Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 89 (2008) 450–455
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Evaluation of the acute effects of amitriptyline and fluoxetine on anxiety using grooming analysis algorithm in rats

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Received 11 October 2007; received in revised form 29 January 2008; accepted 1 February 2008
Available online 8 February 2008

Abstract

The tricyclic amitriptyline and the selective serotonin reuptake inhibitor fluoxetine have distinct actions in animal models of anxiety, though both antidepressants are used against anxiety disorders. Grooming behavioural sequencing, rather than its general "activity" measures, has been suggested to measure effectively the pharmacologically induced anxiolytic and anxiogenic-like effects in rats and mice. In the present study, the acute effects of amitriptyline and fluoxetine on anxiety were re-evaluated by using an analysis algorithm in novelty-induced grooming activity in rats. Additionally, the effects on anxiety-like behaviour in the hole board were examined. Amitriptyline (5 and 10 mg/kg) and fluoxetine (5 and 10 mg/kg) not only affected the traditional gross measures, but also produced changes in incorrect transitions and regional distribution of grooming behaviour. High dose of fluoxetine showed an anxiogenic-like profile by reducing head dipping and rearing in the hole board. Depending on the effects on the behavioural microstructure of grooming activity, present findings imply that amitriptyline may possess anxiogenic and fluoxetine may possess anxiolytic activities. However, measures of hole board do not fully support this suggestion.

Keywords: Amitriptyline; Fluoxetine; Grooming; Hole board; Anxiety; Rat

1. Introduction

Antidepressants are widely prescribed for the chronic treatment of several anxiety disorders (Feighner, 1999; Zohar and Westenberg, 2000). However, increased anxiety has been observed in some patients at the beginning of treatment with some of these drugs (Beasley and Potvin, 1993; To et al., 1999). Acute and/or chronic administrations of antidepressants, on the other hand, mostly produce anxiolytic effect, but also result in ineffectiveness or even in anxiogenic activity in animals (Borsini et al., 2002).

Amitriptyline, the older tricyclic antidepressant used in the treatment of anxiety disorders (Feighner, 1999), is one of the drugs which produces inconsistent effects in experimental anxiety. In the elevated plus-maze test, it has been shown that acute (Parra et al., 2002) and chronic (Everss et al., 2005; Harro et al., 1997) treatments of amitriptyline have no effect. On the other hand, chronic, but not acute, treatment of the drug decreases measures of anxiety-related behaviours (Yau et al., 2002) or increases anxiogenic activity (Weinstock et al., 2002) in the same test. In other tests, amitriptyline was found non-anxiolytic (Bilkei-Gorzo et al., 1998; Hijzen et al., 1995; Simiand et al., 1984), anxiogenic (Bodnoff et al., 1989) or anxiolytic only after chronic treatment (Bodnoff et al., 1988).

There are contradictory findings for the selective serotonin reuptake inhibitor (SSRI) antidepressant drug fluoxetine in various anxiety models also. The inconsistencies have been particularly well illustrated in the elevated plus-maze test. Acute fluoxetine produces anxiolytic-like effect when animals were tested 24 h, but not 30 min after drug administration (Griebel et al., 1999). There is no evidence for anxiolytic-like activity following chronic treatment (Griebel et al., 1999; Silva and Brandao, 2000). Both acute (Drapier et al., 2007; Silva et al., 1999; Silva and Brandao, 2000) and chronic (Silva et al., 1999; Uz et al., 2004) treatments of the drug produce anxiogenic activity. Chronic, but not acute, fluoxetine attenuates escape

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doi:10.1016/j.pbb.2008.02.001

behaviour in unstable elevated exposed plus-maze, a novel model of extreme anxiety (Jones et al., 2003). In other tests, the drug exerts clear acute anxiogenic actions which disappear after chronic treatment (To et al., 1999; To and Bagdy, 1999) or produce anxiolytic effect after single (de Angelis, 1996; Nowakowska et al., 1996, 2000) and prolonged (Nowakowska et al., 1996) treatments.

Grooming is an innate stereotyped behaviour found in most animal species (Spruijt et al., 1992). As being considered a response to stress (D'Aquila et al., 2000; Moody et al., 1988; Rodriguez Echandia et al., 1983), novelty-induced grooming has long been studied in neurobehavioural stress research in mice and rats (Barros et al., 1994; O'Callaghan et al., 1982; Whyte and Johnson, 2007). In these studies, general characteristics of grooming behaviour (latency to onset, frequency and duration) have been described. Few reports, however, analyze its organization (patterning) in different stressful situations (Audet et al., 2006; Kametani et al., 1984; Komorowska and Pellis, 2004). It has been suggested that the traditional 'quantitative' measures of grooming may be insufficient for correct data interpretation and analysis, and that additional grooming measures (such as its 'qualitative', or patterning characteristics) are also necessary in order to assess animals' stress-evoked behaviours (Kalueff and Tuohimaa, 2004). Concurrently, a grooming analysis algorithm which uses differential registration and analysis of grooming behavioural microstructure was designed for both mice and rats. This algorithm demonstrating clear impairments of grooming patterning in anxious animals was suggested to detect anxiolytic activity more clearly (Kalueff and Tuohimaa, 2004, 2005a).

In view of the findings mentioned above, the present study investigated whether the analysis of microstructure of novelty-induced grooming behaviour in rats could provide a clarification for the effects of amitriptyline and fluoxetine on anxiety. Anxiety-like behaviour in the hole board was also assessed to discuss the anxiolytic efficacy of the drugs thoroughly.

2. Methods

2.1. Animals

Inbred male Wistar albino rats (Animal Care Unit, Department of Pharmacology and Clinical Pharmacology) were used. The animals were 6–8 months old and weighing 320–370 g. They were housed under standard laboratory conditions for at least 1 week prior to experimentation and were allowed to free access to both food and water. All animal studies carefully conformed to the guidelines outlined in *Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education* issued by the New York Academy of Sciences (1988).

2.2. Procedures

The animals were randomly divided into 5 groups (11–14) and injected i.p. with saline (control), amitriptyline (5 or 10 mg/kg)

Table 1
Behavioural alterations in grooming activity after amitriptyline and fluoxetine administrations in rats

Grooming measures	Control	Amitriptyline		Fluoxetine	
		5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg
Traditional gross measures					
Latency to start grooming (s)	49±11	40±11	65±23	45±7	55±13
Total number of bouts	3.7±0.4	3.2±0.7	2.6±0.6	2.5±0.3*	2.2±0.2**
Total time spent grooming (s)	130±16	58±11**	56±17**	69±13**	66±13*
Average duration of a single bout (s)	43±5	22±4**	19±3**	32±7	30±5
Patterns					
Total number of patterns	38±4	16±3**	13±3***	19±4**	17±4**
Number of interruptions of grooming	1.5±0.3	0.8±0.2	0.6±0.3	1.4±0.4	0.7±0.3
Average duration of a single pattern (s)	3.5±0.3	4.2±0.7	3.8±0.3	3.7±0.3	4±0.2
Transitions between patterns					
Total number of transitions	41±5	18±4**	15±4***	21±4**	18±4**
Average transitions per patterns	1.1±0	1.1±0	1.1±0	1.1±0	1.1±0
Percent of incorrect transitions (%IT)					
Reversed IT, %IT (r)	29±2	19±4	20±4	19±3*	23±2
Skipped IT, %IT (s)	10±1	3±1***	6±3*	9±2	13±3
Aborted IT, %IT (a)	14±2	26±5*	30±7**	22±3	16±2
Incorrectly initiated, %IT (i)	2±1	1±1	2±1	3±1	11±8
% Total, % IT=IT/T=% IT ($r+s+a+i$)	55±2	49±3	58±5	53±3	63±5
Bouts					
Number of interrupted bouts	0.9±0.2	0.7±0.2	0.5±0.2	0.7±0.1	0.5±0.2
% Interrupted bouts	25±5	22±8	14±6	33±9	24±9
Number of incomplete bouts	3.7±0.4	3.2±0.7	2.6±0.6	2.5±0.3*	2.2±0.2**
% Incomplete bouts	100±0	100±0	100±0	100±0	100±0
Average number of patterns per bout	10.9±1.3	5.9±1.0*	4.9±0.6**	8.2±1.4	7.6±1.4
Average number of transitions per bout	12.1±1.3	6.4±1.0**	5.3±0.7**	8.8±1.4	8.2±1.5
Average interruptions per bout	0.4±0.1	0.4±0.2	0.2±0.1	0.7±0.2	0.3±0.1

Data are expressed as the mean±SEM (n=11–14).
* $P<0.05$, ** $P<0.01$, *** $P<0.001$, difference from control.

or fluoxetine (5 or 10 mg/kg). Twenty-five minutes following treatments (Silva and Brandao, 2000), they were individually placed into a clean glass cylinder (19×19×19 cm) with a plexiglas cover having holes for air entrance and were observed for 5 min for the grooming activity. Then, they were individually placed in a hole board for the assessment of exploratory and locomotor activity for 5 min. The hole board was a wooden apparatus with a plexiglas cover (35×50×20 cm). The floor of the apparatus was divided into 12 equal squares in each of which a hole (3 cm in diameter, 5 cm in depth) was present in the middle. Behaviours of animals were recorded after a minute of adaptation in both tests. Between subjects, the cylinder and the hole board were cleaned with diluted ethanol solution to eliminate any odour traces and were dried with wet cloths.

2.3. Grooming analysis

Novelty-induced grooming activity was evaluated using the following grooming analysis algorithm described by Kalueff and Tuohimaa (2004, 2005a).

The latency to start grooming, the number of grooming bouts (NB) and the total time spent grooming (TS) were evaluated as the gross measures of grooming activity.

Grooming patterns organized in bouts were assessed by the following 6-point scaling system: (0) no grooming; (1) forepaw licking; (2) nose/face grooming (strokes along the snout); (3) head washing (semicircular movements over the top of the head and behind the ears); (4) body grooming/scratching (body fur licking and scratching the body with the hindpaws); (5) hindleg licking and (6) genital grooming (licking of the genital area). Our previous observations have shown that fluoxetine treated rats spent much time in genital grooming. To clarify this effect, a minor modification in scaling system was made by registering genital grooming activity alone, not with tail licking as did Kalueff and Tuohimaa (2004).

The number of grooming patterns (NP), the number of interruptions (NI), the number of interrupted bouts (NIB) and the number of incomplete bouts (NICB) were calculated. A 'complete' bout (CB) consisted of the following sequence of patterns: 0–1–2–3–4–5–6–0, all other bouts were considered 'incomplete' (ICB). A grooming bout was considered 'interrupted' if at least one interruption was recorded within its transitions (interruptions longer than 5 s determined separate, independent grooming bouts). The percentages of interrupted (% IB=NIB/NB) and incomplete bouts (% ICB=NICB/NB) were assessed.

Transitions (T) between grooming patterns were assessed and all 'correct' and 'incorrect' transitions between stages were analyzed. Correct transitions (CT) between the grooming stages included the following cephalocaudal progression: (0–1), (1–2), (2–3), (3–4), (4–5), (5–6), (6–0). Incorrect transitions (IT) included: skipped, $IT_{(s)}$ (e.g. 1–4, 2–5), reversed, $IT_{(r)}$ (e.g. 3–2, 4–1), aborted, $IT_{(a)}$ (e.g. 3–0, 4–0) and incorrectly initiated, $IT_{(i)}$ (e.g. 0–4, 0–5). The numbers of incorrect transitions were assessed. The percentages of incorrect transitions (% IT=IT/T) and incorrect transitions of each type (% $IT_{(...)}$=$IT_{(...)}$/T) were calculated.

The average durations of a single bout (ADB) and pattern (ADP) were assessed (ADB=TS/NB and ADP=TS/NP). The average numbers of transitions per bout (ATB) and pattern (ATP) were calculated (ATB=T/NB and ATP=T/NP).

The regional distribution of grooming was separately analyzed and the percentages of total grooming patterns (% of total number of patterns) were assessed for each anatomical area.

2.4. Hole board analysis

Over a period of 5 min, the number of rearing, head dipping/hole sniffing and area crossing was counted.

Experiments were carried out between 9:00 and 13:00 in a temperature controlled (21±2 °C) quiet room. The animals were acclimatized to the experimental room 1 h prior to experimentation. Observers were blind to the treatments.

2.5. Drugs

Amitriptyline hydrochloride was obtained from Sigma, St. Louis, MO. Fluoxetine hydrochloride was a generous gift from Abdi İbrahim Drug Company (Turkey). Amitriptyline and fluoxetine were dissolved in saline and distilled water, respectively. Both drugs and saline were given intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. Drug dosages were selected based on anxiety (Hijzen et al., 1995; Silva et al., 1999; Silva and Brandao, 2000) and anxiety and depression (Weinstock et al., 2002) studies.

2.6. Statistical analysis

Data obtained from grooming and hole board measures were analyzed by Kruskall–Wallis followed by post hoc Mann–Whitney tests for comparisons between control and drug groups.

3. Results

3.1. The effects of amitriptyline and fluoxetine on grooming behaviour

Tables 1 and 2 show the grooming activity of rats after amitriptyline and fluoxetine administrations.

Table 2
Regional distribution of grooming activity after amitriptyline and fluoxetine administrations in rats

Grooming measures	Control	Amitriptyline		Fluoxetine	
		5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg
Grooming patterns (% of total number of patterns)					
Forepaws	40±2	52±5*	46±3	41±2	35±4
Face/head	47±2	42±4	45±3	44±2	40±5
Rostral grooming (paws+face/head)	87±2	94±2	91±4	85±3	75±7
Body	11±2	6±2	8±4	7±2	7±2
Hindleg patterns	1±1	0±0	1±1	0±0	1±1
Genitals	1±1	0±0	0±0	8±2**	17±8**

Data are expressed as the mean±SEM (n=11–14).
* $P<0.05$, ** $P<0.01$, difference from control.

Kruskall–Wallis analysis revealed that amitriptyline had significant effects on; the duration of grooming ($H=11.77$, $P<0.01$), the average duration of a single bout ($H=13.02$, $P<0.01$), the total numbers of patterns ($H=15.49$, $P<0.001$) and transitions ($H=15.43$, $P<0.001$), the percentages of skipped ($H=12.46$, $P<0.01$) and aborted ($H=9.43$, $P<0.01$) transitions, the average numbers of patterns ($H=11.37$, $P<0.01$) and transitions ($H=13.36$, $P<0.01$) per bout and the percentage of forepaw grooming ($H=6.35$, $P<0.05$).

Kruskall–Wallis analysis revealed that fluoxetine had significant effects on; the duration of grooming ($H=9.10$, $P<0.05$), the total number of bouts ($H=9.27$, $P<0.01$), the total numbers of patterns ($H=12.41$, $P<0.01$) and transitions ($H=12.75$, $P<0.01$), the percentage of incorrect transitions ($H=7.06$, $P<0.05$), the number of incomplete bouts ($H=9.80$, $P<0.01$) and the percentage of genital grooming ($H=10.28$, $P<0.01$).

Analysis of traditional gross measures of grooming activity showed that amitriptyline (5 and 10 mg/kg; $P<0.01$) and fluoxetine (5 and 10 mg/kg; $P<0.01$, $P<0.05$) decreased the total time spent grooming. Amitriptyline (5 and 10 mg/kg; $P<0.01$) decreased the average duration of a single bout and fluoxetine (5 and 10 mg/kg; $P<0.05$, $P<0.01$) reduced the total number of bouts.

Analysis of grooming patterns and transitions showed that amitriptyline (5 and 10 mg/kg; $P<0.01$, $P<0.001$) and fluoxetine (5 and 10 mg/kg; $P<0.01$) decreased the total number of patterns and transitions. Amitriptyline (5 and 10 mg/kg) decreased and increased the percentages of skipped ($P<0.001$, $P<0.05$) and aborted ($P<0.05$, $P<0.01$) transitions, respectively. Fluoxetine (5 mg/kg; $P<0.05$) decreased the percentage of reversed transitions.

Detailed analysis of grooming bouts showed that amitriptyline (5 and 10 mg/kg) reduced the average numbers of patterns ($P<0.05$, $P<0.01$) and transitions ($P<0.01$) per bout. Fluoxetine (5 and 10 mg/kg; $P<0.05$, $P<0.01$) decreased the number of incomplete bouts.

The regional distribution of grooming was also affected by amitriptyline and fluoxetine treatments. Amitriptyline (5 mg/kg; $P<0.05$) increased forepaw and fluoxetine (5 and 10 mg/kg; $P<0.01$) increased genital grooming.

3.2. The effects of amitriptyline and fluoxetine on hole board behaviour

Table 3 shows the activity of rats following amitriptyline and fluoxetine administrations in the hole board. Kruskall–Wallis analysis revealed that fluoxetine had significant effects on head dipping ($H=9.81$, $P<0.01$) and rearing ($H=6.76$, $P<0.05$).

Fluoxetine (10 mg/kg) reduced head dipping ($P<0.01$) and rearing ($P<0.01$).

Table 3
Activity in the hole board after amitriptyline and fluoxetine administrations in rats

Holeboard measures	Control	Amitriptyline		Fluoxetine	
		5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg
Head dipping	3.9±0.9	3.3±1.0	5.2±1.3	1.7±0.5	0.5±0.3*
Area crossed	18.6±2.6	20.4±3.1	19.0±2.7	17.7±2.7	12.6±1.4
Rearing	8.2±0.9	10.1±1.7	10.1±1.7	6.7±1.1	5.2±0.7*

Data are expressed as the mean±SEM ($n=11$–14).
* $P<0.01$, difference from control.

4. Discussion

The aim of the present study was to reassess the effects of amitriptyline and fluoxetine on anxiety in rats. For this purpose, we used a detailed analysis algorithm which allowed differential registration and analysis of grooming behavioural microstructure. Our experiments demonstrated that amitriptyline and fluoxetine not only affected the traditional gross measures, but also altered the behavioural microstructure of grooming activity. Changes in incorrect transitions and regional distribution of grooming behaviour suggest that amitriptyline and fluoxetine may possess anxiogenic and anxiolytic activities, respectively. In the hole board, however, fluoxetine, but not amitriptyline, produced anxiogenic effect.

The effects of two reference compounds on mouse grooming were examined to test the predictive validity of grooming behavioural microstructure as a marker of anxiety (Kalueff and Tuohimaa, 2005b). The findings showed that the anxiolytic diazepam did not alter 'general' activity measures of grooming. But, the drug normalized its behavioural microstructure by decreasing the percentages of incorrect transitions and interrupted bouts. In contrast, anxiogenic pentylenetetrazole increased the duration of grooming, the percentages of incorrect transitions and interrupted bouts. The effects of amitriptyline and fluoxetine on the duration of grooming activity suggest an anxiolytic activity in comparison with the pentylenetetrazole's effect. However, the drugs are not fully effective in normalizing the microstructure of grooming behaviour like diazepam. In fact, the increase in aborted transitions produced by both doses of amitriptyline suggests an anxiogenic activity.

Kalueff and Tuohimaa (2005a) reported shorter latency to start grooming and dramatic increase in grooming frequency and duration as stress-evoked alterations in rats' grooming activity. In addition, the authors showed increased percentages of interrupted bouts and incorrect transitions between different grooming patterns, as well as altered regional distribution of grooming (less caudal, more rostral) as behavioural markers of stress in rats. When compared with the stressed animals, it is rather difficult to interpret our findings. The decrease in the duration of grooming after amitriptyline and fluoxetine treatments suggests that the drugs possess antistress (anxiolytic) efficacy, though a recent study provides opposing evidence with shorter duration of grooming activity and longer latency to start grooming after social isolation stress in rats (Spasojevic et al., 2007). According to the detailed analysis of grooming microstructure, frequent prematurely terminated (aborted) grooming bouts and increased number of reversed transitions are associated to high anxiety in rats (Kalueff and Tuohimaa, 2005a; Komorowska and Pellis, 2004). Consistent with this, increased percentage of aborted transitions in amitriptyline treated rats and decreased percentage of reversed transitions at low dose of fluoxetine imply that the drugs produce anxiogenic- and anxiolytic-like activity,

respectively. The altered regional distribution of grooming; more rostral (forepaw) grooming at low dose of amitriptyline and more caudal (genital) grooming at both doses of fluoxetine seem consistent with these suggestions. On the other hand, increased genital grooming in fluoxetine treated animals appears to be interesting. Whether it reflects antistress activity or simply arises from penile erection (Berendsen and Broekkamp, 1987) may need further evaluation for clarification.

The present study appears parallel to previous studies which demonstrated suppressive effects of amitriptyline on both induced (Skuza et al., 1989; Traber et al., 1982) and spontaneous (Skuza et al., 1989) grooming behaviour in mice and rats. Fluoxetine results, however, appear to not fully confirm the previous findings that the drug had clear anxiogenic effects with an increase in self-grooming in addition to a decrease in total interaction time in social interaction test (To et al., 1999; To and Bagdy, 1999).

Exploratory behaviour is most often studied in rodents as a stress-sensitive parameter to assess anxiety (Crawley, 1985; Prut and Belzung, 2003). Anxiolytic drugs produce anxiolytic-like effects by increasing exploratory activity of novel compartment (Belzung et al., 2001) and the typical anxiogenic drugs decrease head dipping (Takeda et al., 1998) and rearing (Crawley et al., 1997) in novel environments. Amitriptyline was found to reduce locomotor activity in the elevated plus-maze (Parra et al., 2002). Our study, however, demonstrating the ineffectiveness of the drug on the measures of exploratory and locomotor activity in the hole board is consistent with the previous studies which showed the absence of anxiolytic-like effects of amitriptyline in similar tests (Galeotti et al., 2006; West and Weiss, 1998). Nevertheless, hole board findings could not provide referring data for the interpretation of alterations observed in the microstructure of grooming activity for amitriptyline treated animals. Our study also demonstrated that high dose of fluoxetine reduces head dipping and rearing. These effects could not be accounted to sedation, since the drug has no effect on locomotor activity as shown before (de Angelis, 1996). Anxiogenic profile produced by fluoxetine in the hole board, on the other hand, seems not to be correlated with the putative antianxiety-related changes in grooming patterning.

In conclusion, present results suggest that amitriptyline may possess anxiogenic and fluoxetine may possess anxiolytic activities in respect to their effects on the microstructure of grooming behaviour. However, hole board measures imply that fluoxetine, but not amitriptyline may exert anxiogenic effect. Further studies, investigating the effects of long-term treatments may provide more reliable data to discuss the discrepancy between the anxiogenic or anxiolytic profile of the drugs and their therapeutic uses. The analysis of grooming behaviour used in this study, on the other hand, needs re-evaluations for its validity in detecting anxiolytic activity of drugs in rats.

Acknowledgements

This study was presented at the 10-th Jubilee Multi-disciplinary International Conference of Neuroscience and Biological Psychiatry “Stress and Behaviour” in St-Petersburg, Russia, 2007.

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