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REVIEW

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Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression

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Abstract Flinders Sensitive Line (FSL) rats have been proposed as an animal model of depression because they resemble depressed humans in that they have elevated REM sleep, reduced activity, and increased immobility and anhedonia after exposure to stressors. The present paper reviews experiments on the drug treatment of FSL and control Flinders Resistant Line (FRL) rats related to their utility as an animal model of depression, and presents new information. FSL rats exhibited exaggerated immobility in the forced swim test which is counteracted by the tricyclic antidepressants imipramine and desipramine and the serotonin reuptake blocker sertraline; the low immobility exhibited by the FRL rats is generally unaffected by these compounds. In contrast to these "therapeutic" effects of well recognized antidepressants, lithium and bright light treatment did not alter the exaggerated immobility of FSL rats. Novel data indicated that neither FSL nor FRL rats exhibited alterations in swim test immobility following chronic administration of the psychomotor stimulant amphetamine (2 mg/kg) and the anticholinergic scopolamine (2 mg/kg), which typically reduce immobility after acute administration. However, it was found that the calcium channel blockers verapamil (5 and 15 mg/kg) and nicardipine (10 mg/kg) did reduce the exaggerated immobility in FSL rats following chronic administration, suggesting that these compounds need to be evaluated further in humans. Previous studies have indicated no differences between FSL and FRL rats evaluated in the elevated plus maze, either at baseline or after the administration of diazepam, suggesting that the FSL rat may not differ from controls in anxiety-related behavior. Another recently published study showed that the FSL rat also did not differ from normal Sprague-Dawley rats in startle tests, indicating that the FSL rats do not exhibit behaviors shown in animal models of schizophrenia. These findings confirm the utility of FSL rats as an animal model of depression because the FSL rats do not appear to exhibit behaviors analogous to anxiety or schizophrenia and because they respond "therapeutically" to antidepressants and not psychomotor stimulants.

Key words FSL and FRL rats · Animal model of depression · Imipramine · Desipramine · Sertraline · Diazepam · Scopolamine · Amphetamine · Nicardipine · Verapamil · Immobility

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Introduction

There is a wide variety of animal models of depression, and the main focus of most of these models has been to predict the antidepressant potential of new medications; i.e. to establish predictive validity (e.g. Borsini and Meli 1988). However, as Willner (1984, 1990, 1991) has noted, evaluation of such animal models should include a consideration of face validity (i.e., how closely the model resembles the human condition) and construct validity (i.e., how closely the model resembles a specific theoretical position) as well. In a recent review considering the Flinders Sensitive Line (FSL) rat, selectively bred for increased cholinergic function (Overstreet 1993), it was concluded that the FSL rats meet reasonably well face, construct, and predictive validity for an animal model of depression.

In humans, individuals may exhibit symptoms of both anxiety and depression, on the one hand, and depression and schizophrenia, on the other. In fact, comorbidity is becoming a very important issue in psychiatric diagnosis. Despite the increasing recognition of this overlap in psychiatric symptoms, there has been little work in animals which addresses the issue of comorbidity. However, it could be argued that, in considering an animal model of depression, it is just as important to demonstrate that the model does not have "anxious" or "schizophrenic" symptoms, a point frequently not addressed in many reviews. Only a limited number of experiments in the Flin-

ders rats have explored the issue of comorbidity and they suggest that FSL rats do not exhibit "anxious-like" or "schizophrenic-like" behavior (see later for description of experiments). Thus, the FSL rat may be a "pure" animal model of depression.

The psychomotor stimulants, including amphetamine and scopolamine, may produce transient improvement of symptoms in depressed humans, but they are not regarded as effective antidepressant medications. Similarly, amphetamine and scopolamine reduce immobility in rats in the forced swim test when given acutely, and so are regarded as false positives (Borsini and Meli 1988). A few investigators have given these agents chronically and have suggested that there is tolerance development to them, unlike the case of antidepressants, where the therapeutic effect increases with time (Borsini and Meli 1988). In the present paper, we will show that under appropriate experimental conditions, immobility of the Flinders Line rats in the swim test is not affected by scopolamine or amphetamine (see later).

The present paper will summarize the data related to the effects of antidepressants on Flinders rats, discuss the effects of the prototypic anxiolytic, diazepam, summarize some findings on the startle test and the administration of dopaminergic drugs and their relevance to schizophrenia, and present new data on the psychomotor stimulants, amphetamine and scopolamine. In addition, some initial findings on the antidepressant-like effects of the calcium channel inhibitors, verapamil and nicardipine, will be presented.

Characteristics of Flinders Line rats

The Flinders Line rats were developed at Flinders University in Australia by selective breeding for differences in effects of the anticholinesterase, diisopropylfluorophosphate (DFP) on temperature, drinking, and body weight. The Flinders Sensitive Line (FSL) rats are more sensitive to DFP as well as cholinergic agonists and have more brain muscarinic receptors in comparison with the Flinders Resistant Line (FRL) rats (Overstreet 1993, for review). The FSL rats were originally proposed as a genetic animal model of depression (Overstreet 1986) because human depressives are also more sensitive to cholinergic agonists (e.g., Janowsky and Risch 1987). However, the FSL rats also resemble depressed humans in several other characteristics, including elevated REM sleep (Shiromani et al. 1988), appetite and weight changes (Bushnell et al. 1995; Overstreet 1993); reduced activity (Overstreet and Russell 1982), and increased anhedonia after exposure to chronic mild stress (Pucilowski et al. 1993). The latter effect is particularly noteworthy because anhedonia, a reduction in ability to experience reward, is a cardinal symptom in human affective disorders. The exaggerated anhedonia in the FSL rats was observed as a greater decrease in saccharin preference following chronic exposure to a series of mild stressors, a procedure originally developed by Willner et al. (1993).

The exaggerated decrease in saccharin preference seen in the FSL rats is only one of a number of abnormal responses to environmental stressors observed in these rats. For example, FSL rats exhibit a greater short-term decrease in saccharin preference after restraint stress (Pucilowski et al. 1993), a greater decrease in activity following exposure to a brief footshock (Overstreet 1986; Overstreet et al. 1989a), and an exaggerated immobility in the forced swim test (Overstreet 1986, 1993). Thus, they resemble depressed humans in being more reactive to stressors (Anisman and Zacharko 1982). Because the FSL rats differ from the FRL rats on the forced swim test on their first exposure, this test has been used to examine the antidepressant-like effects of well established and putative antidepressant drugs (see next section). Because only a single exposure is employed, there are fewer interpretative problems (e.g., learning). However, the issue of face validity of the forced swim test needs to be addressed. We have observed strain differences in rats which suggest that the forced swim test can differentiate strains with varying "depressive" tendencies. For example, Fawn-Hooded rats resemble depressed humans in having blunted hormonal responses to serotonergic agents (Aulakh et al. 1988) and reduced serotonin transporter binding on platelets (Arora et al. 1983) and they are more immobile in the forced swim test than their Wistar controls (Overstreet et al. 1992a). In addition, the more emotional Maudsley Reactive rat is more immobile in the forced swim test than is the Maudsley NonReactive rat (Abel 1991; Overstreet et al. 1992b). Whether immobility represents "depressive-like" behavior or is simply an adaptive reaction to stressful stimuli is still debatable. A greater utility of this test lies in its ability to predict the antidepressant potential of drugs, as discussed in the next section.

Antidepressant effects

Investigators have used a wide variety of paradigms to detect antidepressant-like effects in animals. Among those which continue to be used with some success are the olfactory bulbectomy model (Richardson 1991; vanRiezen and Leonard 1992), learned helplessness (Thiebot et al. 1992), restraint stress (Curzon et al. 1992; Thiebot et al. 1992), chronic mild stress (Willner et al. 1993), differential reinforcement of low rates (O'Donnell and Seiden 1983; Thiebot et al. 1992) and the forced swim test (Borsini and Meli 1988; Detke et al. 1995). Each of these models has a reasonably high predictive validity, but none of them is perfect. The hyperactivity induced by olfactory bulbectomy is counteracted by chronic but not acute treatment with all classes of antidepressants except monoamine oxidase inhibitors (Richardson 1991); interestingly, however, sertraline is active but fluoxetine is not (vanRiezen and Leonard 1992). In a recent comparative study, Kelly and Leonard (1994) reported that tianeptine, a novel serotonergic compound, and sertraline were both active in the olfactory bulbecto-

my and forced swim test paradigms. The escape failures associated with the learned helplessness paradigm or the reduced activity associated with restraint stress can be counteracted by treatment with the benzodiazepine chlordiazepoxide (Curzon et al. 1992; Thiebot et al. 1992). The more recently developed restraint stress and chronic mild stress paradigms have so far been free of false positives or negatives, but many compounds remain to be tested. Several major concerns about the forced swim test, in comparison with these other paradigms, are the false positives shown by amphetamine and scopolamine, the false negatives shown by serotonin reuptake inhibitors (however, see Cervo et al. 1991; Kelly and Leonard 1994), and the relative paucity of information about chronic drug effects (Borsini and Meli 1988). In the following paragraphs we describe a series of studies in the FSL and FRL rats which overcome these concerns.

As indicated above, the FSL rats exhibit exaggerated immobility in the forced swim test when compared to their controls, the FRL rats, whose scores on the swim test are similar to those of outbred Sprague-Dawley rats, from which the two lines were selected (Overstreet 1986; Overstreet et al. 1986). Because antidepressant drugs must be given chronically to depressed patients, a series of studies were initiated using the FSL and FRL rats to observe their responses to antidepressant drugs. One unexpected finding of this first series of studies was that neither FSL nor FRL rats exhibited any alteration in immobility following the commonly used protocol for examining antidepressants in the forced swim test (Overstreet 1993). Typically, rats (usually outbred rats of various strains) are exposed to the swim test for 15 min and then injected with specific doses of the test compound 24 h, 5 h and 1 h before a 5-min retest session (Porsolt et al. 1977; Borsini and Meli 1988). When desipramine (5 mg/kg) and sertraline (5.7 mg/kg) were administered in this regimen, there were no significant changes in immobility in either the FSL or the FRL rats (Pucilowski and Overstreet 1993). In contrast, chronic treatment with these same drugs (once daily IP for 14 days), significantly reduced immobility in the FSL rats, as summarized in Table 1. Also included in Table 1 are an indication of the effectiveness of the treatment in counteracting the symptoms of endogenous depression (unipolar), the effect of chronic treatment on cholinergic sensitivity, and the reference for the findings.

Like desipramine, imipramine also significantly reduced immobility in the FSL rats (Schiller et al. 1992), and both tricyclics are well recognized and effective antidepressants in humans (Table 1). However, in contrast to its effectiveness in FSL rats, imipramine did not alter immobility in the FRL rats (Schiller et al. 1992). It is not clear whether this negative result is a consequence of the low baseline immobility of the FRL rats or suggests that they are resistant to the effects of antidepressants. Also noted in Table 1 is the observation that both desipramine and imipramine induce cholinergic supersensitivity upon chronic treatment, due to their anticholinergic side effects. These anticholinergic side effects are generally undesirable effects of most tricyclic antidepressants, and it has been postulated that depression following the termination of antidepressant treatment may be related, in part, to unmasked cholinergic supersensitivity (Dilsaver and Greden 1984a,b). Indeed, we found that chronic treatment with amitryptyline, a tricyclic with strong anticholinergic properties, induces an increase in immobility in the forced swim test when the test is carried out 48 h after a period of chronic treatment (Overstreet et al. 1986).

Sertraline, which has been shown to be a very effective antidepressant and was also found effective in reducing immobility in the forced swim test in the FSL rats (Table 1; Pucilowski and Overstreet 1993), is a selective serotonin reuptake blocker. Like fluoxetine, it does not have significant anticholinergic side effects. In unpublished findings, we have found that the cholinergic sensitivity of the FSL rats is unaltered by chronic sertraline treatment. In contrast, however, the exaggerated serotonergic sensitivity of FSL rats (Wallis et al. 1988) is reduced following chronic sertraline treatment (Pucilowski and Overstreet 1993). Because of the pattern of these effects, it is difficult to maintain the original hypothesis that the exaggerated immobility of FSL rats is related to their cholinergic supersensitivity (Overstreet 1986; Over-

Table 1 Effects of recognized or potential antidepressants on affective symptoms in humans and on immobility and cholinergic sensitivity in Flinders rats

Compound	Reduction in affective symptoms	Reduction in Immobility	Cholinergic sensitivity
Imipramine (15 mg/kg/day, 60 days)	Yes	Yes	Increased[a]
Desipramine (2×5 mg/kg per day, 14 days)	Yes	Yes	Increased[b]
Sertraline (2×5.7 mg/kg per day, 14 days)	Yes	Yes	No change[b]
Lithium (0.3% lithium diet, 38 days)	Yes/No	No	Increased/decreased[c]
Bright lights (7,400 lux, 8 days)	Yes/No	No	Decreased[d]
DFP (1 mg/kg + 0.2 mg/kg daily, 21 days)	No (increased)	No	Decreased[a]

[a] Schiller et al. (1992)
[b] Pucilowski and Overstreet (1993)
[c] Shiromani et al. (1990)
[d] Overstreet et al. (1990)

street et al. 1986, 1988). Rather, it is likely that the exaggerated serotonergic sensitivity of the FSL rats (Wallis et al. 1988; Overstreet et al. 1992a) underlies their increased immobility in the forced swim test. Recent findings from our laboratory support this argument. In a cross-breeding study to achieve F1, F2, and backcross progeny, it was found that the strain distribution pattern of oxotremorine-induced hypothermia, a measure of cholinergic sensitivity, was not significantly correlated with the strain distribution pattern for immobility or hypothermia induced by 8-OH-DPAT, a selective serotonin$_{1A}$ receptor agonist, but the latter two were significantly correlated with each other (Overstreet et al. 1994a). In addition, selective breeding for 8-OH-DPAT-induced hypothermia (Overstreet et al. 1994b) has resulted in exaggerated immobility in those rats more sensitive to 8-OH-DPAT (Overstreet et al. 1994c). Further support for an association between serotonergic sensitivity and immobility comes from the extensive literature on antidepressants: most antidepressants induce serotonergic subsensitivity when administered chronically (see Kelly and Leonard 1994).

Table 1 also includes information on several other compounds/treatments which are less well recognized as routine antidepressant treatments for endogenous depression. Lithium is still regarded as preventing relapse in bipolar affective disorder patients and may also have antidepressant effects during depressive episodes in bipolar patients (Goodwin et al. 1972), but is not commonly used to treat acute depression per se. There is, however, considerable evidence for lithium's usefulness in conjunction with conventional antidepressants in the treatment of treatment-resistant depressed patients (Heninger et al. 1983; Schopf 1989). Consequently, the yes/no designation in the table signifies the mixed results with respect to lithium's antidepressant effects. There is also controversy as to lithium's mechanism of action, particularly in relation to the importance of cholinergic mechanisms. There is evidence indicating that lithium treatment potentiates the seizure-producing effects of high doses of pilocarpine, a cholinergic partial agonist (Jope et al. 1986). On the other hand, there are other observations indicating that chronic lithium treatment may blunt cholinergic responses (Casebolt and Jope 1989). Chronically treating FSL rats with a diet including lithium led to a blunted hypothermic response to oxotremorine (Shiromani et al. 1990a), but did not lead to an alteration in immobility in the forced swim test (Shiromani et al. 1990b). Thus, lithium partially counteracted the cholinergic supersensitivity of the FSL rats, but did not have antidepressant-like effects in the tests evaluated.

Bright light therapy has been found to be particularly effective in treatment of patients with seasonal depression (Dilsaver 1989). Although there is one report of small benefit occurring following bright light therapy to patients with endogenous depression, bright light therapy has not been generally recommended as a treatment for endogenous depression (Kripke et al. 1992). Consequently, a yes/no designation has been included in Table 1 for this treatment as well. Since FSL rats are postulated to be "endogenously depressed", bright light therapy may not be effective in counteracting their exaggerated immobility. The pattern of results for this study was similar to that for lithium (Shiromani et al. 1990a,b). Exposure to bright light for 1 week did not alter immobility in the forced swim test but did induce a blunting of the hypothermic response to oxotremorine in the FSL rats (Overstreet et al. 1990). In addition, the locomotor suppressant effects of oxotremorine were unaltered by exposure to bright light (Overstreet et al. 1990). Thus, only the hypothalamically mediated hypothermic response to oxotremorine was blunted by exposure to bright light, and the lack of effect on immobility could, therefore, be a consequence of the lack of change in either nonhypothalamic cholinergic systems (e.g., limbic/striatal for locomotion) or other neurotransmitter systems (e.g., serotonergic mechanisms).

The "negative" findings for lithium and bright lights in the FSL rats (Table 1) suggest that the FSL rats may be related predominantly to the endogenous subtype of the affective disorders. It has been observed that individuals which experience seasonal depression typically have the bipolar constellation of symptoms (i.e., overeating and oversleeping) and may experience hypomanic or manic episodes in the off-season (Goodwin and Jamison 1990). Therefore, bipolar illness and seasonal depression may be similar to each other and different from unipolar illness. The fact that treatments normally used for bipolar illness and seasonal depression do not alter immobility in the FSL rats suggests that the FSL rat is relevant to the unipolar subtype.

The final compound listed in Table 1 is diisopropylfluorophosphate (DFP), an irreversible cholinesterase inhibitor. The FSL and FRL rats were genetically selected from Sprague-Dawley rats on the basis of their responses to DFP, with supersensitive responses being used to select the FSL rats (Russell and Overstreet 1987; Overstreet 1993). DFP was given to human subjects many years ago, and a major effect was the development of depressive-like symptoms (Rowntree et al. 1950). Similar depressive effects have been reported for other cholinesterase inhibitors such as physostigmine (Janowsky and Risch 1987; Janowsky et al. 1994). An interesting feature of DFP is that, because of its ability to inhibit acetylcholinesterase activity irreversibly, it can be administered in low dose regimens to achieve dramatic reductions in enzyme activity without causing apparent behavioral effects (Chippendale et al. 1972; Bushnell et al. 1991). Such treatments also lead to a reduction in the number of muscarinic receptors and in a decrease in responses to cholinergic agonists (see Russell and Overstreet 1987, for review). We took advantage of this knowledge to treat FSL and FRL rats with a chronic regimen of DFP which would reduce cholinergic function. After such treatment, the large differences in immobility between the FSL and FRL rats remained; they were unaffected by the chronic DFP treatment (Schiller et al. 1992). These "negative" findings are entirely consistent

with the conclusion noted above, i.e., that the exaggerated immobility of the FSL rats is not directly related to their cholinergic supersensitivity, but to some other difference.

In summary, FSL rats exhibit reductions in immobility following chronic treatment with the classic tricyclic antidepressants, imipramine and desipramine, and the serotonin reuptake inhibitor, sertraline, but not following chronic treatment with lithium, bright light, or DFP. The pattern of these effects is consistent with the possibility that serotonergic mechanisms underlie the exaggerated immobility of the FSL rats, but rule out any major direct role for cholinergic mechanisms in causing the exaggerated immobility.

Psychomotor stimulants and immobility

Because of the effectiveness of imipramine, desipramine, and sertraline in reducing the exaggerated immobility of the FSL rats in the forced swim test, we re-examined the effects of chronic treatment with amphetamine and scopolamine, which act as "false positives" in the classical swim test paradigm (Borsini and Meli 1988). There had been reports that the anti-immobility effects of these psychomotor stimulants lost their effectiveness during chronic treatment (see Borsini and Meli 1988), but no investigator had examined the effectiveness of these compounds at some time after the final treatment. Because the anti-immobility effects of imipramine, desipramine, and sertraline were examined 24 h after the last treatment (Schiller et al. 1992; Pucilowski and Overstreet 1993), a similar time frame was employed for this study.

The FSL and FRL rats were selected from the viral-free breeding colonies maintained in the Skipper Bowles Center for Alcohol Studies at the University of North Carolina at Chapel Hill. At 70 days of age, the rats were randomly divided into three treatment groups: vehicle, *d*-amphetamine sulfate (2 mg/kg) and scopolamine hydrochloride (2 mg/kg). All compounds were injected intraperitoneally twice daily (0830 and 1630 hours) for 14 consecutive days. Approximately 24 h after the last treatment, the rats were placed individually into a cylinder (18 cm diameter, 40 cm tall) containing enough 25°C water so that the hindpaws could not touch bottom. The degree of immobility (Pucilowski and Overstreet 1993) over a 5-min session was assessed by an observer blind to the treatment received by the rats.

As can be seen in Fig. 1, the FSL rats, regardless of treatment, are two to three times more immobile in the forced swim test than their FRL counterparts, confirming many previous reports (see Overstreet 1993). Neither amphetamine nor scopolamine significantly altered the exaggerated immobility of the FSL rats when evaluated 24 h after the last treatment (Fig. 1). Neither did amphetamine or scopolamine alter the lower immobility exhibited by the FRL rats (Fig. 1). This outcome is consistent with the literature (see Borsini and Meli 1988) suggesting that these drugs are poor or inconsistent antidepressants. Both of these drugs give positive results in the standard swim test paradigm, which involves measuring immobility within 1 h after drug treatment (Borsini and Meli 1988). We have shown that acutely administered scopolamine and amphetamine activate the rats so that immobility scores are very low, and scopolamine retains its anti-immobility effects upon chronic administration if the test is conducted 1 h after the last treatment (Overstreet, unpublished observations 1993). Thus, the present set of conditions (chronic treatment of drugs prior to test; single 5-min exposure to swim test 24 h after last treatment) has permitted us to detect the antidepressant-like effects of compounds appearing as false negatives (sertraline) and to reject compounds appearing as false positives in the standard swim test paradigm (amphetamine and scopolamine).

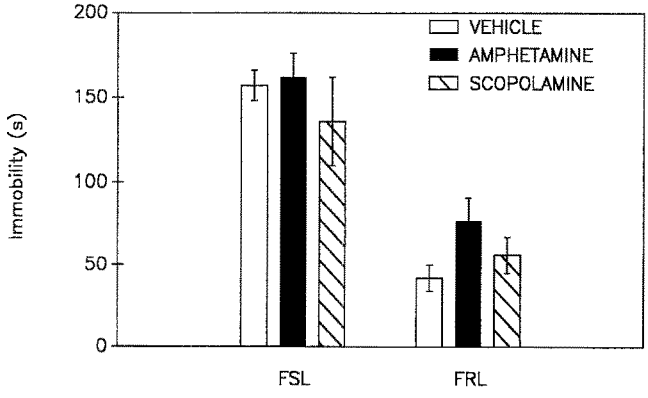

Fig. 1 Effects of chronic scopolamine and amphetamine treatment on immobility of FSL and FRL rats in the forced swim test. Data represent the means+SEM for eight rats. Rats were treated for 14 consecutive days with vehicle (1 ml/kg isotonic saline), amphetamine (2 mg/kg) or scopolamine (2 mg/kg). A single 5-min exposure to the swim test occurred 24 h after the last treatment

Anxiolytic effects

As indicated above, anxiety and depression frequently coexist in psychiatric patients. Related to this, there has also been some question as to whether various animal tests purported to model depression or anxiety, respectively, are truly specific tests. Thus, although earlier pharmacological studies using the forced swim test revealed negative results when benzodiazepine anxiolytics were given (Porsolt et al. 1977; Borsini and Meli 1988), there have been some reports of positive findings (e.g., Fernandez-Teruel et al. 1989). The Maudsley Reactive (MR) rats, selectively bred for high defecation in the open field test (Blizard 1981), exhibit signs of anxiety-like behavior in some tasks but not all (Beardslee et al. 1989; Overstreet et al. 1992b). In addition, MR rats are more immobile in the forced swim test than their control counterparts, the Maudsley NonReactive (MNRA) rats, or outbred Wistar rats, from which they were genetically selected (Abel 1991; Overstreet et al. 1992b). The MR

rats are also less active than are the MNRA rats in the open field test (Blizard 1981; Berrettini et al. 1994). It should also noted that MR rats spend significantly less time in the open arms of the plus maze than the MNRA or Wistar control rats (Overstreet et al. 1992b) suggesting that, as predicted, they are more anxious than their control counterparts. Since, like the MR rats, FSL rats are less active than FRL rats in the open field test (Overstreet and Russell 1982), there is some suggestion that there could be benzodiazepine-GABAergic differences which might underlie these differences in activity and possibly anxiety-related behavior.

An initial study provided evidence for benzodiazepine-GABAergic differences between the two Flinders lines. It was found that FSL rats were more sensitive to the behavioral suppressant effects of diazepam, a benzodiazepine agonist, and muscimol, a $GABA_A$ agonist, than were FRL rats on two different tasks: locomotor activity in the open field and operant responding for water reward (Pepe et al. 1988). In addition, it was determined that the FSL rats exhibited increased levels of benzodiazepine receptor binding in the striatum and hippocampus, regions previously shown to have increased muscarinic receptor binding (Overstreet et al. 1984; Pepe et al. 1988). Thus, there is pharmacological and neurochemical evidence for differences in the benzodizepine-GABAergic system in the FSL and FRL rats.

To address this issue further, we used the elevated plus maze test, a test which had been reported to be sensitive to benzodiazepine anxiolytics (Pellow et al. 1985). This apparatus consists of two elevated closed arms and two elevated open arms. Rats are placed in the maze for a 5-min session and the number of arm entries and time spent in the open arms are recorded. Typically, an "anxious" rat will spend little or no time in the open arms of the maze; thus, the percentage of time a rat spends in the open arms of the maze is a measure of its anxiety-like behavior. In baseline 5-min exposures, FSL and FRL rats spent approximately the same amount of time in the open arms of the plus maze and entered the open arms a similar number of times (Schiller et al. 1991; Overstreet et al. 1992a). It would appear, therefore, that the benzodiazepine-GABAergic differences in these rats reported earlier (Pepe et al. 1988) do not impact upon this task. Moreover, when diazepam (1 mg/kg) was administered, there were similar increases in the amounts of time spent in the open arms of the maze, suggesting that the anxiolytic effects of diazepam were also similar in the Flinders Line rats.

Thus, there do not appear to be differences in the FSL and FRL rats in anxiety-related behavior, although it must be acknowledged that only a single test has been used. Much more work is required before a firm conclusion can be reached.

Dopamine and antipsychotic effects

The cholinergic and dopaminergic systems are linked in the regulation of a number of behaviors and functions, including locomotor activity and temperature regulation and, in humans, mood and psychotic phenomena. For locomotor activity, mood and psychotic phenomena, the cholinergic and dopaminergic systems are opposed, with either cholinergic stimulation or dopaminergic blockade leading to a reduction in the target phenomena. Conversely, dopaminergic stimulation or cholinergic blockade may lead to increased locomotor activity, mood, and/or psychotic effects (see Janowsky and Risch 1987; Tandon and Greden 1989). Because of these known interactions, we compared dopaminergic function in FSL and FRL rats, rat strains with known differences in cholinergic sensitivity and receptors. Relative to the FRL rats, the cholinergically supersensitive FSL rats were less sensitive to the stereotyped behavior induced by the dopamine agonists apomorphine and quinpirole, and, conversely, were more sensitive to the catalepsy induced by the dopamine D_2 antagonist raclopride (Crocker and Overstreet 1991). These findings suggest that a dopaminergic subsensitivity exists in the FSL rats. However, in contrast, the FSL rats were more sensitive to the hypothermia induced by the same doses of apomorphine and quinpirole used to study stereotyped behavior (Crocker and Overstreet 1991; Pucilowski and Overstreet 1993). In addition, investigations of the numbers of dopamine (D_2) receptors in the FSL and FRL rats evaluated by receptor binding and autoradiographic techniques failed to detect any differences between the two lines (Crocker and Overstreet 1991). Because there were no differences in dopamine receptors, it was concluded that the differences in effects of dopaminergic drugs in the two lines can be ascribed to differences in the cholinergic system, which interacts with the dopaminergic system, either in parallel (temperature), or in opposition (locomotor activity) (see Overstreet 1993, for further discussion). Of course, it is also possible that other changes in dopaminergic function or in other neurotransmitters could account for these findings.

If the concept of balance between systems has heuristic value, one may predict that rats as well as humans having deficient dopaminergic function would be supersensitive to cholinergic agonists. There is no literature having a direct bearing on this point in animals. However, there have been reports that rats which have been chronically treated with dopamine antagonists to induce dopaminergic supersensitivity are indeed subsensitive to cholinergic agonists without any changes in muscarinic receptors (Muller and Seeman 1977). In addition, Tandon and Greden (1989) have suggested that cholinergic supersensitivity may be associated with the negative symptoms of schizophrenia. To our knowledge, Tandon and colleagues have not explored whether these same individuals have a dopaminergic deficiency. Therefore, it is not clear at this stage whether cholinergic supersensitivity is a primary change in schizophrenics or whether it is related to changes in some other neurotransmitter which interacts with the cholinergic system, such as dopamine. What is clear, however, is that FSL rats have a cholinergic supersensitivity which parallels that reported for

schizophrenics (Tandon and Greden 1989; Overstreet 1993) and that the functional changes seen in these rats in response to dopaminergic drugs cannot be related to changes in dopamine receptors (Crocker and Overstreet 1991).

Relevant to this issue, an animal model of schizophrenia which has been recently developed and appears to have face, construct and face validity is the prepulse inhibition of startle, a measure of sensory gating in both animals and humans (Braff and Geyer 1991; Swerdlow et al. 1992). Normally, a moderately loud stimulus given before a louder startle stimulus leads to a smaller response, but this is not the case in schizophrenics or in animals treated with dopamine agonists. Normalization of the response occurs in both the schizophrenics and the rats treated with dopamine agonists when they are given dopamine antagonists. Recently, FSL and FRL rats were studied using the startle test paradigm and were compared with randomly bred Sprague-Dawley controls. Although the FRL rats differed from both the FSL rats and Sprague-Dawley controls with regard to startle thresholds and to sensitization, there were no differences in pre-pulse inhibition of startle among all three groups (Markou et al. 1994). Thus, for a task mediated by dopamine and directly related to the deficits seen in schizophrenics, the FSL rats are not different from the FRL rats or from control Sprague-Dawley rats. These "negative" data reinforce the utility of the FSL rats as a specific animal model of depression.

Calcium channel inhibitors and immobility

Because the exaggerated immobility of the FSL rats in the forced swim test was counteracted by chronic treatment with imipramine, desipramine and sertraline (Table 1), this paradigm was used to explore the antidepressant potential of calcium channel inhibitors (CCIs). CCIs are known for their vasodilatory and antiarrhythmic properties (Kiowski et al. 1986). Recently, considerable evidence has accumulated on the psychopharmacological properties of CCIs, and they appear to have a unique profile. There have been reports suggesting that CCIs possess antidepressant, neuroleptic, anxiolytic-like, anticonvulsant and sedative properties (see Pucilowski 1992, for review). Presented is a study we designed to provide further evidence of the antidepressant potential of CCIs by examining the effects of chronic treatment with them on the exaggerated immobility of the FSL rats in the forced swim test.

FSL and FRL rats were selected from the viral free breeding colonies maintained in the Skipper Bowles Center for Alcohol Studies at the University of North Carolina at Chapel Hill. The rats were maintained in groups of four in polycarbonate cages in a room with constant temperature (22°C) and humidity (50%) and under a reversed light:dark cycle (lights out from 1000 to 2200 hours). At 70 days of age, the rats were randomly divided into five groups which received the following treatments (n=8 per group): Vehicle, Amphetamine (2 mg/kg), Scopolamine (2 mg/kg), Verapamil (5 mg/kg) and Verapamil (15 mg/kg). The results of amphetamine and scopolamine treatments have been presented above (Fig. 1). In a second experiment, 100-day-old FSL rats only were treated with vehicle, 2.5 or 10 mg/kg nicardipine (n=8 per group). Because FRL rats did not exhibit any changes in the first experiment, they were not used in the second experiment.

All treatments were injected intraperitoneally twice daily (0830 and 1630 hours) for 14 consecutive days. Approximately 24 h after the last treatment the rats were placed individually into a cylinder (18 cm diameter, 40 cm tall) containing enough 25°C water so that the hindpaws could not touch bottom. The degree of immobility (Pucilowski and Overstreet 1993) over a 5-min session was assessed by an observer blind to the treatment received by the rats.

Verapamil significantly reduced the immobility in the 70-day old FSL rats, as illustrated in Fig. 2. The vehicle-treated groups, repeated from Fig. 1, clearly illustrate the large differences between the FSL and FRL rats. Note also that the although the immobility of the FSL rats has been significantly reduced by verapamil, the immobility has not been lowered to the level of the FRL rats. Finally, the immobility of the FRL rats was not significantly affected by chronic verapamil treatment (Fig. 2).

Thus, Fig. 2 shows that verapamil significantly reduces immobility of only the FSL rats in the forced swim test. However, this phenylalkylamine CCI has significant serotonin reuptake inhibition properties (Pucilowski 1992), and it could be this property of the compound which is responsible for its anti-immobility effects. Therefore, as mentioned above, we conducted a second study with nicardipine, a dihydropyridine CCI which does not inhibit serotonin reuptake and for which there is

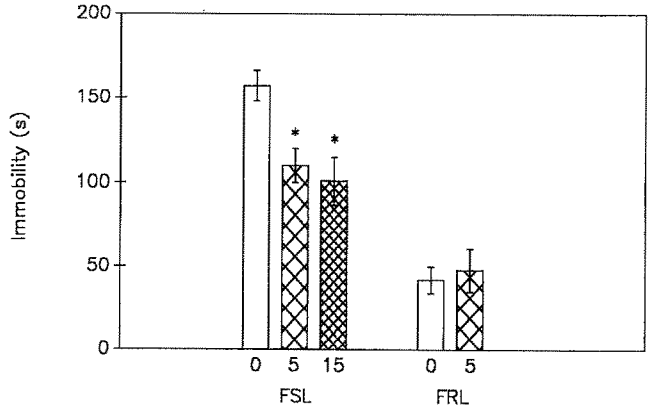

Fig. 2 Effects of chronic verapamil treatment on immobility of FSL and FRL rats in the forced swim test. Data represent the means+SEM for eight rats. Rats (70 days old) were treated for 14 consecutive days with vehicle (1 ml/kg isotonic saline) or verapamil (5 and 15 mg/kg in FSL rats; 5 mg/kg in FRL rats). A single 5-min exposure to the swim test occurred 24 h after the last treatment. *Significantly different from vehicle-treated group, $P<0.05$, Newman-Keuls test

limited animal data with respect to immobility in the forced swim test (Bidzinski et al. 1990). FSL rats (n=8) were injected twice daily with saline or 2.5 or 10 mg/kg nicardipine for 14 consecutive days beginning at 100 days of age and were tested in a 5-min forced swim test 24 h after the last injection. One-way ANOVA and Newman-Keuls analyses indicated that the saline-treated group exhibited high immobility (227+11 s), which was counteracted by the higher dose of nicardipine (98+10 s), but not by the lower dose (232+13 s). The values for the saline-treated rats are higher than those for saline-treated FSL rats in Fig. 2 because the rats were older. Nevertheless, these data indicate that nicardipine dose-dependently reduced immobility of the FSL rats in the forced swim test, suggesting that the CCI properties might be the basis of the anti-immobility effects.

Both nicardipine and verapamil are L-type CCIs and have been reported to suppress serotonin$_2$ receptor mediated head twitch responses in mice and to inhibit competitively the binding of ketanserine, a serotonin$_2$ receptor antagonist, to brain membranes (Green et al. 1990). Since both chronic tricylic (Peroutka and Snyder 1980; Goodwin et al. 1984) and serotonin$_2$ receptor antagonist (Blackshear et al. 1983) treatments induce a down-regulation of serotonin$_2$ receptors, it is possible that the two CCIs may be having their antidepressant-like effects in the FSL rats by down-regulation of these receptors rather than by interacting with L-type calcium channels. It has also been reported that several atypical antidepressants such as mianserin, and trazodone and potent serotonin$_2$ receptor antagonists (Nemeroff 1994). In fact, these two mechanisms may be synergistic. There is evidence that serotonin$_2$ receptors are coupled to the phosphatidyl inositol/phospholipase C system, which mobilizes calcium (Affolter et al. 1984). It has also been reported that serotonin-stimulated calcium responses are increased in depressed patients (Kusumi et al. 1991). By both inhibiting serotonin$_2$ receptors and blocking calcium channels, the CCIs could be particularly effective antidepressants. Further studies with CCIs without 5-HT properties are required to evaluate the relative contribution of calcium channel and/or serotonin receptor blockade to these antidepressant-like effects.

Because well recognized antidepressants had anti-immobility effects (Table 1) but amphetamine and scopolamine did not (Fig. 1), the anti-immobility effects of verapamil and nicardipine are likely to reflect antidepressant potential. The fact that both verapamil and nicardipine were effective suggests that the L-type calcium channel itself might be the site of action of their antidepressant-like effects. Verapamil is known to have fairly strong serotonin reuptake blocking properties, but nicardipine does not (Pucilowski 1992). In addition, as described above, both compounds can block serotonin$_2$ receptors. However, the affinity of nicardipine and verapamil for the serotonin$_2$ receptor (Green et al. 1990) is in the micromolar range, while that of the atypical antidepressants is in the nanomolar range (Nemeroff 1994). Therefore, these findings must still be regarded as preliminary. There are a wide range of other CCIs, both those interacting with L-type calcium channels as well as those interacting with other types of calcium channels, which need to be tested before we can arrive at a definitive conclusion. Even so, the present findings are encouraging. The use of CCIs to treat affective disorders has another advantage: the CCIs generally have very low side effect profiles, so if they do have antidepressant effects in humans, they could potentially be better tolerated than more classical antidepressants, particularly in groups such as the elderly who are very sensitive to the related side effects (Raftery 1984).

Discussion

The present paper has summarized previous research on the effects of chronic antidepressant treatment on immobility of Flinders Line rats in the forced swim test and concluded that the FSL rats respond to clinically effective antidepressants by reducing their exaggerated immobility. However, FSL rats do not exhibit a therapeutic response following chronic administration of lithium or DFP or exposure to bright light, treatments not normally used as a first option in depressed patients. The novel data presented in this paper indicate that neither FSL nor FRL rats exhibit changes in immobility following chronic treatment with scopolamine or amphetamine 24 h after the last treatment (Fig. 1). Therefore, the use of a genetic animal model with exaggerated immobility, a chronic treatment protocol, and testing occurring at 24 h after the end of chronic treatment has led to conditions where both the false positive treatments amphetamine and scopolamine and the false negative treatment sertraline have been eliminated. It should be emphasized that sertraline has also been reported to be effective in the standard swim test protocol (Cervo et al. 1991; Kelly and Leonard 1994). The predictive validity of the FSL rat could be strengthened further if it can be shown that the exaggerated immobility of the FSL rats is counteracted by monoamine oxidase inhibitors, atypical antidepressants such as trazodone, electroconvulsive shock, and other particularly effective antidepressant treatments in humans.

The fact that CCIs were "active" under these conditions reinforces the previous preclinical literature suggesting that these compounds may act as antidepressants (Bidzinski et al. 1990; Pucilowski 1992). Although a number of CCIs have been used clinically to manage manic-depressive disorders and verapamil has been reasonably successful as a prophylactic treatment for mania (Hoschl 1991; Dubovsky 1993), clinical trials of CCIs as specific antidepressants have met with mixed results (see Dubovsky 1993). Unfortunately, the most recent clinical study on the antidepressant effects of CCIs examined a population of treatment-resistant patients and reported negative results (Adlersberg et al. 1994).

These negative clinical findings suggest at least two possibilities. The first is that the CCIs are false positives

in the FSL-forced swim test paradigm. If this is the case, then further clinical trials with the CCIs will continue to reveal negative findings. A second possibility is that the CCIs produce a true antidepressant-like effect in the FSL rats and that further clinical trials with CCIs will detect their efficacy in the treatment of affective disorders. The fact that the FSL-forced swim test paradigm correctly excluded amphetamine and scopolamine as antidepressants (Fig. 1) provides some hope that the latter alternative might be correct. In addition, the fact that there is one report indicating an antidepressant-like effect for the CCI nimodipine in the learned helplessness model (Martin et al. 1989) supports the suggestion that the anti-immobility effect of CCIs reflects antidepressant effects.

Further studies are needed to define the efficacy of CCIs in depressed patients, although CCIs do appear to be effective in treating mania and rapid cycling bipolar patients (Dubovsky 1993). More information is also needed on the mechanisms by which CCIs exhibit their antidepressant-like effects in rats; further studies of the FSL rats may provide some insights. The possibility that CCIs may augment the action of classical antidepressants, as found by Martin et al. (1989) in the learned helplessness paradigm, has recently received some attention in Poland (Vetulani 1993).

The present paper has also summarized data indicating that the FSL rats exhibits altered sensitivity to the locomotor suppressant effects of diazepam. However, anxiolytic effects of diazepam are similar in the FSL and FRL rats and there are no differences in their anxious-like behavior (time spent in the open arms of the elevated plus maze). Therefore, FSL rats appear to be an animal model of depression without a significant anxiety component. However, it must be emphasized that only one anxiety-related test has been conducted, so this conclusion of the lack of differences in anxious-like behavior between the FSL and FRL rats should be regarded as only preliminary, particularly in view of the fact that the Maudsley rat strains differ on some but not all tests of emotionality (Overstreet et al. 1992b).

A similar tentative conclusion can be reached regarding dopaminergic mechanisms and schizophrenia. Although the FSL rats exhibit altered responses to dopamine agonists and antagonists, they do not have altered D_2 receptors. Neither do they exhibit altered prepulse inhibition of startle, as do schizophrenics and dopamine-stimulated animals. Therefore, FSL rats do not exhibit any model schizophrenic-like behavior. Again, however, only a limited number of studies of dopaminergic function and/or model schizophrenic-like behavior have been conducted in the FSL and FRL rats, so this conclusion must also be regarded as preliminary.

In conclusion, not only does the FSL rat fulfill the criteria of face, construct, and predictive validity for an animal model of depression. It also does not exhibit any anxiety- or schizophrenic-like behaviors, at least in the limited testing conducted to date. It should also be stressed that the FSL rats will not voluntarily drink much alcohol, unlike some depressed individuals (Overstreet et al. 1989b) and the Fawn-Hooded rats (Overstreet et al. 1992a). Thus, the FSL rat may very specifically model "pure" unipolar or endogenous depression, which may make it a useful model for understanding the psychopharmacology and psychobiology of this subtype of affective disorders.

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