemic administration of imipramine and fluoxetine produced a number of changes in antipredator defense which may be related to modulation of fear/anxiety or panic behaviors. In particular, these results show that chronic treatment with SRIs produce complex, but relative similar, patterns of behavioural changes and, as will be discussed below, that these changes differ from those seen with other anxiolytics such as chlordiazepoxide and 5-HT_{1A} receptor ligands (Griebel et al 1994a, 1995a).

Effects preceding and following predator exposure: "contextual fear defense"

Previous findings from this laboratory have shown that the traditional BZP chlordiazepoxide as well as the novel anxiolytic gepirone strongly counteracted the potentiation of escape attempts after the removal of the predator, while the panicolytic compound alprazolam, given acutely or chronically, failed to produce such an effect (Griebel et al. 1994a, 1995a). In the present study, neither acute nor chronic treatment with SRIs was able to counter the potentiation of escape attempts during the post-predator period. However, both acute and chronic fluoxetine as well as chronic imipramine produced at certain doses a reliable reduction in the occurrence of escape attempts, compared to those of saline-treated animals. In the absence of a sedative action of such treatment (line crossing, wall rearing and maximum flight speed were unchanged), these escape reductions appear to reflect a rather specific action of the treatments. However, taken as a whole, these results indicate that short (in the case of fluoxetine) as well as long-term treatment of imipramine and fluoxetine only weakly reduced contextual fear/defense responses. Furthermore, in view of the ability of the anti-GAD agents chlordiazepoxide and gepirone to prevent a post-rat increase in escape attempts, our results are in line with clinical data showing weak effects of SRI treatment in the management of GAD (Nutt and Glue 1991; Murphy et al. 1993).

Drug effects during exposure to the predator

Flight. Following single administrations, imipramine and fluoxetine tended to potentiate flight reactions in response to an approaching or chasing predator. Thus, both treatments reliably increased the prey-predator distance at which flight occurred. In addition, acute imipramine also increased escape distance as well as overall flight speed, indicating a strong action of the drug on the potentiation of flight responses. By contrast, after chronic treatment with either drug, flight-facilitating effects were not seen and in fact, a strong reduction in avoidance distances was obtained. Chronic injection of imipramine also reduced the frequency of escapes. In light of the suggestion that panic symptoms are due to pathological and spontaneous activation of neuronal mechanisms underlying flight reactions (Graeff 1990; Deakin and Graeff 1991; Deakin et al. 1991), taken together with the observation that SRIs are effective in the alleviation of panic attacks, the present data strongly suggest that the MDTB provides measures that serve as an effective experimental model of PD. This view is further supported by recent findings in the MDTB that the panic-promoting drug yohimbine potentiates flight reactions (Blanchard et al. 1993b), while the panicolytic agent, alprazolam, given on a repeated basis, markedly reduced the prey-predator distance of which flight occurred (Griebel et al. 1994a). In addition, the specific anti-GAD compounds chlordiazepoxide and gepirone were found to be devoid of any flight-modulating action (Griebel et al. 1994a, 1995a). Furthermore, our findings of a potentiation in flight reactions after single acute doses of either SRI, are in agreement with the well described exacerbation in the severity and frequency of panic attacks at the beginning of SRI medications, often accompanied by anxiety-related symptoms described as racing thoughts,

nervousness, tremor, jitteriness or emotional discomfort (Saletu and Grüberger 1985; Gorman et al. 1987; Kahn et al. 1987; Van Praag 1988; Westenberg and Den Boer 1988, 1993 Humble et al. 1989; Giesecke 1990; Kahn and Moore 1993). Finally, comparisons of drug effects on the flight/avoidance data from situations in which the subject was actually exposed to a predator, and on contextual defense measures such as wall-climbing and jump-escape responses, are in agreement with previous findings suggesting that the former, but not the latter, respond to panic-altering drugs. Although the panicogenic agent yohimbine increased some wall climbing measures in a post-predator test, it did so against a background in which these measures were not increased by exposure to a predator. In fact, in the inescapable and confined situation used in that study, cat exposure increased immobility, and yohimbine decreased it. Thus drug treatment might have released wall climbing by its diminution of crouching/freezing. However, in the same study yohimbine also potentiated flight to a predator (Blanchard et al. 1993b). Moreover, a known panicolytic agent, alprazolam, administered on a repeated basis, failed to decrease such contextual defense or escape responses in tests/situations identical to those of the present study, but did reduce flight responses to an approaching predator (Griebel et al. 1994a).

Predator assessment in the chase/flight and the straight alley test. Previous studies in the MDTB have shown that classic anxiolytics (e.g. chlordiazepoxide) differentially modulate predator assessment activities than does the 5-HT_{1A} anti-anxiety agent gepirone (Griebel et al. 1994a, 1995a). Thus, BZPs only reduced predator assessment responses when the subject was chased, without affecting animals' behavior in the straight alley test. This was in contrast to the 5-HT_{1A} ligand, gepirone which strongly reduced active predator assessement in the straight alley without altering this activity in the chase/flight test. In the present acute experiments, only the 10 mg/kg dose of fluoxetine altered only a single and indirect measure of predator assessment, increasing maximum prey-predator distance in the straight alley, without changing approaches/withdrawals to the predator, the more direct predator assessment measure in the same test. This pattern fails to suggest any substantive anxiolytic effect of acute administration of fluoxetine.

In chronic experiments, when the predator chased the subject, both imipramine and fluoxetine tended to reduce predator assessment responses, with a great potency of the former as it reduced both the number of stops and the occurence of orientations toward the oncoming rat. This behavioral profile closely resembles the one we recently obtained with chlordiazepoxide and with chronic alprazolam and thus indicated an anxiolytic-like action of chronic SRI treatment (Griebel et al. 1994a). These results are also in line with previous findings in rats demonstrating that chronic imipramine treatment significantly reduced behaviors associated with risk assessment during presentation of a cat odour stimulus, along with other changes suggesting an "anxiolytic profile" for imipramine, given on a chronic basis (Blanchard et al. 1993a).

In the straight alley test, both drugs appeared to decrease freezing, but this effect was only reliable for chronic fluoxetine. The apparent, albeit nonsignificant, imipramine effect is consistent with a recent finding in rats showing that chronic imipramine treatment reduced freezing in response to cat presentation (Blanchard et al. 1993a). As mentioned above, freezing baselines for animals receiving repeated injections were dramatically increased compared to the acute control groups. Thus, the lack of effect of acute drug treatments on freezing might be merely due to the fact that baseline means were too low to be further decreased: certainly results for the acute fluoxetine group appear to suggest some decreased immobility, although from a much lower baseline. These possible reductions in immobility (significant for chronic fluoxetine) contrast with the finding that 8-OH-DPAT and gepirone increased immobility time in the same test. However, it might be noted that these effects occurred at higher doses of these 5-HT_{1A} ligands such that involvement of some aspects of the mouse 5-HT syndrome was quite likely (Griebel et al. 1995a).

Defensive threat and attack. When contact was forced between the predator and the subject, acute treatment with both SRIs reliably potentiated defensive attacks, increasing biting to the rat, and (imipramine only) jump attacks. By contrast, after the drugs has been given repeatedly, neither altered jump attacks, while both reliably and markedly reduced biting at all doses. This reduced defensive threat/attack is very similar to that obtained in previous studies in rats and mice with traditional (e.g. BZPs) and atypical (5-$HT_{1A/2A}$ receptor ligands) anti-anxiety agents (Blanchard et al. 1988, 1989; Griebel et al. 1994a, 1995a), a patterning suggesting that defensive threat/attack is a defensive behavior sensitive to a range of anxiolytic drugs. In fact, the only drug with efficacy against anxiety which has not given an unequivocal reduction in this measure is alcohol, which produces an increase in defensive attack at intermediate doses, with reductions at a higher dose (Blanchard et al. 1990). Also, the finding that both of the present drug reliably increased the occurrence of defensive attack after a single acute dose is consonant with other data (from the avoidance paradigm and the chase/flight test) suggesting an anxiogenic-like profile following a single administration of these compounds.

In conclusion, present findings indicate a consistent fear/anxiety reduction following chronic treatment with imipramine and fluoxetine, along with consistent evidence of a potentiation of fear/anxiety level for

both drugs when administered on an acute basis. Interestingly, although imipramine and fluoxetine somewhat differ in their neuropharmacological specificity (e.g. Horn 1980; Klimek et al. 1994), they displayed generally similar effects in the MDTB. We can tentatively assume that common neuronal mechanisms of both drugs (e.g. 5-HT reuptake inhibition) are more specifically involved in the modulation of fear/anxiety responses of mice confronted with a natural predator. In addition, these effects are in agreement with the increase in anxiety observed clinically after the initial administration of SRIs, as well as with the improvement of symptoms of anxiety seen after several weeks of SRI medication. Finally, the marked effect of both SRIs on flight reactions, in light of previous findings with the panic-modulating drugs yohimbine and (chronic) alprazolam of these behaviors, suggests that the MDTB is an effective experimental procedure for the investigation of panicogenic and panicolytic properties of psychoactive drugs.

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