Telemetry as a Tool to Measure Sedative Effects of a Valerian Root Extract and Its Single Constituents in Mice

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Abstract

Valeriana officinalis L. is a popular herbal treatment for mild sleep disorders. Clinical and non-clinical studies found contradictory results for valerian extracts and single constituents regarding the influence on sleep parameters. It was the aim of this study to investigate the sedative effects of a valerian root extract. Therefore, locomotor activity and core body temperature were recorded in male mice using radiotelemetry. A 70% ethanolic extract prepared from the roots of V. officinalis (s.l.) and some of its single constituents, valerenic acid, linarin, and apigenin, were tested for effects on locomotion and body temperature over 180 minutes after oral administration. The extract was tested in a dose range of 250–1000 mg/kg, and only a dose of 1000 mg/kg valerian extract showed a mild short-term sedative effect with reduced locomotor activity between 66–78 minutes after administration. Paradoxically, an increased activity was observed after 150 minutes after gavage. A dose of 1 mg/kg valerenic acid produced an intermittent stimulation of activity. However, a mild short-term sedative effect was found for linarin at 12 mg/kg and apigenin at 1.5 mg/kg. Considering the cumulative locomotor activity over the observation period of 180 min, it is concluded that neither the extract nor one of the compounds had considerable sedative effects. More precisely, the observed short-term changes in activity pattern indicate that valerian extract as well as the flavonoids linarin and apigenin are rather effective to reduce sleep latency than to act as a sleep-maintaining agent.

Introduction

An estimated 10 to 15% of the adult population suffers from chronic insomnia (≥ 1 month) and an additional 25 to 35% from transient or occasional insomnia (< 1 month) [1]. These high prevalence rates connected with pharmacological treatments cause significant economic and clinical costs. Traditionally, preparations obtained from the roots of Valeriana officinalis L. (Valerianaceae) are used as an herbal remedy because of their putative sleep promoting effects. In most countries, valerian preparations are a marketed over-the-counter product with remarkable success. One reason for this is that herbal products and dietary supplements are often considered as more natural and safer than prescription or other nonprescription drugs and therefore are very popular [2]. Not only are extracts from Valeriana officinalis L. the best known, but also the most studied herbal treatment for insomnia. Clinical studies of its sleep-inducing and sleep-promoting effects are contradictory [2–7]. Although sedative and anxiolytic effects have been observed in nonclinical studies [8,9], literature provides insufficient evidence to imply beneficial effects on sleep. Several compounds have been isolated and identified from valerian root preparations, but it is still unclear which of them are responsible for the recorded activities. Some of the active compounds found in commonly used extracts are the sesquiterpenic acids, especially valerenic acid, which was recently identified as a GABAA receptor modulator [8,10]. Recently, sedative or sleep-enhancing properties were detected for the flavonoids: linarin (acacetin-7-O-rutinoside) [11], 6-methylapigenin (4’,5,7-trihydroxy-6-methylflavone) [12,13], and hesperidin (25(−)-7-rhamnoglucosyl-hesperetin) [12]. More recently, lignan derivatives, in particular 4’-O-beta-D-glucosyl-9-O-’(6’-deoxyacetyl)olivil found in V. officinalis, were shown to bind the adenosine A1 receptor, which is linked to sleep induction [14].
In animal studies, sleep parameters are optimally evaluated under familiar and undisturbed conditions where usual routine is not interrupted. Therefore, the measurement of sleep-inducing effects in nonclinical studies is complicated by handling-related disturbances or unfamiliar measuring instruments. For this reason, changes in locomotor activity are used as a surrogate parameter to quantify the effect of valerian root, and experiments are conducted with methods such as open field [15], rotator test [16], and elevated plus maze [8,9]. But these approaches require special apparatuses, equipped with a particular measuring system (e.g., photocells and detectors, infrared beams and sensors, electromagnetic field, or video cameras). Moreover, long-term studies in animals are not possible using these methods, and therefore results are inadequate for the investigation of sleep-inducing effects (plus handling effects which cause stress). Sleep-wake cycle and body temperature are strongly influenced by each other [17]. While core temperature in humans is rapidly rising in the morning and falling in the evening, activity and body temperature in mice are subject to an inverted circadian rhythm. It is known that individuals normally fall asleep when core body temperature is decreasing [18]. Thus, it is indicated to consult both, activity and core temperature, to evaluate possible effects on sleep.

The present study represents an innovative, objective approach to measure sedative effects with minimized handling-related stress and remote data collection. The animals can be left undisturbed during the recording period and long-term data acquisition is possible. Most importantly, effects on locomotion can directly be quantified. For the first time, radiotelemetry was used to investigate a 70% ethanolic extract of Valeriana officinalis L. in mice. Additionally, selected constituents with reported positive pharmacological activity, namely valerenic acid, linarin, and apigenin [8,10,11,19], were tested. Locomotor activity and body temperature were recorded and analyzed for possible acute and medium-term sedative effects.

Material and Methods

Extract and single compounds
Valerian extract was manufactured by Finzelberg GmbH & Co. KG, with 70% ethanol as the solvent and a drug:extract ratio (DER) of 3–6:1 (91% native extract, batch #: 08016674). Its content of sesquiterpenic acids, calculated as valerenic acid, was 0.32% (w/w). Valerenic acid (purity ≥ 98.5%) was purchased from PhytoLab GmbH & Co. Linarin (purity ≥ 98.5%) and apigenin (purity ≥ 99%) were purchased from Indofine Chemical Company, Inc.

Chemicals and drugs
Midazolam hydrochloride 50 mg/10 mL solution was purchased from Bedford Laboratories™. Zolpidem tablets (5 mg) were purchased from Henry Schein, Inc. and comminuted by mortar and pestle for preparation as a suspension for oral administration. Caffeine (purity ≥ 99%) was purchased from Sigma-Aldrich, Inc.

Drug preparation
All preparations for administration were made on the morning of the experimental run. Compounds for oral administration were dissolved, suspended or diluted in a vehicle of deionized water containing 0.5% propylene glycol (Fisher Scientific, Inc.). Compounds were administered by feeding needle in a volume of 10 mL/kg in the following concentrations: midazolam 5 mg/kg, zolpidem 5 mg/kg, caffeine 5 mg/kg, valerian extract 250–1000 mg/kg, valerenic acid 0.5–5.0 mg/kg, linarin 2.0–12 mg/kg, and apigenin 1.5–6.0 mg/kg. The concentration of each compound was chosen based on published literature. Control animals only received vehicle of the same volume.

Animals
Non-fasted male C57BL/6J mice (supplied by Harlan Sprague Dawley, Inc.) weighing 22–30 g (between 8–12 weeks old) were singly caged in a temperature controlled (20 ± 2°C) quiet room and maintained on a nonreversed 12-h light/dark cycle (lights on at 6 a.m. and lights off at 6 p.m.). The animals had unlimited access to food (Harlan Teklad LM-485, standard diet) and water. From the day of the E-Mitter implantation, all mice were handled daily before the treatment in order to reduce potential handling stress. Each mouse was considered available for experimental runs after a minimum of 7 days post-transmitter implantation. Animals were randomly assigned to the different treatment groups (n = 8 per group). Experiments were conducted between 9 a.m. and 1 p.m. All animal experiments were performed according to the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville, USA (NIH publication #85-23), study protocol # 200801768 (approved on 09/25/2008).

G2 E-Mitter implantation
The surgical implantation of the G2 E-Mitters was performed under aseptic conditions according to the University of Florida, Animal Care Services Guidelines for Telemetry Implants and Guidelines for Survival Surgery in Mice. Before surgery, meloxicam (Sigma-Aldrich, Inc.) 1 mg/kg was administered orally for analgesia, and anesthesia was maintained by 1–3% isoflurane delivered by nose cone. Twenty-four hours before implantation, the E-Mitters were sterilized with 2.4% activated glutaraldehyde (Cidex®, Johnson & Johnson, Inc.). The abdomen was opened by making a 2-cm incision along the linea alba. The intestines and colon were gently reflected to allow insertion of the E-Mitter into the abdominal cavity along the sagittal plane, placing it in front of the caudal arteries and veins, but dorsal to the digestive organs. The positioning is important to acquire accurate temperature data. In order to prevent migration of the E-Mitter within the peritoneal cavity, transmitters were anchored to the abdominal cavity wall by the silastic suture sleeve. The peritoneal incision was closed using 4/0 caliber monofilament polypropylene nonabsorbable sutures with reverse cutting needle in a simple interrupted stitch pattern. The skin incision was closed with a series of 9-mm surgical staples which were removed 7 days after surgery. Rehydration at the end of the surgery was assured by subcutaneous administration of sterile isotonic saline (10 mL/kg body weight). Wound recovery was checked regularly.

Telemetry system
A data acquisition system, the VitalView® from Mini Mitter, a Respironics Company (Series 4000), was used to acquire animal body temperature and activity data. VitalView® is a software and hardware system specially designed for controlling data collection in laboratory monitoring of physiological parameters without the need for animal handling and prior habituation to the test environment or the presence of the experimenter. E-Mitters capture energy from the field of radio waves emitted by coils of the ER-4000 Energizer/Receiver, allowing the E-Mitter devices to measure sedative effects with minimized handling-related stress and remote data collection. The animals can be left undisturbed during the recording period and long-term data acquisition is possible. Most importantly, effects on locomotion can directly be quantified. For the first time, radiotelemetry was used to investigate a 70% ethanolic extract of Valeriana officinalis L. in mice. Additionally, selected constituents with reported positive pharmacological activity, namely valerenic acid, linarin, and apigenin [8,10,11,19], were tested. Locomotor activity and body temperature were recorded and analyzed for possible acute and medium-term sedative effects.

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to be free from battery requirements. The signal returned to the receiver consists of sequences of pulses whose period is dependent on temperature. The VitalView® system records the average rate and the receiver converts this pulse frequency into a serial bit stream using calibration values specific to each device. For the quantification of locomotor activity, movement of the implanted E-Mitter results in fluctuations in signal strength, which are detected by the ER-4000 Receiver via telemetry and noted as activity counts. The total number of these counts during a sampling period interval is recorded. After a minimum 5-day washout period following administration of the test substance, each mouse was administered its matched control vehicle for a comparison run. At least two corresponding control values were matched with every test value. On the day of the experiment, the cages were placed on the receivers at least one hour before dosing. At 9:29 a.m. EST, dosing was started for the first four mice and at 9:35 a.m. EST for the second four mice. To assure and control constant environmental conditions, experiment runs were always accompanied by control animals.

Statistical analysis
All statistical procedures were calculated with GraphPad Prism® statistical software package, version 5.00 (GraphPad Software, Inc). Locomotor activity and body temperature data were collected in 6-minute intervals and are displayed as mean ± SEM. Comparative cumulative locomotor activity between groups was analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. Additionally, temperature and activity pattern differences between control and each treatment group were evaluated using the unpaired two-tailed t-test. A probability level of p < 0.05 was set as statistically significant.

Results

Fig. 1A shows the depressant effect of midazolam (5 mg/kg, p.o.) and illustrates the rapid onset of action within the first minutes. Locomotor activity was significantly lower at 12 minutes (control: 104.86 ± 16.48, midazolam: 17.36 ± 4.55; p < 0.001, two-way ANOVA) and 18 minutes (control: 109.45 ± 20.85, midazolam: 24.73 ± 11.33; p < 0.001, two-way ANOVA) after administration. As shown in Fig. 1B, body temperature showed a sig-
nificant drop of 0.78 °C 12 minutes after dosing (control: 37.19 ± 0.15 °C, midazolam: 36.41 ± 0.18 °C; p < 0.05, two-way ANOVA). The mean body temperature for the initial 30 minutes was significantly lower in the midazolam group compared to vehicle (control: 37.13 ± 0.09 °C, midazolam: 36.66 ± 0.12 °C; p < 0.05, unpaired two-tailed t-test).

Similarly to midazolam, zolpidem (5 mg/kg, p.o.) decreased the activity in the first 30 minutes (Fig. 1C). Mean body temperature at 12 minutes after administration was significantly lower in the zolpidem group (35.96 ± 0.13 °C) than in the control group (36.43 ± 0.07 °C; p < 0.01, unpaired two-tailed t-test) (Fig. 1D).

The total activity for 180 minutes after administration of 5 mg/kg caffeine was increased to 126% compared to the control group (Fig. 1E). More precisely, locomotion was significantly stimulated only during the initial 30 minutes with a high significance at 12 minutes (control: 72.40 ± 10.86, caffeine: 176.00 ± 31.76; p < 0.001), 18 minutes (control: 42.71 ± 10.38, caffeine: 146.43 ± 36.90; p < 0.001) and 24 minutes (control: 30.09 ± 7.23, caffeine: 140.29 ± 32.96; p < 0.001) indicating a rapid onset of action. Body temperature was not considerably affected (Fig. 1F). The cumulative locomotor activity of midazolam, zolpidem, and caffeine are shown in Table 1.

Fig. 2A and C show no major effect on locomotor activity after oral administration of 250 and 500 mg/kg valerian extract, respectively.
spectively, and body temperature remained mostly unaffected in these concentrations (Fig. 2B, D). In a higher concentration of 1000 mg/kg, valerian extract did not induce a significant difference in the overall locomotor activity over 180 minutes (Table 1), but altered the pattern of activity with nonsignificant short-term decreases between 60 and 90 minutes (Fig. 2E) and increases in the extract group between 132 and 150 minutes. Body temperature remained unchanged during the whole recording period except with a nonsignificant increase between 132 and 180 minutes (control: 35.68 ± 0.05 °C; extract: 35.96 ± 0.10 °C) (Fig. 2F).

No major effects were observed for valerenic acid in a low concentration (0.5 mg/kg) (Fig. 3A, B). One mg/kg of valerenic acid considerably altered the locomotion pattern (Fig. 3C). After a prominent increase at 78 minutes, a subsequent decrease occurred. Between 138 and 180 minutes, activity was significantly lower in the valerenic acid group. Simultaneously, body temperature was significantly decreased by 0.34°C during the same period (Fig. 3D; control: 35.82 ± 0.09 °C; valerenic acid: 35.48 ± 0.10 °C; p < 0.05, unpaired two-tailed t-test). In higher concentrations (2 mg/kg and 5 mg/kg, respectively), valerenic acid did not induce any statistically significant changes in locomotion (Fig. 3E, G) or body temperature (Fig. 3F, H). Overall, valerenic acid had no effects on the cumulative activity from 0–180 min (Table 1).

Fig. 3 Changes in locomotor activity (A, C, E, G) and in body temperature (B, D, F, H) after oral administration of valerenic acid (0.5, 1.0, 2.0, and 5.0 mg/kg, respectively) or vehicle. Values are expressed as mean ± SEM, n = 8 per group. Zero min represents time of dosing.

Fig. 4A and 4B illustrate that linarin at a concentration of 2 mg/kg did not considerably affect locomotion or body temperature. After oral administration of 6 mg/kg linarin, a significant increase in locomotor activity during the initial 30 minutes compared to vehicle was observed. Fig. 4C shows the significantly higher activity values at 12 minutes (control: 72.40 ± 10.86, linarin: 150.20 ± 35.00; p < 0.05), 18 minutes (control: 42.71 ± 10.38, linarin: 152.80 ± 41.04; p < 0.001) and 24 minutes (control: 30.09 ± 7.23, linarin: 110.80 ± 40.42; p < 0.01). Body temperature was not affected over the whole observation period (Fig. 4F). In a dose of 12 mg/kg, linarin significantly reduced locomotor activity between 60 and 132 minutes (Fig. 4E) whereas no effect on body temperature was observed (Fig. 4F). Considering the effects on
the cumulative activity from 0–180 min, linarin had no significant effect (Table 1).

Oral administration of 1.5 mg/kg apigenin induced a significant reduction in locomotor activity (Fig. 5A). The evaluation indicates significantly lower activity between 90 and 120 minutes after gavage. The overall activity during 180 minutes did not significantly differ between the groups after oral administration of 3 mg/kg apigenin (Fig. 5C). However, locomotion was slightly lowered between 84 and 114 minutes. Locomotion pattern was also not considerably altered after administration of 6 mg/kg apigenin (Fig. 5E). However, the total cumulative activity remained unaffected (Table 1). No considerable effect on body temperature was observed for all concentrations (Fig. 5B, D, F).

**Discussion**

It is generally acknowledged that the quality of physiological measurements collected from conscious unstressed animals possesses the highest validity and best reflects the normal state of the animal [20–22]. The radiotelemetry system therefore represents an adequate method and has proven its justification in modern pharmaceutical research thanks to a minimum of handling-related or restraint stress to the animal [17, 23, 24]. In light of the above, the present study was interested in possible sedative effects of a 70% ethanolic valerian extract and some of its single constituents (valerenic acid, linarin, and apigenin), which are assumed to be mainly responsible for valerian’s activity.

The outcomes of the positive (midazolam, zolpidem) and negative (caffeine) controls are supported by existing literature data and validate the used method [23, 25–29].

The effects of valerian root on sleep parameters are only sparsely investigated in animals. Wagner et al. [30] observed a sedative action of a fresh valerian tincture at 1000 mg/kg p.o. in mice, whereas an aqueous extract induced a slight decrease in spontaneous motility at 10 mg/kg p.o. Similar findings are reported by Leuschner et al. [15] who found pronounced sedative properties after oral administration of 20 and 200 mg/kg of an aqueous alkaline extract. While assessing these results, it must be taken into consideration that statistical specification or significance is absent in these studies [15, 30]. Additionally, enhancing of barbiturate sleeping time is demonstrated in several studies [15, 31] and considered to be evidence for sedation. But its relevance for proving sedative properties remains unclear since barbiturates have been shown to interfere with CYP P450 enzymes [32], and therefore an increased sleeping time due to interactions of valerian with the barbiturate metabolism in mice cannot be excluded.

Tokunaga et al. [33] reported significant shortening of sleep latency in rats after oral administration of 1000 mg/kg valerian extract without affecting total time of wakefulness, non-REM sleep, and REM sleep. These results are not supported by the present study, which did not find any significant effect on total cumula-
tive activity over 180 minutes. However, 1000 mg/kg altered the activity pattern of the mice and induced a nonsignificant intermittent drop in activity (66–78 min). This short-term depressant effect is not sufficient to ascribe positive effects on sleep, since a stimulating activity was observed thereafter. In contrast, 250 mg/kg and 500 mg/kg did not affect locomotor activity at all. Hatte-sohl et al. [9] drew a similar conclusion since they reported no effect on locomotion for several valerian extract preparations after acute and subacute (19 days) oral administration.

Temperature data indicate that valerian extract produces a mild dose-dependent hyperthermic effect in higher doses. This finding is contradictory to Hendriks et al. [34] who reported a hypothermic effect for the essential oil of V. officinalis and several constituents, including valerenic acid. In contrast, Hiller and Zetler [31] did not observe any influence on body temperature after administration of 50 and 100 mg/kg ethanolic valerian extract (i.p.), whereas diazepam produced a significant hypothermic effect. No marked effects on temperature have been observed in the present study. In conclusion, considering our results on locomotor activity and body temperature obtained with telemetry, it seems unlikely that valerian has any significant sedative actions. None of the tested concentrations of valerenic acid did significantly lower the activity over 180 minutes. However, at a concentration of 1 mg/kg the activity pattern was considerably altered. A short-lasting increase was observed between 78 and 102 minutes after administration followed by a reduction in locomotion between 138 and 180 minutes. These findings are in line with results from recent studies. Benke et al. [8] reported no indication for sedative activity for 10 mg/kg p.o. and 30 mg/kg i.p. (elevated plus-maze). A similar conclusion was drawn by Fernandez et al. [11]. They observed no in vivo effects in mice at low doses up to 15 mg/kg i.p. (sodium thiopental-induced sleeping time and holeboard test). In contrast, Hendriks et al. [34] found valerenic acid to decrease motility of mice at a concentration of 50 mg/kg i.p. and higher. The same authors reported in a later study a decrease in rotarod performance in mice (100 mg/kg i.p.) and a dose-related increase in pentobarbital-induced sleeping time (50 and 100 mg/kg i.p.) [16]. Furthermore, a significant decrease of rectal temperature in mice at concentrations of 100, 200, and 400 mg/kg was observed. Paradoxically, after administration of 50 mg/kg, a mild increase in body temperature occurred [16]. Overall, the authors concluded that this compound has central depressant properties. However, considering the extremely high and unphysiological doses, the relevance of these results has to be questioned.

In the present study, linarin induced only at a higher concentration (12 mg/kg) a significant reduction of locomotor activity between 60 and 132 minutes; however, when considering the cumulative activity, no significant changes were detected. Therefore, the findings of Fernandez et al. [11], could partly be confirmed. The authors observed depressant effects at doses of 4 and 7 mg/kg i.p. in the holeboard test. Furthermore, 7 and
14 mg/kg i.p. significantly prolonged the thiopental-induced sleeping time in mice. Hence it was concluded that linarin possesses sedative and sleep-enhancing activity. In contrast to the findings of Fernandez et al. [11] we found an initial stimulating effect after oral administration of 6 mg/kg linarin.

While in the present study all substances were administered orally, Fernandez et al. [11] used intraperitoneal injection. It is likely that the different routes of administration caused different effects on locomotor activity. Consequently, results obtained using different routes of administration should not be compared directly.

In the present study, apigenin showed mild sedative effects at low concentrations. Locomotor activity was significantly reduced between 90 and 120 minutes (1.5 mg/kg) after gavage. The observed effect was indeed short-lasting since after 120 minutes activity began to rise to control levels. In higher doses, apigenin did not significantly reduce activity or body temperature. Viola et al. [35] suggested apigenin to act as a benzodiazepine partial agonist and to possess anxiolytic activity at low doses (3 mg/kg). In the same study, sedative effects were only observed after i.p. administration of high doses (30 and 100 mg/kg) in mice (holeboard and locomotor activity test), but not for doses up to 10 mg/kg i.p. Similarly, Avallone et al. [36] reported potent sedative properties for high doses (25 and 50 mg/kg i.p.) in rats as well. However, in most of the previously reported studies apigenin was tested for anxiolytic properties and not particularly for sedation. Therefore, the presented results reveal for the first time the potential beneficial effect of apigenin on sleep.

In summary, the present findings indicate that the radiotelemetry system is suitable for the investigation of sleep-modifying effects. Considering all aspects, it is concluded that neither the extract nor one of the compounds had significant sedative activities over 180 min. However, positive short-term sedative effects were found for valerian extract 1000 mg/kg, linarin 12 mg/kg, and apigenin 1.5 mg/kg whereas valerenc acid (0.5, 1, 2, and 5 mg/kg) had no significant effect on locomotor activity or body temperature.

The fact that mild sedative effects of the extract and the single compounds lasted only for a very short period suggests an interesting pharmacokinetic profile probably pointing to a short half-life of all compounds. Since pharmacokinetic data about valerian compounds are only sparsely available, this assumption presently is speculative. However, pharmacokinetic data are an important issue to link data with pharmacological assays. Our findings, obtained with telemetric recordings of locomotor activity and body temperature, do not support the use of valerian root for the treatment of sleep disorders in general. However, the observed short-term effects presented for the flavonoids linarin and apigenin indicate that these compounds could be effective to reduce sleep latency rather than to act as sleep-maintaining agents. Future studies therefore should focus on the investigation of other flavonoids from valerian as sleep-inducing agents.

References

1 Benca RM. Diagnosis and treatment of chronic insomnia: a review. Psychi- atr Serv 2005; 56: 332–343
4 Bristroem A. Scientific evidence for a fixed extract combination (ZE 91019) from valerian and hops traditionally used as a sleep-inducing aid. Wien Med Wochenscr 2007; 157: 367–370
17 Mailliet F, Gallasp P, Poisson D. Comparative effects of melatonin, zolpi- dem and diazepam on sleep, body temperature, blood pressure and heart rate measured by radiotelemetry in wistar rats. Psychopharma- cology (Berl) 2001; 156: 417–426
19 Campbell EL, Chebib M, Johnston GA. The dietary flavonoids apigenin and (−epigallocatechin gallocatechinate enhance the positive modulation by diazepam of the activation by GABA of recombinant GABA(A) recep- tors. Biochem Pharmacol 2004; 68: 1631–1638
22 Taylor SC, Little HJ, Nutt DJ, Selles R. A benzodiazepine agonist and con- tracontagion have hypothermic effects in rodents. Neupharmacology 1985; 24: 69–73
23 Elliot EE, White JM. The acute effects of zolpidem compared to diaze- pamin and lorazepam using radiotelemetry. Neuropharmacology 2009; 56: 174–175
24 Matthews CB. Telemetry augments the validity of the rat as a model for heat acclimation. Ann NY Acad Sci 1997; 813: 233–238
29 Yang JN, Tiselius C, Dare E, Johansson B, Valen G, Fredholm BB. Sex differences in mouse heart rate and body temperature and in their regulation by adenosine A(1) receptors. Acta Physiol (Oxf) 2007; 190: 63–75