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Mouse grooming microstructure is a reliable anxiety marker bidirectionally sensitive to GABAergic drugs

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Abstract

Grooming is an important part of rodent behavioural repertoire, representing a complex hierarchically ordered cephalo-caudal sequence of patterns sensitive to stress and various drugs. Gamma-aminobutyric acid (GABA) is involved in the regulation of both anxiety and grooming behaviours. This study investigated the predictive validity of grooming behavioural microstructure as a marker of anxiety, by examining the effects of two GABAergic reference compounds, anxiolytic diazepam (0.1 and 0.5 mg/kg i.p.) and anxiogenic pentylenetetrazole (5 and 10 mg/kg i.p.) on mouse grooming. Our data suggest that the percentage of pattern transitions not fitting to the cephalo-caudal progression, and the percentage of interrupted grooming bouts are more reliable behavioural markers of stress bidirectionally sensitive to GABAergic anxiogenic and anxiolytic drugs, compared to the frequency and duration scores. Our study also confirms that detailed ethological analyses of grooming microstructure can be a useful tool in behavioural pharmacology of anxiety.

Keywords: Grooming behaviour; Mice; Anxiety; GABA; Diazepam; Pentylenetetrazole

1. Introduction

Self-grooming is an ancient innate behaviour that is represented across most animal species (Berridge et al., 1987; Aldridge and Berridge, 1998). It is an important, complex and hierarchically ordered part of rodent behavioural repertoire (Berridge and Aldridge, 2000) generally proceeding in cephalo-caudal direction: paw licking–nose and face wash–head wash–body wash and fur licking–leg licking–tail/genitals licking and wash (Fentress, 1977; Berridge and Whishaw, 1992; Cromwell and Berridge, 1996). In rodents, grooming is particularly sensitive to stress and various endo- and exogenous manipulations (Van Erp et al., 1994, 1995; Moyaho et al., 1995; Moyaho and Valencia, 2002). It has long been known that rodents' grooming activity can be generally increased in two opposite situations: in high and low stress (Kalueff and Tuohimaa, 2004a). Low-stress comfort grooming is a spontaneous body care ritual which occurs as a transition from rest to activity; it is a typical behavioural marker of low or no stress, usually going uninterrupted in a "relaxed fashion" from paw licking to tail/genitals wash (Fentress, 1977). Similarly, stress has long been known to induce grooming in rodents (Moyaho et al., 1995; Moyaho and Valencia, 2002). However, this stress-evoked grooming ethologically differs from low-stress comfort grooming, and is characterised by frequent bursts of rapid short grooming activity with abnormal progression and frequent incomplete and interrupted bouts (Fentress, 1977).

Many neuromediators and hormones as well as multiple brain regions are involved in the regulation of comfort and stress-evoked grooming (Dunn et al., 1987; Van Erp et al., 1994; Cromwell et al., 1998; Eguibar et al., 2003). Importantly, several peptides released under stress conditions (e.g. adrenocorticotropic hormone (ACTH), corticoliberin (CRH), prolactin, vasopressin, oxytocin, bombesin) stimulate grooming in rodents, mimicking the effects of stressors on grooming behaviour (Drago et al., 1980, 1983;

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Gispen and Isaacson, 1981; Caldwell et al., 1986; Bertolini et al., 1988; Dunn et al., 1988; Traber et al., 1988; Lumley et al., 2001; Amico et al., 2004). These observations further strengthen the link between stress and grooming behaviour in rodents.

Gamma-aminobutyric acid (GABA) is the primary mediator of inhibitory transmission in the mammalian central nervous system (Sieghart et al., 1999). GABA and its $GABA_A$ receptors are involved in the regulation of a number of normal and pathological brain mechanisms, such as sleep, epilepsy, memory, emotions and various behaviours (Kalueff and Nutt, 1997; Sieghart et al., 1999; Nutt and Malizia, 2001), including grooming (Barros et al., 1992, 1994). At the same time, dysfunctions of the GABAergic system have long been associated with stress and anxiety spectrum disorders (Nutt and Malizia, 2001). In both humans and animals, positive modulators of $GABA_A$ receptors, such as benzodiazepines, possess robust anxiolytic activity while negative modulators, such as chloride channel blockers, have been reported to produce anxiogenic effects (Kalueff and Nutt, 1997; Belzung, 2001; Korpi et al., 2002). Given the role of GABA in the regulation of anxiety and grooming, and the influence of stress on grooming behaviours, it is therefore of interest to assess the effects of GABAergic anxiolytic and anxiogenic drugs on grooming behaviours.

Although grooming in rodents plays a very important role, in many anxiety studies this behaviour has merited little scrutiny, frequently addressed only cursorily among other measures (for details, see Kalueff and Tuohimaa, 2004a; Kalueff et al., 2004). In general, traditional grooming measures include the latency to onset, the number of bouts and the duration of grooming bouts. Although several GABAergic drugs have been reported to affect the duration and frequency of grooming (Barros et al., 1992; 1994), we note that grooming is a complex ethologically "rich" behaviour (Fentress, 1977), and therefore its behavioural organization (patterning), in addition to activity measures, also needs experimental investigation in detail. The present study sought to extend the available literature on the behavioural pharmacology of anxiety by presenting a detailed ethological analysis of the effects of GABAergic anxiolytic and anxiogenic drugs on grooming in mice. In the present study, we used as reference compounds anxiolytic benzodiazepine diazepam and anxiogenic chloride channel blocker pentylenetetrazole at systemic doses traditionally known to induce specific anxiolytic and anxiogenic effects in rodents, respectively.

Since our work is the first study to analyse the effects of these drugs on grooming sequencing, and given its high sensitivity to various manipulations, we wanted to avoid possible non-specific effects of these drugs. For example, C57BL/6 mice used in the present study have long been known as a mouse strain highly sensitive to diazepam. Indeed, acute i.p. administration of 1 mg/kg diazepam produced strong sedative effects in these mice (Ohl et al., 2001a). Therefore, we chose a non-sedating 0.5 mg/kg dose as a maximal diazepam dose for our experiments (also see (Hascoet and Bourin, 1998) noting that anxiolytic effects of diazepam at 1 mg/kg coincide with the onset of sedation in mice). Importantly, at this dose, diazepam has been reported to exert reliable anxiolytic non-sedating effects in many rodent anxiety tests, including the light–dark and social defeat tests in mice (Lepicard et al., 2000; Lumley et al., 2000), elevated plus maze in hamsters and rats (Yannielli et al., 1996; Bert et al., 2001), devibrissation and the open field tests in rats (Kozlovskii and Prakh'e, 1995; Bert et al., 2001). Moreover, Merali et al. (2003) have recently reported a robust anxiolytic effect of 0.25 mg/kg diazepam in a novel sensitive palatable food consumption mouse paradigm. Collectively, these data justify the doses of diazepam used in the present study (0.1 and 0.5 mg/kg) to reduce anxiety in C57BL/6 mice without affecting locomotory functions in mice (which are crucial for grooming). The doses of pentylenetetrazole (5 and 10 mg/kg) were chosen based on its strong anxiogenic profile in the elevated plus maze in mice at 15–20 mg/kg (Rodgers et al., 1995), and on our own data showing that both doses used here elicited marked anxiogenic effects in the elevated plus-maze and the light–dark tests in rats (Kalueff, 2002).

Choosing the observation period for our study (5 min), we note that grooming is the immediate rodents' response to stress (Moyaho et al., 1995; Moyaho and Valencia, 2002), and therefore anxiety-related grooming alterations have to be analysed shortly after the stressor (novelty) is applied. Furthermore, given the impact of within-trial habituation on grooming (File et al., 1988), and since both drugs might affect grooming, habituation or both, we wanted to minimize the role of these additional factors in our study. Therefore, a short 5-min test (rather than a longer observation period) seemed to be appropriate to assess grooming in the present study. Finally, the pre-treatment time for our experiments (45 min) has been chosen based on numerous data showing that both drugs are able to exert their clear-cut behavioural effects 30–45 min after i.p. administration (Rodgers et al., 1995; Lumley et al., 2000; Ohl et al., 2001a; Kalueff, 2002).

Overall, here we analyse (1) grooming traditional cumulative measures and (2) grooming behavioural microstructure, following treatment with anxiolytic and anxiogenic GABAergic compounds, and using our grooming analysis algorithm already reported to be a reliable tool in neurobehavioural stress research in mice and rats (Kalueff and Tuohimaa, 2004a, in press(b)).

2. Materials and methods

2.1. Animals

Experiments were carried out on adult male mice (C57BL/6 strain; 25–30 g; Tampere University, Finland). All animals were experimentally naïve, housed 4 per cage

and kept in a controlled environment maintained at a constant temperature (24±1 °C) and humidity (60%), with free access to food and water. Lights were turned on at 0600 h and off at 1800 h. Animal care procedures were conducted in accordance with guidelines set by the European Community Council Directives (86/609/EEC).

2.2. Apparatus and procedures

Behavioural testing was always conducted between 1400 and 1800 h. On test days, the animals were transported to the dimly lit laboratory and left undisturbed for 2 h prior to testing. The mice were administered the drugs intraperitoneally, placed individually in the clean plastic box (30×30×30 cm), and observed by an experienced investigator (intra-rater reliability >0.9) for a period of 5 min. The observer was blind to the treatment groups tested in a counter-balanced, random order. Between subjects, the box was thoroughly cleaned with wet and dry cloths. Non-grooming behavioural measures included vertical exploration (the number of vertical rears) and defecation (the number of defecation boli deposited).

2.3. Grooming analysis

The observer recorded the duration and the number of grooming behaviours, using specially designed protocol sheets. In addition, the following grooming stages were recorded for each bout: paw licking, nose/face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind ears), body grooming/scratching (body fur licking and scratching the body with the hind paws), leg licking and tail/genitals grooming (licking of the genital area and tail). Overall, five ethological measures of grooming activity were evaluated in our experiments: frequency (the number of grooming bouts); duration (total time spent grooming, s); average duration of a single grooming bout (s) calculated as total time spent grooming divided by the number of bouts; total number of transitions between grooming stages and average number of transitions per bout, calculated as total number of transitions divided by the number of bouts.

To analyse grooming patterning, the following scaling system was used in the present study: no grooming (0), paw licking (1), nose/face/head wash (2), body grooming (3), leg licking (4), and tail/genitals grooming (5). A grooming bout was considered "interrupted" if at least one interruption was recorded within its transitions. Interruptions greater than 6 s determined separate grooming bouts. Grooming interruptions and correct vs. incorrect transitions between stages were analysed using the transition matrix, according to Moyaho et al. (1995) and Kalueff and Tuohimaa (2004a). Correct transitions between grooming stages included the following progressive transitions: 0–1, 1–2, 2–3, 3–4, 4–5, 5–0. Incorrect transitions were chaotic and characterised by skipped (e.g. 0–5, 1–5, etc.) or reversed (e.g. 3–2, 4–1, 5–2, etc.) stages. Overall, two ethological measures of grooming behavioural patterning were evaluated in our experiments: the percentage of incorrect transitions (of total transitions) and the percentage of interrupted grooming bouts (of total number of bouts), according to Kalueff and Tuohimaa (2004a, in press(b)). The procedures used in this study were in strict accordance with the European legislation (86/609/EEC) and approved by the Ethical Committee of the University of Tampere.

2.4. Drugs

Diazepam and pentylenetetrazole were obtained from Sigma-Aldrich (Finland). Diazepam was dissolved in saline (to which a drop of Tween 80 per 5 ml saline had been added) 1 h prior to administration. Pentylenetetrazole was dissolved in saline 1 h prior to administration. Injection volumes of all drugs were 5 ml/kg body weight. Diazepam (0.1 and 0.5 mg/kg) and pentylenetetrazol (5 and 10 mg/kg) were injected intraperitoneally 45 min prior to testing. Control groups received the appropriate vehicle treatment.

2.5. Statistical analysis

All results are expressed as mean±S.E.M. Data were analysed by Kruskal–Wallis followed by a post hoc Mann–Whitney tests for comparisons between control and experimental groups. A probability of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of diazepam

Diazepam exerted clear anxiolytic effects on non-grooming behaviours, significantly increasing vertical rears (14±3 (saline) vs. 26±4 (0.1 mg/kg, $P<0.05$) and 36±5 (0.5 mg/kg; $P<0.05$)) and predictably decreasing defecation boli (5±1 (saline) vs. 2±0.4 (0.1 mg/kg, $P<0.05$) and 2±0.2 (0.5 mg/kg, $P<0.05$)).

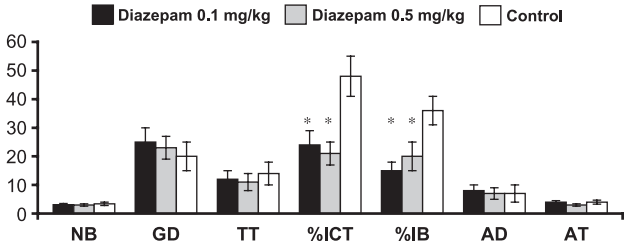

Fig. 1. Effects of diazepam on grooming behaviour in mice (n=8 in each group). Data are expressed as mean±S.E.M. *$P<0.05$ compared to control group. NB—number of grooming bouts; GD—total duration of grooming activity, (s); TT—number of transitions between grooming stages; %ICT—percentage of incorrect transitions (of total transitions); %IB—percentage of interrupted bouts (of total grooming bouts); AD—average duration of a single bout; AT—average number of transitions per bout.

Fig. 1 shows grooming activity of mice following diazepam administration. In this experiment, the number of grooming bouts, the duration of grooming and total transitions between grooming stages were not altered by diazepam at any dose given. The drug also did not change the average duration of a single bout and the number of transitions per bout (Fig. 1). However, using a detailed grooming ethological analysis, we found that diazepam at both non-sedating anxiolytic doses produced a robust shift in grooming, "normalizing" its behavioural microstructure by decreasing the percentages of incorrect transitions (diazepam: 24±5% (0.1 mg/kg); 21±4% (0.5 mg/kg); saline: 48±7%; $P<0.05$) and interrupted bouts (diazepam: 15±3% (0.1 mg/kg); 20±5% (0.5 mg/kg); saline: 36±5%; $P<0.05$) (Fig. 1).

3.2. Effects of pentylenetetrazole

Pentylenetetrazole exerted clear anxiogenic effects on non-grooming behaviours in mice, significantly decreasing vertical rears (18±4 (saline) vs. 9±1 (5 mg/kg, $P<0.05$) and 8±1 (10 mg/kg; $P<0.05$)), while predictably increasing defecation boli (4±1 (saline) vs. 7±2 (5 mg/kg, NS) and 8±1 (10 mg/kg, $P<0.05$)).

Fig. 2 shows the behavioural effects of pentylenetetrazole on mouse grooming activity. In this experiment, the drug-treated mice showed similar number of grooming bouts but spent significantly more time engaged in grooming activity than did their control counterparts (pentylenetetrazole: 43±7 s (5 mg/kg); 49±6 s (10 mg/kg); saline: 26±4 s; $P<0.05$). The drug also had no effects on the average duration of a single bout and the number of transitions per bout (Fig. 2). However, a detailed ethological analysis of grooming microstructure in this experiment showed that pentylenetetrazole-treated mice, compared to the control group, generally display "high arousal" grooming patterns, including frequent incorrect transitions (pentylenetetrazole: 77±8% (5 mg/kg); 82±10% (10 mg/kg); saline: 52±6%; $P<0.05$) and interrupted bouts (pentylenetetrazole: 62±6% (5 mg/kg); 54±7% (10 mg/kg); saline: 31±6%; $P<0.05$).

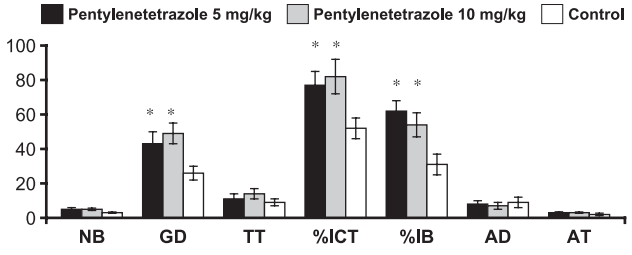

Fig. 2. Effects of pentylenetetrazole on grooming behaviour in mice (n=8 in each group). Data are expressed as mean±S.E.M. *$P<0.05$ compared to control group. NB–number of grooming bouts; GD–total duration of grooming activity, (s); TT–number of transitions between grooming stages; %ICT–percentage of incorrect transitions (of total transitions); %IB–percentage of interrupted bouts (of total grooming bouts); AD–average duration of a single bout; AT–average number of transitions per bout.

4. Discussion

The main aim of these studies was to pharmacologically assess the mouse grooming behavioural microstructure by testing standard anxiolytic and anxiogenic GABAergic drugs. The experiments with two classical reference compounds, diazepam and pentylenetetrazole, show that grooming behavioural sequencing, rather than its general "activity" measures, can effectively measure pharmacologically induced anxiolytic and anxiogenic-like effects. Indeed, diazepam did not alter grooming activity measures, but decreased the percentage of incorrect transitions and uninterrupted bouts. In contrast, both doses of pentylenetetrazole, increasing the duration of grooming, also predictably increased the percentages of incorrect transitions and interrupted bouts. In addition, both drugs affected non-grooming behavioural indices of anxiety (vertical rears, defecation boli), producing clear anxiogenic (pentylenetetrazole) or anxiolytic (diazepam) effects.

Notably, these results differ from several previously published studies examining the effects of some GABAergic drugs on rodent novelty-induced grooming. For example, anxiolytic drugs that increase GABAergic function (aminooxiacetic acid, valproate, barbital and chlorazepam) have been shown to decrease grooming activity measures in the open field (Barros et al., 1992, 1994). Non-sedating anxiolytic doses of diazepam and chlordiazepoxide inhibited novelty-induced grooming and blocked the increase in grooming produced by i.c.v. administration of stress-related peptides bombesin and $ACTH_{1-24}$ (Crawley and Moody, 1983; Moody et al., 1988). In the open field test, diazepam inhibited mouse grooming in the "thigmotactic" areas—corners and walls (Choleris et al., 2001), while doramectin, a novel compound with GABAergic anxiolytic/anticonvulsant properties, reduced grooming duration and frequency in rats (De Souza Spinosa et al., 2000). In contrast, several anxiogenic drugs that inhibit central GABAergic transmission have been shown to increase grooming activity. For example, $GABA_A$ receptor chloride channel blockers pentylenetetrazole, picrotoxin and benzylpenicillin all dose-dependently elicited grooming activity in rats (Adams et al., 1993; Kalueff, 2002; Giorgi et al., 2003). Together, this allowed Barros et al. (1994) to suggest that grooming scores can be useful to detect the effects of GABAergic anxiolytic/anxiogenic drugs and manipulations. Supporting this notion, recent drug-free factor analysis studies have shown that grooming cumulative measures in the holeboard and the elevated plus maze positively correlate with "anxiogenic" arousal and negatively—with "low anxiety" exploratory motivation (Ohl et al., 2001b).

However, there are numerous published data contradicting this "high GABA–low stress–low grooming" theory. For example, microinjection of anxiogenic $GABA_A$ receptor antagonist bicuculline (50–100 μg/μl) into the rat brain produced a decrease in spontaneous

grooming (Perier et al., 2002); also see similar dose-dependent trend in the elevated plus maze in mice (Dalvi and Rodgers, 1996). Perfusion of the preoptic area with $GABA_A$ receptor agonist, anxiolytic muscimol (10–100 μM) induced a dose-dependent increase in grooming activity which was attenuated by co-perfusion with bicuculline (Osborne et al., 1993). In mice, the anxiolytic drug ethanol increased grooming (see File et al., 1988 for details), whereas a dramatic 10-fold increase was observed in the elevated plus maze following anxiolytic treatment with 15 mg/kg chlordiazepoxide (Rodgers et al., 2002). In line with this, diazepam has been recently shown to increase grooming in aversive "non-thigmotactic" areas of the open field (Choleris et al., 2001) in mice. Collectively, these data clearly negate the hypothesis that activation of GABAergic system always leads to reduced grooming activity.

Does anxiety always lead to increased grooming frequency or duration? Although this is a widely accepted view, there are many data showing the opposite phenomena. For example, "anxiolytic" sudden darkness (Nasello et al., 2003) is seen together with increased time spent grooming, while Lawler and Cohen (1988) reported pawgrooming activation induced by positive stimuli in rats. In line with this, our recent studies in mice have shown no clear correlation between grooming scores and anxiety. For example, lower grooming activity was seen in more anxious 129S1 mice, compared to non-anxious C57BL/6 mice (Kalueff and Tuohimaa, 2004c), see also similar data in Hossain et al. (2004), whereas essentially the same grooming activation was seen in C57BL/6 mice in both low-stress (novel box) and high-stress (social encounter with an unfamiliar male) situations (Kalueff and Tuohimaa, 2004a). Finally, more stressful situation (exposure to the elevated plus maze) produced less grooming in mice than did a relatively weak stressor such as exposure to a familiar box (Kalueff and Tuohimaa, 2004a). Taken together, these findings indicate that the effects on grooming behaviours produced in rodents by stress, as well as GABAergic anxiolytic and anxiogenic drugs, are rather complex and not yet fully understood; see also File et al. (1988) and Moyaho and Valencia (2002) for discussion.

In contrast, while grooming levels varied inconsistently in these studies, the behavioural microstructure of grooming was consistently impaired in more anxious animals: 129S1 vs. non-anxious C57BL/6 mice, anxious Vitamin D receptor knockouts vs. 129S1 wild-type mice, stressed vs. non-stressed C57BL/6 mice (Kalueff and Tuohimaa, 2004a, 2004c). Collectively, this confirms that behavioural patterning of grooming is indeed a very sensitive marker of stress in rodents; see similar data obtained in rats (Komorowska and Pellis, 2004; Kalueff and Tuohimaa, in press(b)) using different ethological approach. Therefore, it is possible to assume that "qualitative" characteristics of grooming may also be sensitive to various anxiogenic or anxiolytic pharmacological manipulations. As such, it is necessary to more fully assess GABAergic behavioural pharmacology of grooming and its relation to anxiety.

As already mentioned, all previously published works analysed the effects of GABAergic drugs on grooming by measuring changes in its traditional "quantitative" parameters, such as the frequency and the duration. To the best of our knowledge, the present work is the first study focusing on detailed "in-depth" ethological analyses of changes in grooming patterning ("quality") following administration of reference GABAergic anxiolytic and anxiogenic drugs. Overall, our data show that the number of bouts and, at a lesser extent, the duration of grooming, may not detect anxiety, if taken alone. In contrast, grooming behavioural microstructure is particularly sensitive to GABAergic anxiolytic/anxiogenic drugs (Figs. 1 and 2), and can therefore represent a reliable grooming-related behavioural marker of anxiety.

In general, the idea of analysing grooming separately instead of focusing on grooming demonstrated only during other anxiety tests (such as the open field or elevated plus maze, as done in the majority of studies (Barros et al., 1994; Moyaho and Valencia, 2002; Rodgers et al., 2002)) is important for several reasons. Grooming scores taken during these tests are generally low (being masked by alterations in other, non-grooming behaviours), and their analysis can therefore be extremely difficult. In contrast, more specific analysis of mouse grooming in an observation box enables not only higher grooming scores, but also the identification of more "pure" stress- or drug-evoked grooming, not confounded by other (non-grooming) behaviours (Kalueff and Tuohimaa, 2004a). In general, this allows obtaining several parallel sets of data, including: (a) behavioural measures taken in these initial anxiety tests, and (b) grooming measures obtained using this current protocol, which can then both be used for a detailed behavioural analysis, such as reported here.

Importantly, the attractiveness of the present approach is the ease of behavioural analysis and its potential good predictive validity in respect to GABAergic drugs, although future studies using other GABAergic agents are necessary to further examine this notion. Moreover, clear neurobiological rationale behind stress-evoked "high-arousal" alterations in grooming behavioural microstructure (Kalueff and Tuohimaa, 2004a, in press(b)) indicates that this approach to measure anxiety may also have good construct validity. Indeed, similar phenomenon of stress-evoked disruption of the cephalo-caudal patterning of grooming has been recently reported in rats (Komorowska and Pellis, 2004), further supporting the utility of our approach to assess stress-evoked alterations in grooming patterning, including testing anxiolytic and anxiogenic drugs. Furthermore, we can suggest that our approach is also suitable for use with rats and other laboratory rodents, given the similarity in patterning of their grooming behaviours (Kalueff and Tuohimaa, 2004a, in press(b)). Finally, although not directly tested in this study, it is

possible to speculate that our method may be sensitive to other, non-GABAergic anxiotropic pharmacological agents. Given earlier data showing that antidepressant (amitriptyline, nomifensine, mianserin) and neuroleptic (haloperidol, clozapine) drugs influence stress-evoked grooming in rats (Traber et al., 1988), it is possible to assume that these drugs may also affect grooming patterning in rodents. Clearly, this promising research direction will almost certainly require further investigation, and may represent an important potential application of our findings.

Given the results of our study, assessment of grooming may also be useful in assisting with more accurate interpretation of other (non-grooming) behavioural data, especially in situations when conflicting data have been obtained, and there is a need for more detailed and in-depth behavioural analysis. For example, it may be extensively used when studying different pharmacological manipulations with mixed or unclear effects, or interpreting drug-induced behaviours of novel mutant mice with unknown or unclear behavioural phenotype.

In conclusion, our results not only confirm the link between the GABAergic system, anxiety and grooming, but also pharmacologically validate the shifts in grooming behavioural microstructure as a sensitive measure of anxiety in mice (Kalueff and Tuohimaa, 2004a). This grooming-oriented ethological approach can be a novel tool in behavioural pharmacology of anxiety, used to complement the existing mouse tests. We can also suggest that ethological dissection of grooming, such as reported here, may be used for the search of novel antianxiety antistress psychotropic drugs.

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