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Autonomic Changes Associated with Enhanced Anxiety in 5-HT_{1A} Receptor Knockout Mice

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5-HT_{1A} receptor knockout (KO) mice have been described as more anxious in various anxiety paradigms. Because anxiety is often associated with autonomic changes like elevated body temperature and tachycardia, radiotelemetry was used to study these parameters in wild type (WT) and KO mice in stress-/anxiety-related paradigms. Basal body temperature (BT), heart rate (HR), and their diurnal rhythmicity did not differ between well-adapted WT and KO mice. In a simple stress-test, the Stress-induced Hyperthermia (SIH), injection-stress resulted in an exaggerated stress-response in KO mice. Furthermore, the 5-HT_{1A} receptor agonist flesinoxan dose-dependently antagonized SIH and stress-induced tachycardia in WT, but not in KO, mice. In both genotypes, diazepam blocked SIH, but not stress-induced tachycardia. Finally, KO mice displayed an exaggerated stress response in HR and BT to novelty stress; this was supported by behavioral indications of enhanced anxiety. The present findings show that 5-HT_{1A} receptor KO mice display a more "anxious-like" phenotype not only at a behavioral, but also at autonomic levels.

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KEY WORDS: *5-HT1A receptor knockout mice; Anxiety; Behavior; Diazepam; Flesinoxan; Hyperthermia; Novelty; Radiotelemetry; Stress-induced hyperthermia; Tachycardia*

Among the 14 different serotonergic (5-HT) receptors in the brain, particularly the 5-HT_{1A} receptor is thought to play an important role in the etiology of anxiety and depression (Deakin 1993; De Vry 1995; Hoyer et al. 1994; Olivier et al. 1999).

Recently, mice lacking 5-HT_{1A} receptors have been generated in different genetic backgrounds (C57BL/6J strain: Heisler et al. 1998; Swiss-Webster × 129/Sv strain: Parks et al. 1998; 129/Sv strain: Ramboz et al. 1998), and results indicate that these mice display a more anxious phenotype than their corresponding wild types, predominantly in approach-avoidance conflict paradigms, such as elevated plus maze (Ramboz et al. 1998), open field (Heisler et al. 1998; Parks et al. 1998), and novelty suppressed feeding (Zhuang et al. 1999).

In humans, anxiety and stress disorders are often associated with autonomic nervous system changes and include tachycardia and heart palpitations (Friedman

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and Thayer 1998a) and elevated core body temperature (Lesch 1991; Murphy et al. 1991). In animals, experimentally induced stress elicits tachycardia (Bouwknecht et al. 2000; Nijsen et al. 1998a) and increases body temperature (Borsini et al. 1989; Bouwknecht et al. 2000; Olivier et al. 1998). Moreover, conditioned fear also elicits tachycardia in freely moving rats (Nijsen et al. 1998b) and mice (Stiedl and Spiess 1997; Stiedl et al. 1999). Interestingly, a preliminary study indicated that 5-HT_{1A} receptor KO mice showed increased tachycardia compared with wild type (WT) mice in response to footshock also accompanied by increased freezing (Gross et al. 2000).

Therefore, the purpose of the present experiments was to further investigate whether the putative anxious phenotype of 5-HT_{1A} receptor knockout (KO) mice is also reflected in autonomic nervous system disturbances and paradigms differing from approach-avoidance conflict ones that are easily subject to confounding influences of laboratory environments (Crabbe et al. 1999) and largely seem to measure exploration, instead of anxiety-related behavior (Dulawa et al. 1999).

First, in a radiotelemetry setup, it was investigated whether the putative enhanced anxiety of KO mice is reflected in differential levels, or basal 24-h variation, of core body temperature, heart rate, and activity. Second, based on the putative higher levels of anxiety in KO mice, exaggerated heart rate and temperature responses in the stress-induced hyperthermia (SIH) paradigm were expected (Van der Heyden et al. 1997). The anxiogenic 5-HT_{2C} receptor agonist, mCPP (Griebel 1995), and the anxiolytics flesinoxan, a 5-HT_{1A} receptor agonist (Schipper et al. 1991) and diazepam were tested in this SIH procedure. Finally, as it has been suggested that KO mice display altered exploratory activity or reactivity in response to a novel environment (Ramboz et al. 1998), the effects of switching mice to a novel environment were investigated, which in rats previously has been shown to induce tachycardia (Nijsen et al. 1998a) and possibly corresponds with increased anxiety.

MATERIALS AND METHODS

Subjects

Male homozygote 5-HT_{1A} receptor KO and WT mice were bred within the laboratory animal facilities of the Utrecht University (GDL, Utrecht, The Netherlands). The breeding founders were originally obtained from Dr. R. Hen (Columbia University, New York, USA) and were derived from established colonies from the 129/Sv strain (Ramboz et al. 1998). Mice were generated by breeding homozygote KO and WT mice with the same 129/Sv genetic background.

At the start of the experiment, animals were 12 weeks old, weighed approximately 25 g and were individually housed in standard Macrolon cages (22 × 16 × 14 cm) enriched with a gray plastic tube (length 13.5 cm; diameter 5 cm) and nesting material (EnviroDri, BMI, Helmond, The Netherlands). Animals were housed under nonreversed 12 h light – 12 h dark cycle conditions (lights on from 7.00 am–7.00 pm) at controlled room temperature (21 ± 2°C) and relative humidity of 60 ± 15% with standard rodent food pellets (Hope Farms, Woerden, The Netherlands) and water freely available.

All experiments were carried out with the approval of the Animal Ethics Committee of the Faculties of Pharmacy, Chemistry and Biology, Utrecht University, The Netherlands.

Surgery

Mice were first injected with the antibiotic Baytrill® (Bayer, Mijdrecht, The Netherlands; 2.5% enrofloxacin, 0.25 μl, SC; 20 min prior to anesthesia) to prevent possible infections and were subsequently deeply anesthetized with a cocktail of fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium), midazolam hydrochloride (Dormicum®, Hoffman-LaRoche, Mijdrecht, The Netherlands), and sterile water (ratio 1:1:2; 3.3 ml/kg body weight, subcutaneously). ECG transmitters (Data Sciences International™, type TA10ETA-F20, St. Paul, MN, USA) were implanted in the abdominal cavity with two electrodes placed subcutaneously as described previously (Kramer et al. 1993). One electrode was subcutaneously guided and sutured on the muscle of the lower right chest of the animal, and the other on the muscle bundle of the left shoulder. Transmitters consist of a hermetically sealed plastic housing with a biocompatible silastic coating, are 2.1 cm in length, weigh approximately 4 g, and have a volume of approximately 1.9 cm^3. Body weight and wounds were controlled daily to monitor the recovery from surgery, and in the case of severe weight loss, animals were subcutaneously administered 1 ml of 0.9% physiological saline. In addition, during the first 7 days after surgery, a solid energy drink (Triple A Trading, Otterlo, The Netherlands), soaked food pellets, water, and a 5% glucose solution were freely available. Experiments were performed 3 weeks after surgery, when mice had regained presurgery weight (excluding transmitter weight). Initially, there was no difference in body weight between WT and 5-HT_{1A} receptor KO mice (WT: 25.7 ± 0.5 gram, 5-HT_{1A} KO mice: 26.8 ± 0.5 gram). Nevertheless, after 3 weeks of recovery, 5-HT_{1A} KO mice were heavier compared with WT mice, and this difference lasted throughout the entire experiment (WT: 29.4 ± 0.4 gram, 5-HT_{1A} receptor KO mice: 31.3 ± 0.4 gram; $F(1,21) = 11.84$, $p < .05$). After the experiments, all mice were sacrificed under CO_2 euthanasia, and the abdominal cavity was visually inspected for infections. None of the animals showed any signs of infection.

Radiotelemetry System

The radiotelemetry system consisted of an implantable transmitter (model TA10ETA-F20) with two flexible leads, a telemetry receiver (model RLA1020), a Data Exchange Matrix collecting input from the receivers, and an in-line analog ECG adaptor, all obtained from Data Sciences International™ (St. Paul, MN, USA) connected to an IBM Pentium II computer. Each transmitter was equipped with a magnetically activated switch, which allows the device to be turned on and off. Transmitters pass the ECG signal to a receiver beneath the animal cage via a radio signal, which in turn transforms this into a digital signal. Digital information from the telemetry receivers was collected by the datamatrix and relayed to the computer. Raw data were collected and analyzed by a software package Dataquest A.R.T. version 1.02 (Data Sciences International™, St. Paul, MN, USA).

Drugs

Flesinoxan hydrochloride provided by Solvay Pharmaceuticals (Weesp, The Netherlands) and m-chlorophenylpiperazine hydrochloride (RBI, Natick, MA, USA) were dissolved in 0.9% physiological saline. Diazepam base (Brunschwig Chemie B.V., Amsterdam, The Netherlands) was suspended in a 0.5% gelatin/5% mannitol solution. All drug solutions were freshly prepared each test day and injected subcutaneously in a volume of 10 ml/kg. Doses of the drugs were based on the salt or base.

General Experimental Protocol Radiotelemetry Studies

Data sampling started the afternoon of the day prior to the start of each experiment. Sampling was performed at 1-min intervals, and for the entire series of experiments the position of cages was balanced across genotype and treatment. To avoid conditioning, the test-order of mice also varied per day.

Baseline. To determine basal 24-h diurnal variation in 5-HT_{1A} receptor KO mice and WT mice, core body temperature (BT), heart rate (HR), and general activity (GA) were recorded during 6 consecutive days in total. Only on days 2, 4, and 6, the experimenter entered the test room (from 9.00 am until 9.15 am) to check for abnormalities. Data obtained during the "undisturbed" days (days 1, 3, and 5) were used.

Stress-induced Hyperthermia. Mice were injected subcutaneously with either drug or vehicle 60 min before the temperature measurement (stressor), measured by inserting a thermistor probe for a length of 2 cm into the rectum (Digital Thermometer, Type 871A, Tegam Inc., Geneva Ohio, USA). The probe, dipped into silicon oil before inserting, was held in the rectum until a stable rectal temperature had been obtained for at least 10 s (T_1: stressor). This mild stressor causes an increase in temperature of about 1–1.5°C when measured 10 min later (T_2). The stress-induced hyperthermia is determined as the difference between those temperatures ($\Delta T = T_2 - T_1$). In our setting, temperature (BT) was measured every min by the transmitter, and mice were exposed only to the first rectal measurement, whereas the SIH effect was further determined by radiotelemetry as described in the data analysis section. All animals received each dose of a drug, and the various doses of a single drug were counterbalanced across and within genotype. Mice were tested twice a week, and between the various drugs tested in the SIH paradigm (flesinoxan and diazepam), animals were allowed to recover for 1 week. The sequence of testing drugs was (1) flesinoxan, (2) mCPP, and (3) diazepam.

Novel Environment. To assess the effects of a novel environment on BT, HR, and GA, animals were placed in a clean standard macrolon cage (22 × 16 × 14 cm), with only clean bedding. Testing started by placing the animals in the middle of the cage facing the left wall, and animals were left there undisturbed for 10 min, while BT, HR, and GA were determined and behavior recorded with a camera (Sony, model PVM-145E) and videorecorder (Panasonic, model AG-5700-E). The novel environment was placed in a ventilated, custom-built wooden cubicle (75 × 75 × 75 cm) and was dimly lit by a 40 W light bulb (± 150 lux). Behavior was analyzed afterward using The Observer® (Noldus Inc., Wageningen, The Netherlands). Frequencies, latencies, and duration of the following parameters were scored: locomotion, rearing, grooming, exploration (i.e., sniffing, scanning), stretched approach posture, immobility, and burying.

Statistical Analysis

Baseline. Twenty-four hour data of BT, HR, and GA from the undisturbed days (i.e., days 1, 3, and 5) were averaged to a single 24 h period per mouse, after which group values were further averaged to 8 blocks of 3 h values as reported earlier (Boutrel et al. 1999). Subsequently, data were analyzed by means of a repeated measures analysis of variance (ANOVA) with genotype as between subject factor and 3 h blocks of BT, HR, and GA as within subjects factor.

Stress-Induced Hyperthermia. Data of BT and HR were first averaged over 5 successive 1-min samples for each treatment, after which mean group values ± SEM were calculated for the period of injections and SIH exposure. Data and figures represent individual mouse data refitted to injection and SIH time. Statistical analysis compared individual mean values across relevant time windows for each manipulation of interest as described previously (Bouwknecht et al. 2000) and are also shown in Figure 1 (gray areas). To determine intrinsic drug

effects on BT and HR, undisturbed baseline values (−90 to −80 min) were compared with postinjection values (−30 to −20 min). The latter time window was chosen in relation to drug absorption and distribution, and in addition prior to the second disturbance in which the experimenter was present again to expose mice to the rectal procedure. Finally, the effect of SIH exposure was determined by comparing undisturbed baseline values after drug treatment (−30 to −20 min) with SIH response levels. Different response latencies for BT and HR were used, partly adapted from the original nontelemetric SIH paradigm for temperature (Van der Heyden et al. 1997). The fast responding parameter HR was determined over the first 5 min after rectal temperature measurement, whereas BT, which is a slower responding parameter, was analyzed with a delay (+10 to +15 min).

To determine overall reactivity toward the effects of the injection alone and SIH exposure alone, data of flesinoxan, mCPP, and diazepam under vehicle conditions (0 mg/kg) were collapsed and subsequently analyzed. For injection effects on BT and HR, undisturbed values (−90 to −80 min) were compared with injection stress values (−50 to −45 min for BT and −50 to −55 min for HR, respectively). For SIH exposure effects on BT and HR proper, postinjection values (−30 to −20 min) were compared with SIH exposure effects (+10 to +15 min for BT and 0 to +5 min for HR, respectively). Repeated measures ANOVA with genotype as between-subjects factor and dose as within-subjects factor was used to analyze data.

Novel Environment. Mean BT, HR, and GA data were calculated over successive 1-min blocks for both genotypes. For HR, also heart rate variability (HRV), a parameter for autonomic control of heart rate (Friedman and Thayer 1998a; b) was computed as the mean standard deviation of HR (Stiedl and Spiess 1997). Data were analyzed by means of an ANOVA for repeated measures, with genotype as between-subjects factor, and BT, HR, and GA in 1-min blocks as within-subjects factor. Data of individual behaviors were scored with The Observer®, and were subsequently calculated as frequency (i.e., total number of occurrences of scored behavior), latency (time in seconds from start until scored behavior), and total time of the behavior. Subsequently, these data were analyzed by means of a one-way ANOVA with genotype as independent variable and behavior as dependent variable.

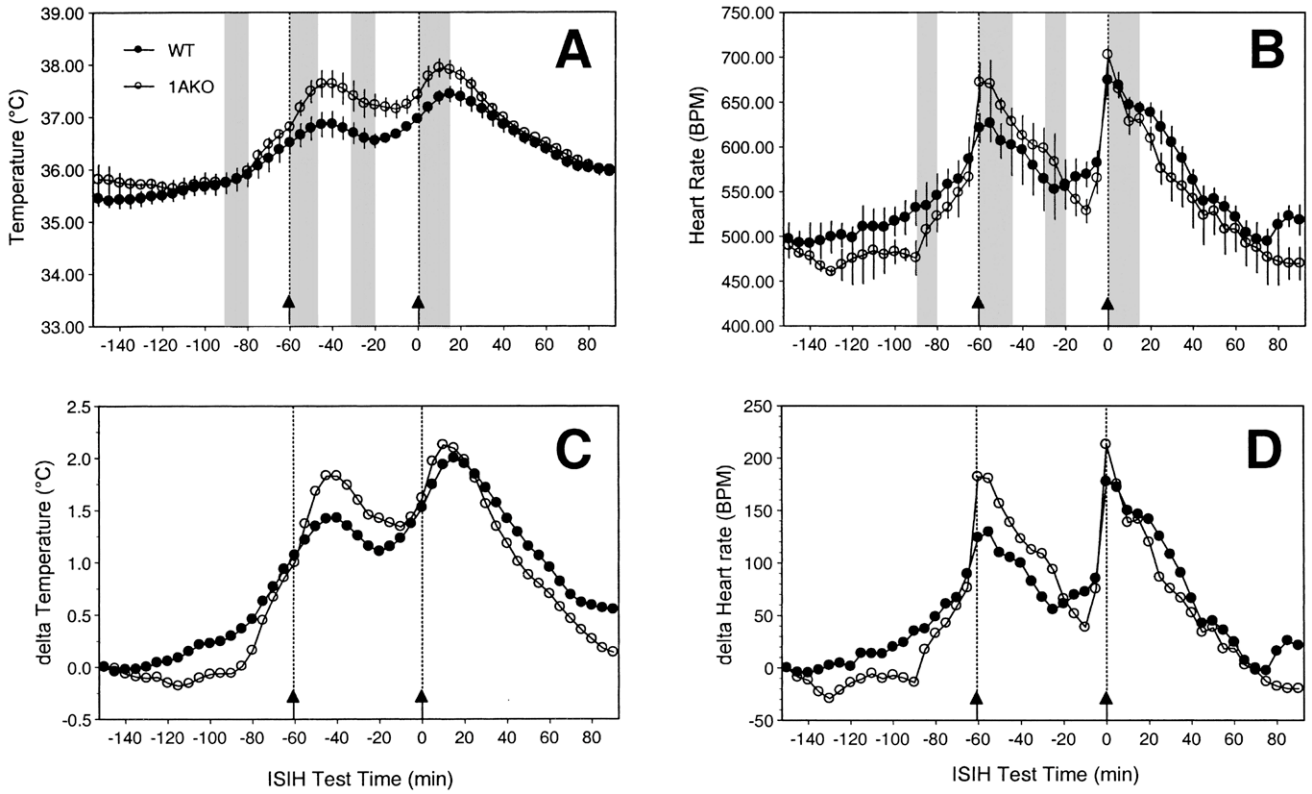

Figure 1. Effects of two stressors, a vehicle injection (first arrow) and a rectal temperature procedure (second arrow) on core body temperature (A) and heart rate (B) in wild type (WT; n = 11; closed circles) and 5-HT$_{1A}$ receptor knockout mice (KO; n = 11; open circles). Figures C and D show differences in BT and HR over the same time window, taking time–150 min as the zero value, and expressing all data as changes (deltas) compared with that time point. Data represent mean group values, averaged over 5-min periods ± SEM.

In general, in case of statistical significance, Bonferonni corrected *t*-tests post-hoc comparisons were used for subsequent analyses. The level of significance was set at $p < .05$. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows version 9.0 (SPSS, Chicago, Ill, USA).

RESULTS

Baseline

Diurnal variation, as shown in Figure 2, did not differ between WT and KO mice regarding absolute levels of body temperature, BT (Figure 2A: $F(1,21) = .034$, $p = .86$, NS), heart rate, HR (Figure 2B: $F(1,21) = 1.46$, $p = .24$, NS), and general activity, GA (Figure 2C: $F(1,21) = 2.54$, $p = .13$, NS), indicating no basal differences between genotypes on these measures. To determine differences in light versus dark periods, analysis of the data over time blocks indicated a significant effect of time block in all measures (BT: $F(7,147) = 9.72$, $p < .005$; HR: $F(7,147) = 57.45$, $p < .005$; GA: $F(7,147) = 23.73$, $p < .005$). Further analyses showed that in both genotypes, BT, HR, and GA were higher during time blocks in the dark period, compared with time blocks in the light period.

Injection Stress

To determine injection effects proper on BT and HR responses, data of vehicle condition of the three drug studies (flesinoxan, mCPP, and diazepam) were collapsed and subsequently analyzed. The injection itself resulted in increased BT (Figure 1A: $F(1,20) = 100.46$, $p < .001$) and HR (Figure 1B: $F(1,20) = 46.59$, $p < .001$) and, interestingly, this increase was significantly higher in KO mice compared with WT mice for BT (time × genotype effect: $F(1,20) = 6.85$, $p < .05$) and HR (time × genotype effect: $F(1,20) = 7.13$, $p < .05$). There was no main effect of genotype itself (BT: $F(1,20) = 2.09$, $p = .16$, NS; HR: $F(1,20) = .09$, $p = .77$, NS).

SIH Effect

The SIH (rectal temperature procedure) also resulted in increased BT ($F(1,20) = 74.60$, $p < .001$) and HR ($F(1,20) = 39.82$, $p < .001$) in both genotypes. Nevertheless, there were no differential responses between genotypes (stress × genotype interaction effect: BT $F(1,20) = 1.12$, $p = .30$, NS; HR $F(1,20) = .76$, $p = .39$, NS), and there was no main effect of genotype (BT: $F(1,20) = 3.40$, $p = .08$, NS; HR: $F(1,20) = .20$, $p = .66$, NS).

Flesinoxan/ Intrinsic Effects

Analysis of the intrinsic effects of flesinoxan, comparing baseline values from −90 to −80 min, with values after injection at −30 to −20 min, revealed a multiple interaction of time × dose × genotype on BT (Figure 3 A–B: $F(3,80) = 3.74$, $p < .05$) and a significant interaction of time × genotype on HR (Figure 3C–D: $F(3,80) = 13.40$, $p < .001$). Thus, BT was differentially affected over time by flesinoxan between genotypes, whereas HR was differentially affected over time in WT, but not in KO mice. Post-hoc analyses revealed that in WT mice, 1.0 and 3.0 mg/kg flesinoxan caused hypother-

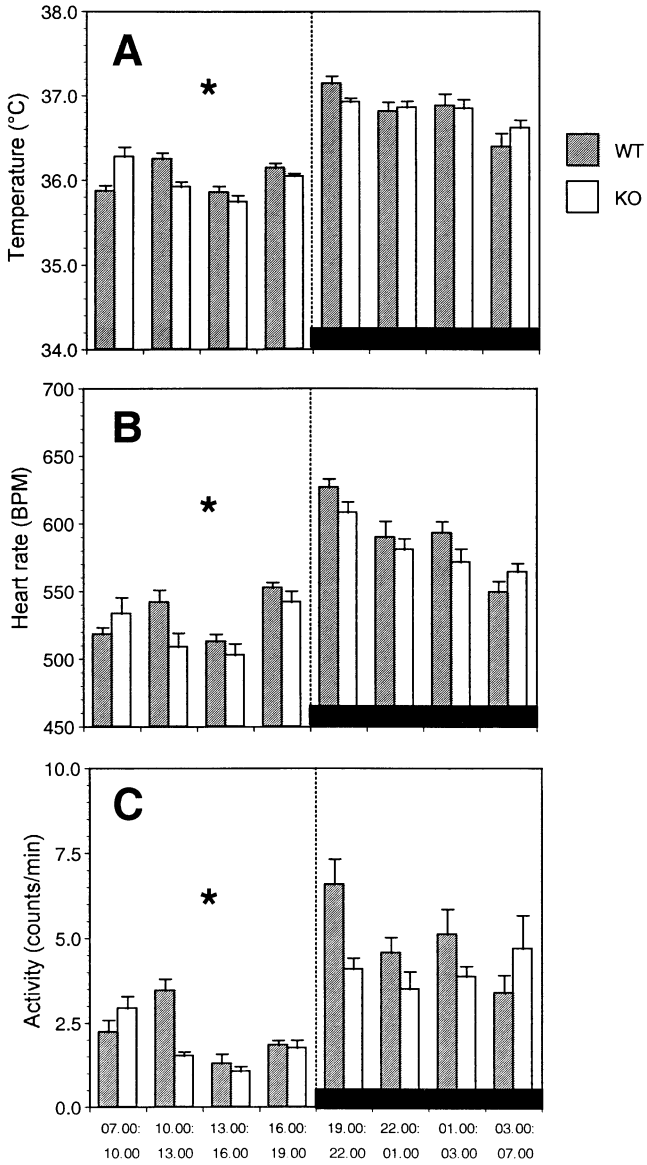

Figure 2. Core body temperature (A), heart rate (B) and general activity (C) over a 12 h light/ 12 h dark cycle (light on from 7:00 am to 7:00 pm) in wild type (WT; n = 12; gray bars) and 5-HT_{1A} receptor knockout mice (KO; n = 12; open bars). Data represent mean group values in 3-h blocks ± SEM.
*Significant lower core body temperature, heart rate and general activity during 3 h blocks in the light period compared with 3 h blocks in the dark period.

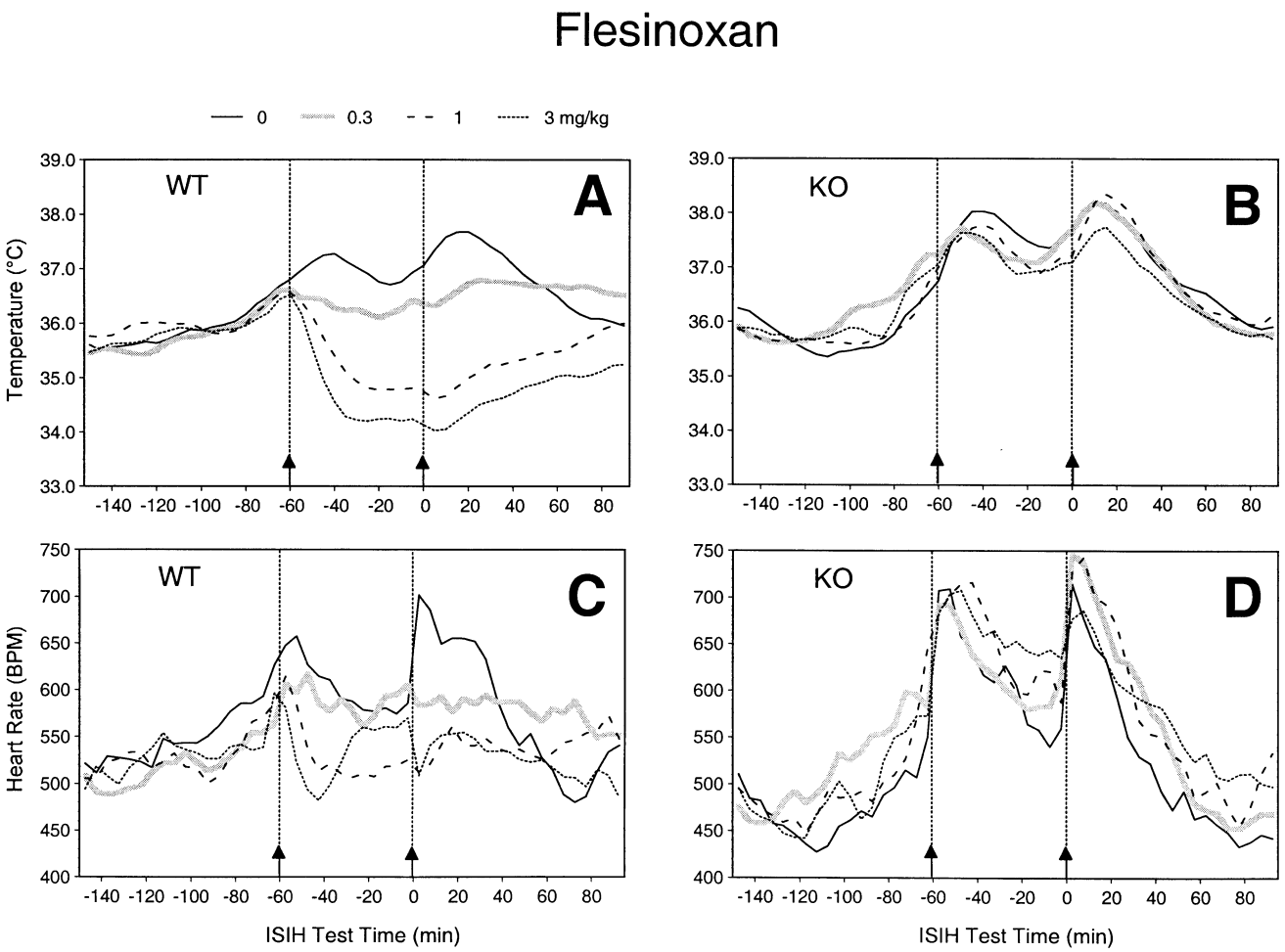

Figure 3. Effects of vehicle (0 mg/kg) or flesinoxan (0.3; 1; 3 mg/kg) treatment and the rectal temperature procedure (SIH) on core body temperature (A/B) and heart rate (C/D) in wild type (WT; n = 11) and knockout mice (KO; n = 12). Times of flesinoxan injection (−60 min) and rectal temperature measurement (0 min) are indicated by vertical lines plus arrows. Data represent mean group values, averaged over 5-min periods. For clarity no SEM is presented.

mia, whereas none of the flesinoxan doses affected BT in KO mice. Although flesinoxan decreased HR in the WT immediately after the injection (−60 to −30 min), comparing its effects at the window chosen (−90 to −80 min vs −30 to −20 min) did not reveal significant effects on HR. Flesinoxan had no effects in KO mice.

Flesinoxan/Stress-Induced Hyperthermia and Tachycardia

Analysis of the effects of flesinoxan on the stress responses revealed a multiple interaction of dose × stress × genotype (Figure 3A–B BT: $F(3,80) = 4.03$, $p < .01$; Figure 3C–D HR: $F(3,80) = 5.33$, $p < .005$). Thus, BT and HR responses, 10 and 5 min, respectively, after the rectal temperature procedure depend on the dose of flesinoxan and genotype. In vehicle-treated mice the rectal temperature procedure increased BT ($F(1,20) = 27.59$, $p < .001$) and HR ($F(1,20) = 23.28$, $p < .001$). This increase was not differentially affected by genotype (BT: $F(1,20) = 2.84$, $p = .11$, NS; HR: $F(1,20) = .04$, $p = .85$, NS). Further post-hoc analyses showed that in WT mice all doses of flesinoxan (0.3, 1.0 and 3.0 mg/kg, SC) antagonized SIH, as well as stress-induced tachycardia compared with vehicle. In contrast, in KO mice none of the flesinoxan doses (as expected) affected SIH, nor the stress-induced tachycardia.

mCPP. None of the tested doses of mCPP (0.3; 1; 3 mg/kg, SC) had intrinsic effects on BT and HR, nor effects on the SIH, and results were completely comparable with vehicle administration in either genotype. Therefore data are not shown.

Diazepam/Intrinsic Effects

Analysis of the intrinsic effects of diazepam revealed an interaction of time × dose × genotype on BT (Figure 4 A–B: $F(3,80) = 3.74$, $p < .05$) with a main effect of geno-

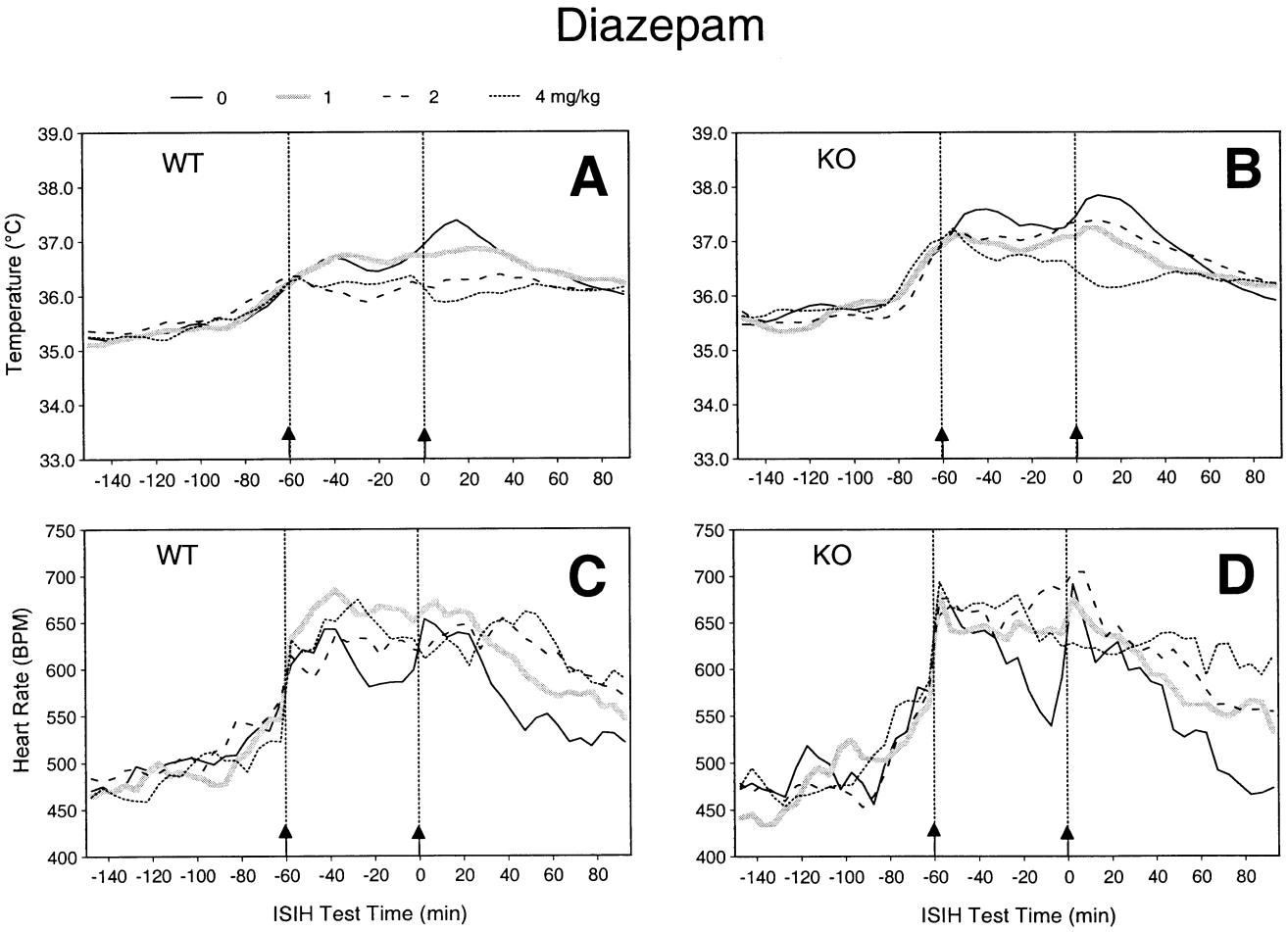

Figure 4. Effects of vehicle (0 mg/kg) or diazepam (1; 2; 4 mg/kg) treatment and the rectal temperature procedure (SIH) on core body temperature (A/B) and heart rate (C/D) in wild type (WT; n = 11) and knockout mice (KO; n = 12). Times of diazepam injection (−60 min) and rectal temperature measurement (0 min) are indicated by vertical lines plus arrows. Data represent mean group values, averaged over 5-min periods. For clarity no SEM is presented.

type ($F(1,80) = 8.76$, $p < .005$). Thus, BT was higher after the injections (−30 to −20 min = post injection) compared with undisturbed baseline conditions (−90 to −80 min), and this was higher in KO compared with WT mice. Furthermore, diazepam affected this temperature increase, but post-hoc analyses revealed no differences between the various doses of diazepam and genotypes. Diazepam had no intrinsic effects on HR (time × dose × genotype: $F(3,80) = 1.55$, $p = .21$, NS; genotype: $F(1,80) = .013$, $p = .91$, NS; dose: $F(3,80) = 2.58$, $p = .06$, NS).

Diazepam/Stress-Induced Hyperthermia and Tachycardia

Analysis of the effects of diazepam challenge on stress responses revealed an interaction effect of dose × stress [Figure 4A–B BT: $F(3,80)] = 14.80$, $p < .001$; Figure 4C–D HR: $F(3,80) = 7.88$, $p < .001$). Thus, BT and HR responses 10 and 5 min, respectively, after the rectal temperature measurement depend on the dose of diazepam. Nevertheless, post-hoc analyses revealed that in both WT and KO mice only 4.0 mg/kg diazepam blocked SIH, whereas none of the doses blocked stress-induced tachycardia.

Novel Environment. During exposure to a novel environment BT increased over time in both genotypes [Figure 5A: $F(9,180) = 98.376$, $p < .001$]. Moreover, BT was overall higher in KO compared with WT mice [$F(1,20) = 6.07$, $p < .05$]. There was, however, no significant time × genotype interaction effect [$F(9,20) = .69$, $p = .72$, NS], indicating that the increase in BT over time did not differ between genotypes. Similarly, also a genotype difference was found in HR [Figure 5B: $F(1,20) = 11.17$, $p < .005$], with no time × genotype interaction effect [$F(9,180) = 1.20$, $p = .30$, NS], indicating that between genotypes the tachycardia did not change over time. Interestingly, the mean standard deviation of HR, averaged over the whole 10 min period, was significantly lower in KO mice compared with WT mice [Figure 5B, inlay: $F(1,18) = 25.33$, $p < .001$].

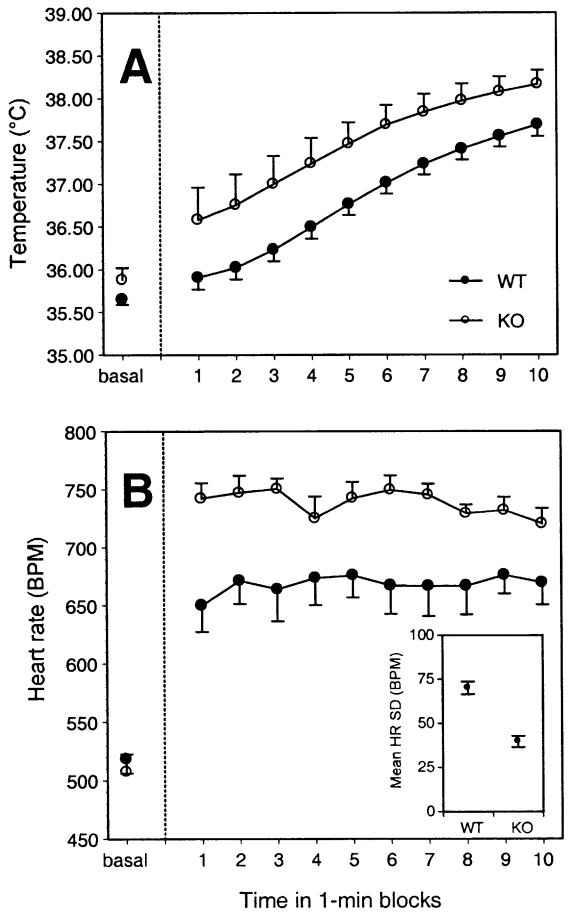

Figure 5. Effects of 10-min exposure to a novel cage on core body temperature (A), heart rate, and mean standard deviation of the heart rate (B) in wild type (WT; n = 11) and 5-HT$_{1A}$ receptor knockout mice (KO; n = 11). Data represent mean group values in successive 1-min blocks ± SEM.
Note that the basal value of core body temperature (A) and heart rate (B) is adapted from the 9:00 am time point of the baseline study (Figure 2) and is only displayed to show basal core body temperature and heart rate during the light period.

Table 1. Individual Behaviors During Exposure to the Novel Cage In 5-HT$_{1A}$ Receptor Knockout and Wild Type Mice

Behavior	Wild Type Mice	Knockout Mice
FREQUENCY		
Locomotion	52.6 ± 5.3	41.5 ± 5.4
Exploration	63.3 ± 3.6	61.8 ± 3.5
Stretched approach	14.3 ± 3.5	23.5 ± 2.6 *
Rearing	18.5 ± 4.7	7.5 ± 2.1 *
Grooming	5.2 ± 0.8	3.7 ± 0.5
Immobility	5.8 ± 1.6	4.2 ± 1.6
Burying	17.6 ± 3.2	34.5 ± 3.2 **
TOTAL TIME (sec)		
Locomotion	179.2 ± 18.0	107.3 ± 13.0 **
Exploration	291.1 ± 30.8	275.2 ± 27.3
Stretched approach	14.9 ± 4.0	27.9 ± 3.1 *
Rearing	28.0 ± 7.5	9.9 ± 2.7 *
Grooming	16.1 ± 1.3	12.3 ± 1.8
Immobility	8.0 ± 2.4	7.6 ± 3.1
Burying	62.7 ± 14.5	159.8 ± 21.1 **
LATENCY (sec)		
Locomotion	0 ± 0	0 ± 0
Exploration	14.7 ± 2.8	5.1 ± 1.2 **
Stretched approach	107.8 ± 45.2	26.7 ± 4.7
Rearing	99.0 ± 57.5	281.0 ± 50.3 *
Grooming	148.3 ± 24.1	135.9 ± 20.5
Immobility	182.4 ± 52.2	290.6 ± 52.9
Burying	216.3 ± 31.7	77.0 ± 14.4 **

Data are represented as means ± SEM. WT = wild type.
* $p < 0.05$ compared with WT mice.
** $p < 0.005$ compared with WT mice.

General activity (data not shown), as measured telemetrically, in the novel environment did not differ between WT and KO mice [F(1,20) = .03, $p = .87$, NS], and did also not differ over time [time × genotype interaction effect: F(9,180) = .99, $p = .44$, NS]. Table 1 shows the individual behaviors of the same mice displayed during exposure to the novel cage. The frequency of stretched approach postures and number of burying episodes was higher in KO mice [F(1,21) = 4.44, $p < .05$ and F(1,21) = 13.98, $p < .005$; respectively], whereas the frequency of rearing was decreased in KO compared with WT mice [F(1,21) = 4.66, $p < .05$]. KO mice spent more time on burying [F(1,21) = 14.35, $p < .005$] and stretched approach postures [F(1,21) = 6.57, $p < .05$], whereas KO mice spent less time on locomotion [F(1,21) = 10.51, $p < .005$] and rearing [F(1,21) = 5.21, $p < .05$]. Total time exploration, grooming, and immobility did not differ between genotypes. In addition, latencies to exploration and burying were shorter [F(1,21) = 10.19, $p < .005$ and F(1,21) = 9.94, $p < .005$; respectively], whereas latency of rearing was longer in KO mice compared with WT mice [F(1,21) = 5.57, $p < .05$].

DISCUSSION

The present experiments show that the anxious phenotype of mice lacking the 5-HT$_{1A}$ receptor is not associated with changes in basal 24 h variation in core BT, HR, and GA in well-adapted animals. Diurnal variation, at least measured under standard 12 h light – 12 h dark schedule, was normal in KO mice compared with WT mice. Core BT, HR, and GA showed the normal distribution, viz. higher during the dark period compared with the light period, as found earlier in mice equipped without (Boutrel et al. 1999), or with radiotelemetry transmitters (Kramer et al. 1998; Bouwknecht et al. 2000). Moreover, no absolute differences in levels of BT, HR, or GA were found between genotypes. Taken together, in addition to data that show normal home cage activity in 5-HT$_{1A}$ receptor KO mice (Heisler et al. 1998; Ramboz et al. 1998), the present results indicate that under basal, nonstress conditions the presumed anxious phenotype in KO mice is neither reflected in an altered

diurnal rhythmicity, nor in changed HR, core BT, or GA in any time period.

Interestingly, SIH experiments combined with radiotelemetry showed a differential reactivity upon a physical stressor, i.e., an injection, in KO mice compared with WT mice. This was not found, however, upon a second sequential physical stressor, i.e., the rectal temperature procedure. Thus, upon a first stressor, a subcutaneous injection, both BT and HR increased more in KO mice compared with WT mice, whereas a subsequent stressor, the rectal procedure, triggered comparable responses. Recently, it has been shown that a mild footshock also triggered an exaggerated HR response in KO mice compared with WT mice, using a different technique to measure HR (Gross et al. 2000). Apparently, the anxious phenotype of KO mice is reflected in the increased reactivity upon the first stressor in the SIH, and not upon a second stressor as only revealed by using radiotelemetry. This also may explain previous findings in nontelemetered KO mice in the SIH paradigm, where no differential reactivity between genotypes was found upon the rectal temperature procedure (Pattij et al. 2001). Nonetheless, it is unknown whether the comparable reactivity upon the second stressor is caused by habituation in KO mice, or alternatively whether the rectal procedure is a less severe stressor compared with an injection in KO mice. Habituation to injection stress has been shown in mice, but only after repeated daily vehicle injections for 1 week (Ryabinin et al. 1999).

Pharmacological challenges with the 5-HT_{1A} receptor agonist flesinoxan and $GABA_A$-benzodiazepine receptor agonist diazepam in the SIH experiments partly confirm previous findings in nontelemetered mice. First, the intermediate and high dose of flesinoxan (1.0 and 3.0 mg/kg, respectively) caused hypothermia in WT mice. This is in agreement with previous studies showing hypothermia in mice after a challenge with 5-HT_{1A} receptor agonists, like 8-OH-DPAT (McAllister-Williams et al. 1999) or flesinoxan (Olivier et al. 1998). As expected, the hypothermic effect of flesinoxan was completely absent in 5-HT_{1A} receptor KO mice. In addition, flesinoxan dose-dependently antagonized SIH and stress-induced tachycardia in WT mice, but not in KO mice. The 5-HT_{2C} receptor agonist, mCPP, has a variety of behavioral effects (Griebel 1995) and increases, among others, anxiety in rats (Kennett et al. 1989; Whitton and Curzon 1990) and humans (Murphy et al. 1991). Moreover, mCPP induces hyperthermia in rats (Mazzola-Pomietto et al. 1996), and antagonistic interactions have been described between the central 5-HT_{1A} and 5-HT_{2C} receptor systems in rats (Salmi and Ahlenius 1998). In the latter study, the hyperthermic effects of the 5-$HT_{2A/2C}$ receptor agonist, DOI, were attenuated by pretreatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT. In line with these findings, we expected a stronger hyperthermic effect of mCPP in 5-HT_{1A} receptor knockout mice. Nonetheless, in the present study, mCPP did neither cause a hyperthermic effect, nor did mCPP affect the SIH and the stress-induced tachycardia in either genotype.

The anxiolytic diazepam blocked the stress-induced hyperthermia at the highest dose (4.0 mg/kg) in line with earlier reports (Groenink et al. 1996; Van der Heyden et al. 1997; Zethof et al. 1995; Olivier et al. 2001) in both WT and KO mice. Stress-induced tachycardia was not affected by diazepam in either genotype, as also reported before in rats (Tornatzky and Miczek 1995).

Results with diazepam (this study; Pattij et al. 2001) indicate that the $GABA_A$-benzodiazepine receptor complex sensitivity as measured in the present SIH paradigm in our 5-HT_{1A} receptor KO mice (strain: 129/Sv) is not altered, as the results of diazepam in WT and KO mice were comparable. This contrasts a recent study indicating that in 5-HT_{1A} receptor KO mice on a Swiss-Webster $\times$ 129/Sv background, $GABA_A$-benzodiazepine receptors were deviant from those in corresponding WT mice (Sibille et al. 2000). Further experiments with various $GABA_A$-benzodiazepine receptor ligands in the SIH paradigm in our laboratory confirm comparable responses in KO and WT mice (unpublished observations). Several options are available to explain these discrepancies in $GABA_A$-benzodiazepine receptor findings between the 5-HT_{1A} receptor KO mice in different background strains (also reviewed in Olivier et al. 2002). It is possible that the population of $GABA_A$-benzodiazepine receptors involved in diazepam's effects on SIH is different from the population measured in the 5-HT_{1A} receptor KO mice of Sibille and coworkers (2000). Second, it might be that the 5-HT_{1A} receptor KO mice made on a pure 129/Sv background has no aberration in the $GABA_A$-benzodiazepine receptor complex, in contrast with a Swiss-Webster background. In addition to the present data, Gross et al. (2000) also suggest that their animals have no changes in the $GABA_A$-benzodiazepine receptor complex in 5-HT_{1A} receptor KO mice on a pure 129/Sv background. Further research and direct comparisons of both types of 5-HT_{1A} receptor KO mice in different benzodiazepine sensitive paradigms are in progress.

The results in the novel cage support recent data, suggesting an altered exploratory activity or reactivity to a novel environment in 5-HT_{1A} receptor KO mice (Ramboz et al. 1998). In general, basal HR in unrestrained mice is in the range of 500–580 bpm (this study; Bouwknecht et al. 2000; Kramer et al. 1998; Stiedl and Spiess 1997; Stiedl et al. 1999). Exposing mice to a novel cage elicited a strong tachycardic response of approximately 150 bpm in WT mice. This tachycardic response was even higher in KO mice, with an increase of approximately 250 bpm immediately at the start of the experiment. The difference between the genotypes ($\sim$100 bpm) remained present during the 10 min test. Together with the observed lower HR variability in KO mice compared with WT mice, this might

point to a ceiling effect of HR in KO mice, or alternatively to altered HR dynamics in KO mice. Interestingly, in mice subjected to a fear conditioning procedure also lower HR variability was found (Stiedl and Spiess 1997), and in humans a link has been proposed between low HR variability and risk for anxiety disorders (Friedman and Thayer 1998a). The strong tachycardic response found in KO mice was accompanied by a comparable higher increase in core BT of approximately 1°C over WT mice. General activity, however, was similar in KO and WT mice and can therefore not easily explain the higher tachycardia and core BT in KO mice. Thus, the observed tachycardia does not necessarily reflect increased anxiety. Although the majority of animal studies focuses on the effects of aversive situations on autonomic responses, e.g., foot-shock in mice (Gross et al. 2000), fear-conditioning in rats and mice (Marchand and Kamper 2000; Nijsen et al. 1998b; Stiedl and Spiess 1997; Stiedl et al. 1999), novelty in rats (Nijsen et al. 1998a), increased tachycardia after appetitive situations has also been reported, e.g., after feeding in pigs (Schouten et al. 1991), and reinforcing intracranial brain stimulation in rats (Burgess et al. 1993).

Therefore to further investigate the "emotional direction" of the observed enhanced tachycardia in KO mice, behavior was also monitored. In addition to the autonomic changes, behavioral observations show that locomotion and rearing are decreased in KO mice, whereas behaviors reflecting increased conflict, i.e., burying behavior (Pinel and Treit 1978) and stretched approach postures (Rodgers and Johnson 1995) were increased in KO mice. Together with the observed tachycardia and hyperthermia, the increased burying and stretched approach postures are indicative for an enhanced fear/anxiety response in KO mice. Moreover, burying behavior and stretched approach postures have been shown to be sensitive to treatment with known anxiolytics, such as benzodiazepines (Cole and Rodgers 1993) and partial and full 5-HT_{1A} receptor agonists (De Boer et al. 1991; Cole and Rodgers 1994; Rodgers et al. 1994).

It is concluded that the 5-HT_{1A} receptor KO mouse shows enhanced autonomic and behavioral responses in reaction to physical stressors (injection) and novelty (novel environment).

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