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Research report

# Drug-, dose- and sex-dependent effects of chronic fluoxetine, reboxetine and venlafaxine on open-field behavior and spatial memory in rats

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## HIGHLIGHTS

- Chronic fluoxetine and venlafaxine were primarily anxiogenic.
- Reboxetine was less anxiogenic than the other two drugs.
- Any anxiogenic effects largely depended on dose and sex.
- All drugs impaired spatial memory for male rats only.
- Overall, reboxetine was the least behaviorally effective drug.

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## ABSTRACT

In an effort to address the need to include both sexes in studies of effects of the SSRI fluoxetine, the NRI reboxetine and the SNRI venlafaxine on anxiety-related behavior and memory along with the use of chronic drug administration, male and female PVG/c rats were fed diets containing two doses of each drug for 21 days. The rats' anxiety level was then assessed in an open field. Short-term spatial memory for a brightness change in a Y maze was also measured. While there was little evidence of anxiolytic effects of any of the drugs, both fluoxetine and, to a lesser extent, venlafaxine appeared to be mainly anxiogenic in their action depending on both dose and sex. Reboxetine was relatively ineffective in this respect. Ability to locate the Y-maze arm that had changed (from white to black) seemed to be impaired for male (but not female) rats by both fluoxetine and venlafaxine and, to a much lesser extent, by reboxetine. Given the relative ineffectiveness of reboxetine in either test, it is possible that the effects of the other two drugs on both anxiety and memory were mainly due to their serotonin reuptake inhibiting properties. The differences that occurred between males and females in responsiveness to all three drugs supported the long-held view that both sexes should be investigated in studies of this sort, especially in view of reports of sex differences in effects of clinically prescribed antidepressants.



## 1. Introduction

In recent years there has been increasing use of newer antidepressant drugs for the treatment of a number of anxiety disorders especially those of a more severe nature such as post-traumatic stress disorder, generalized anxiety disorder or agoraphobia [1]. Amongst other effects, these drugs act on levels of brain serotonin and norepinephrine by specifically inhibiting the re-uptake of either transmitter, or both, thereby reversing their depletion which is believed to underlie symptoms of both depression and anxiety. The first of these newer drugs to be developed were the serotonin-specific re-uptake inhibitors (SSRIs), such as fluoxetine and paroxetine. Since their appearance there have also been produced norepinephrine re-uptake inhibitors (NRIs), such as reboxetine and viloxazine, and serotonin-norepinephrine re-uptake inhibitors (SNRIs) including venlafaxine and duloxetine. Although drugs from each of these groups are routinely used for treating various anxiety disorders as well as depression [2,3], there has been debate concerning their efficacy [4] and effects on other processes, such as memory [5]. Nevertheless, it is generally accepted that both the SSRI fluoxetine and the SNRI venlafaxine are useful for treating several anxiety disorders [3,6,7] with

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venlafaxine possibly being the more effective in some cases [8]. However, there is less agreement about the value of the NRI reboxetine's effects on anxiety symptoms [9,10] consistent with the more recent conclusion that it is relatively ineffective and may even be potentially harmful in the treatment of major depression [11]. Unlike the earlier benzodiazepine anxiolytics, all antidepressants have the disadvantage of requiring regular administration for two or more weeks for any anxiety-reducing action to become effective [12]. This property is particularly relevant to preclinical studies performed with animals that have typically involved acute rather than chronic treatment thereby significantly limiting their predictive value for clinical practice [13]. Nevertheless, there are some notable examples of effects of chronic antidepressant treatment on anxiety-related behavior described below.

A major aim of the present study was to compare the effects on several anxiety-related responses in laboratory rats of chronic oral treatment with a representative SSRI, NRI and SNRI, namely fluoxetine, reboxetine and venlafaxine respectively. Although chronic fluoxetine failed to produce evidence of anxiolysis in high anxiety Wistar-Kyoto rats [14,15], it has more recently been shown that the drug reduced anxiety in stressed rats as determined by behavior in an elevated T but not a plus maze [16]. A similar outcome occurred with chronic reboxetine and venlafaxine. Further evidence for fluoxetine's anxiolytic action is found in observations that, through effects on retention, chronic exposure to the drug can cause a long-term loss prevention of re-instatement of conditioned fear memory [17–19] possibly because of synaptic protein changes [20]. However, it had been earlier shown that chronic fluoxetine increased (rather than reduced) anxiety in rats observed in an elevated plus maze [21]. In spite of the higher prevalence of anxiety disorders and treatment with anxiolytic drugs among women [22], animal research involving antidepressants is overwhelmingly predominated by male-only studies [5], as typifies most areas of behavioral pharmacological and neuroscience research [23,24]. The obvious need to include both sexes in such research is illustrated by clinical reports of men responding more favorably than women to fluoxetine prescribed for both depression [25] and anxiety [26]. However, the opposite outcome was suggested by one of the few animal studies on record involving both sexes which revealed a reduction in fear for female but not for male rats chronically treated with fluoxetine [27].

One of the effects of chronic treatment with antidepressant drugs that has resulted in conflicting findings concerns their possible modification of memory. For example, while some studies have shown improved memory with the SSRI fluoxetine in depressed patients [28] or non-depressed patients with mild cognitive impairment [29], others have suggested fluoxetine-related impaired memory [30,31]. However, it has been more recently concluded that, with the possible exception of paroxetine, SSRIs appear to have negligible effects on memory in either depressed or non-depressed subjects [32]. On the other hand, the NRI reboxetine has been reported to either improve memory deficits in both depressed [33] and non-depressed subjects [34,35], or have no effect on each respective subject group [32,36] or schizophrenic patients [37]. While it has been suggested that there is some weak and rather limited evidence for improved memory with the SNRI venlafaxine [32], there are also cases where the drug was ineffective in this respect. For example, it has been reported that venlafaxine had no effect on memory in either healthy volunteers [38] or elderly depressed patients [39]. Overall, there seems to be a lack of consistency in effects on memory of fluoxetine, reboxetine or venlafaxine in both depressed patients and non-depressed volunteers. This situation is confirmed by the latest review of antidepressant drug effects on cognitive functioning in which the authors also highlight the difficulties of interpretation in many cases because of methodological differences between studies [40]. Research with animals has been characterized by similar inconsistent findings for all three drugs. For example, chronic fluoxetine has been reported to improve [41], impair [42] or have no effect [43] on rodent memory determined by a number of different tests. Although there are significantly fewer examples on record of effects of chronic reboxetine on animal memory, what information is available tends to resemble the situation with human research, namely improved clomipramine-induced impaired memory [44], exacerbation of stress-induced impaired memory [45] or no effect [46]. In a similar fashion, venlaxine has been shown to improve [47,48], impair [49] or have no effect on rodent memory [50,51]. Therefore, in addition to investigating effects of chronic oral treatment with fluoxetine, reboxetine and venlafaxine on anxiety-related behavior in female as well as male rats, the present study aimed to compare the three drugs' effects on short-term spatial memory taking account of some of the issues raised by Monleón et al. [5]. The particular behavioral tests chosen comprised speed of emergence from a small, darkened chamber into a brightly lit open field, subsequent responses emitted in the field, and responsiveness to a brightness change in a Y maze. Increased latencies to emerge have long been viewed as reflecting higher levels of anxiety [52] as is the case with lower levels of ambulation, rearing and center occupancy, and higher levels of grooming, corner occupancy and defecation in an open field [52–54]. Rats' ability to detect and explore the arm of a Y maze that has changed in brightness from what it was during a preceding acquisition trial has been shown to involve curiosity-motivated spatial memory dependent on the influence of both egocentric and allocentric cues [55].

In order to reduce the stress of drug administration via repeated injection or gastric lavage as well as to also more closely approximate how humans normally experience the three antidepressants to be investigated, they were mixed with the rats' food in amounts designed to achieve two target doses of each based on previous research i.e., 7 and 18 mg/kg of fluoxetine [56,57], and 10 and 20 mg/kg of both reboxetine [58,59] and venlafaxine [60,61].

## 2. Materials and methods

### 2.1. Animals

The subjects were 70 male and 70 female PVG/C hooded rats bred in the Animal Facility of the Department of Psychology, the University of Canterbury. They were weaned when 30 days old and housed in 560 mm × 350 mm × 215 mm opaque plastic cages in same-sex groups of 2–4 individuals with ad libitum food (until drug treatment) and water on a 12 h light/dark cycle (lights on at 08:00) at an ambient temperature of 22 ± 2 °C, and humidity of 48 ± 10%. All testing occurred during the light phase of the cycle.

The care and experimental treatment of the rats conformed to requirements of Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Testing and Teaching) of the New Zealand Animal Welfare Act, 1999, and had been approved by the Animal Ethics Committee of the University of Canterbury.

### 2.2. Drugs

The three antidepressants, fluoxetine (Prozac, Eli Lilly, (±)-*N*-methyl-y-[4-(trifluoromethyl)-phenoxy] benzenepropanamine), reboxetine (Edronax, Pfizer, (±)-(R*,R*)-[2-[alpha-(2-ethoxyphenoxy)benzyl]morpholine methanesulfonate) and venlafaxine (Efexor, Pfizer, (±)-1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol) were purchased from CDC Pharmaceuticals (Christchurch, New Zealand) as clinically prescribed tablets or capsules. Tablets of reboxetine (4 mg) were pulverized with a mortar and pestle and appropriate weights of the resulting powder