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Interactions between whole-body heating and citalopram on body temperature, antidepressant-like behaviour, and neurochemistry in adolescent male rats

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ABSTRACT

Evidence suggests that affective disorders are associated with altered thermoregulation, and it has been hypothesized that therapeutic strategies targeting body-to-brain thermosensory systems may be effective for treating depression. Consistent with this hypothesis, a recent randomized, double blind, placebo-controlled clinical trial has suggested that infrared whole-body hyperthermia has therapeutic potential for the treatment of depression. Preclinical models may help uncover the mechanism(s) underlying the antidepressant-like effects of whole-body heating. We have previously shown that exposure to whole-body heating potentiates antidepressant-like behavioural responses following administration of a behaviourally subthreshold dose of the selective serotonin reuptake inhibitor citalopram, but the neurochemical and behavioural interactions between whole body heating and *behaviourally effective* doses of citalopram are not known. In these experiments, we examined the effects of whole-body heating, either with or without treatment of a suprathreshold dose of citalopram (20 mg/kg, s.c.), on body temperature, antidepressant-like behavioural responses in the forced swim test, and tissue concentrations of serotonin and its metabolite, 5-hydoxyindoleacetic acid (5-HIAA), in the prefrontal cortex of adolescent male Wistar rats. Although whole-body heating did not potentiate the behavioural effects of suprathreshold citalopram, citalopram was observed to increase body temperature and potentiate the effects of whole-body heating on body temperature. Whole-body heating, by itself, decreased serotonin concentrations in the infralimbic cortex to a level similar to that observed following treatment with citalopram, suggesting that these treatments have convergent effects on a mesolimbocortical system innervating the medial prefrontal cortex, an effect that was correlated with effects of treatment on body temperature.

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; ANOVA, analysis of variance; CIT, citalopram hydrobromide; DR, dorsal raphe nucleus; DRI, dorsal raphe nucleus, interfascicular part; FST, forced swim test; HPLC, high performance liquid chromatography; IL, infralimbic cortex; MDD, major depressive disorder; pnd, postnatal day; PrL, prelimbic cortex; s.c., subcutaneous; SSRI, selective serotonin reuptake inhibitor; T_b, core body temperature; WBH, whole-body heating

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1. Introduction

Depression is highly prevalent and has significant socioeconomic costs. Recent estimates suggest that the global population with depression is estimated to be 4.4% [1] and major depressive disorder (MDD) accounts for 8.2% of the global years lost to disability [2]. While pharmacotherapy for depression can be effective [3], only a subset of patients respond with remission following antidepressant treatment, and antidepressant treatment is often associated with significant side effects, indicating that novel therapeutics for the treatment of affective disorders are needed.

Several lines of evidence have suggested that affective disorders are associated with altered thermoregulatory processes (for review, see [4]), and recent novel therapeutic strategies have therefore targeted these systems [5–7]. Interestingly, individuals with depression show increased night time temperature compared with healthy controls, which resolves following effective treatment [8]. Similarly, increased nighttime body temperature is observed in the depressed phase of bipolar disorder [9] and during the winter months in seasonal affective disorder [10]. Exposure to whole-body hyperthermia decreases depression ratings among individuals being treated for cancer [11], and in an open trial of whole-body hyperthermia for patients with depression, a single treatment induced a rapid and sustained decrease in patient-reported depressive symptoms [5]. Importantly, the clinical effectiveness of the whole-body hyperthermia was negatively correlated with core body temperature measured prior to treatment, suggesting that patients with a higher core temperature before treatment were more likely to respond to whole-body hyperthermia. Furthermore, decreases in core body temperature measured five days following treatment were associated with decreased depressive symptoms, suggesting that exposure to whole-body hyperthermia may have altered thermoregulatory processes. Finally, a recent randomized, double blind, placebo-controlled clinical trial has suggested that infrared whole-body hyperthermia has therapeutic potential for the treatment of MDD [6]. Individuals that received the active treatment showed decreases in clinician rated depressive symptoms that extended at least six weeks after treatment.

Preclinical studies may be helpful in understanding the systems/circuits that may underlie the clinical benefit of whole-body hyperthermia. Functional neuroanatomical evidence has demonstrated that exposure to warm ambient temperature in rats increases activation of a network of brain regions, including brainstem, thalamic, and hypothalamic regions that are thought to control sensory discriminative and thermoregulatory responses to temperature [12]. Interestingly, warm ambient temperature exposure also activates limbic forebrain regions, such as the basolateral and central amygdala, as well as the cingulate cortex, which are thought to control emotional responses to stimuli [12]. We have shown that exposure to whole-body heating (WBH) activates topographically organized populations of serotonergic neurons in the dorsal raphe nucleus (DR) of rats [13], including a group of serotonergic neurons in the interfascicular part of the DR (DRI), an area that has been associated with antidepressant-like behavioural effects [14]. Consistent with this observation, we have recently demonstrated that exposure to WBH potentiates the antidepressant-like effects of a subthreshold dose of the selective serotonin reuptake inhibitor (SSRI), citalopram. The interactions between WBH and a suprathreshold, or behaviourally active, dose of citalopram to alter thermoregulatory and behavioural responses are not known.

Here, we exposed rats to WBH, either with or without treatment with a suprathreshold dose of the SSRI, citalopram (20 mg/kg, s.c.), and monitored body temperature via biotelemetry and antidepressant-like behavioural responses in the forced swim test, a standard behavioural screen for antidepressants. In order to examine the contribution of brain serotonergic systems in these processes, rats were exposed to WBH, either with or without treatment with a suprathreshold dose of citalopram, and tissue concentrations of serotonin (5-hydroxytryptamine; 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA, the major metabolite of serotonin) were measured in the prefrontal cortex.

2. Materials and methods

2.1. Animals

Our previous studies showing that WBH activates serotonergic neurons in the DR and synergizes with a subthreshold dose of citalopram to induce antidepressant-like behavioural effects were conducted using adolescent male rats [13,15]; therefore, we used adolescent male rats in the present study. Adolescent male Wistar rats (*Experiment 1, N* = 32; *Experiment 2, N* = 23; approximately 85 g, 24 days old, at the time of arrival, 25 days old at the time of surgery; 39–43 days old [approximately 200 g] at the time of behavioural testing; HSD-WI, Harlan Laboratories, Indianapolis, IN, USA) were used. Adolescence in rodents consists of early adolescence (prepubescent or juvenile, postnatal day [pnd] 21–34), mid- adolescence (periadolescent, pnd 34–46), and late adolescence (pnd 46–59) time periods [16]. Consequently, rats were in the juvenile period (pnd 24) upon arrival and mid-adolescence at the time of behavioural testing (pnd 39–43). Rats were single housed in transparent Plexiglas® cages (47.6 cm L × 26 cm W × 20.3 cm H) using standard cage bedding (Teklad Laboratory Grade Aspen Bedding, Harlan, Madison, WI, USA). The rats were single-housed for a number of reasons: first, to be consistent with our previous research [13,15]; and second, as the rats underwent the WBH procedure individually (i.e., not in cages containing multiple conspecifics), it was important that they were habituated to individual housing. This was done to avoid body-to-body contact associated with nesting behaviours, which would elevate skin temperatures. Both food (Cat No. 2018, Teklad Global 18% Protein Rodent Diet, Harlan, Madison, WI, USA) and tap water were provided ad libitum for the duration of the experiment. Rats were kept on a standard 12 h:12 h light/dark cycle (lights on 0600). The experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*, Eighth Edition (Institute for Laboratory Animal Research, The National Academies Press, Washington, D.C., 2011) and were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee. All possible efforts were made to minimize the number of animals used and their suffering. For an illustration of the experimental timelines, see Fig. 1.

2.2. Surgical procedures

Telemetric probes were implanted into the peritoneal cavity to allow continuous measurement of body temperature using in vivo biotelemetry. The surgery was performed under inhaled isoflurane anesthesia (5% initial and 2% maintenance) as previously described [17]. Briefly, following the onset of anesthesia, the rat was placed onto its back, its abdominal hair was shaved, and its skin was disinfected with a 10% povidone-iodine solution (Qualitest Pharmaceuticals, Huntsville, AL, USA), and with 70% ethanol. A 1-cm midline incision was made through both the skin and abdominal muscle wall, starting just caudal to the xiphoid process of the sternum. A telemetric probe (Model No. TA-40; Data Sciences International (DSI), St. Paul, MN, USA) was sterilized with Actril (Cat. No. 176-02-046, Minnetech, Minneapolis, MN, USA) and implanted into the peritoneal cavity. Following probe implantation the abdominal wall was sutured using 3-0 non-absorbable silk surgical suture (Cat. No. MV-684, Med-Vet International, Mettawa, IL, USA) in a simple interrupted pattern (3 stitches). The skin was rejoined using two sterile stainless steel wound clips (Cat. No. 12032-09, Fine Science Tools Inc., Foster City, CA, USA). Antibacterial wound wash (Nolvasan, Fort Dodge Animal Health, Fort Dodge, IA, USA) was applied following application of the wound clips, and the rat was placed on a 37 °C heating pad until fully recovered. Upon recovery, a 5 mg/kg dose of the post-surgical analgesic carprofen (Rimadyl®, Pfizer Animal Health, New York, NY, USA) was administered subcutaneously. After

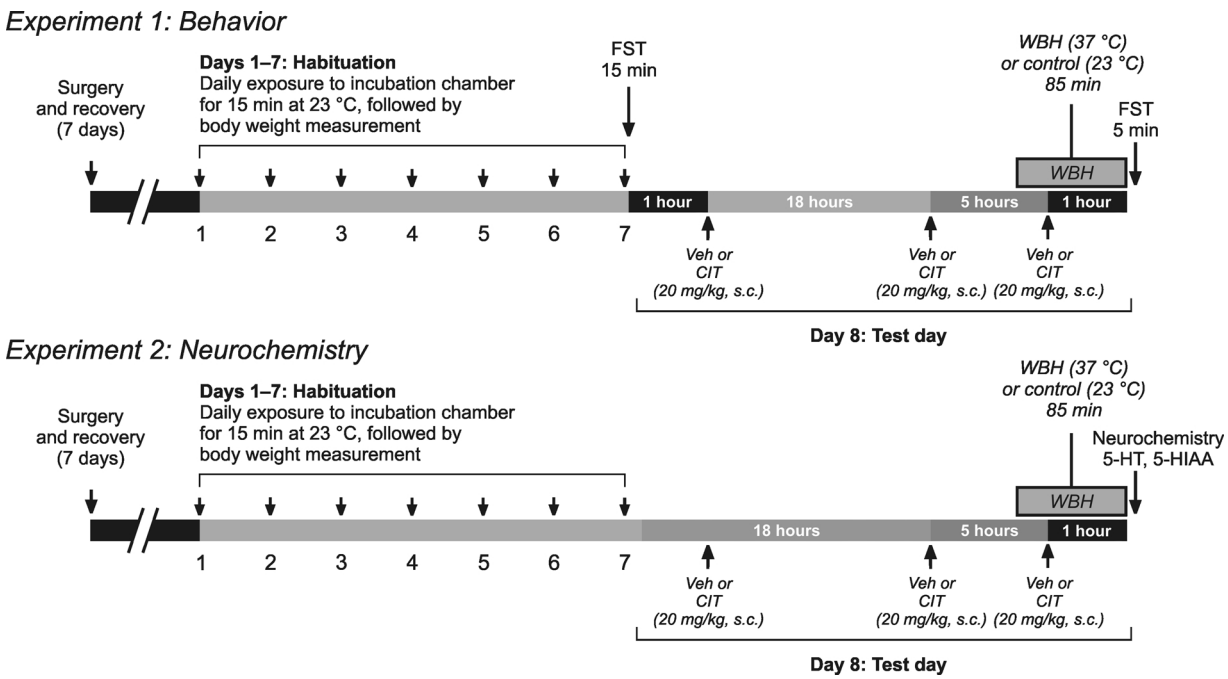

Fig. 1. Schematic illustration of experimental timelines. Abbreviations: CIT, citalopram; FST, forced swim test; s.c., subcutaneous; Veh, vehicle (0.9% saline); WBH, whole-body heating.

the surgery, all rats were single-housed in clean cages with fresh bedding. Rats were allowed to recover from surgery for at least 7 days post-surgery before the beginning of the habituation stage of the experiment.

2.3. Biotelemetric recording of core body temperature

Core body temperature (°C; T_b) was monitored continuously at a frequency of 128 Hz during 5 s samples in 1-min intervals. In *Experiment 1*, T_b was recorded for a 10-min baseline period before, and the 15-min period during, the habituation incubation period on days 1–7 as well as during the 15-min FST on day 7. On day 8 T_b was recorded for a 10-min baseline period before the exposure to the incubation chamber and throughout the 85 min exposure to acute whole body heating (37 °C) or 23 °C control conditions, as well as during the 5-min FST, and throughout the 25-min home cage recovery period. Telemetric receivers (DSI Physiotel® Receiver, Model No. RPC-1) were placed below the smaller rat cages (see below) during baseline periods and during the periods inside the incubation chambers for telemetric recording. They were also used for telemetric recording before and after the FST sessions. During the FST sessions, T_b was recorded with the receivers placed below the forced swim cylinder. In *Experiment* 2, T_b was recorded for a 10-min baseline period before, and throughout the 15-min period during, the habituation incubation period on days 1–7 and for a 60-min period after the first and second injection of either vehicle or citalopram. On day 8, T_b was recorded for a 10-min baseline period before the exposure to the incubation chamber and throughout the 85-min exposure to acute WBH (37 °C) or 23 °C control conditions. Telemetric signals were processed and recorded using the software "Dataquest ART" (version 3.0, DSI).

2.4. Drugs

Rats received three injections of either citalopram hydrobromide (Sigma-Aldrich, Cat No. C7861, St. Louis, MO, USA; 20 mg/kg in 0.9% sterile saline) or vehicle (0.9% sterile saline). This dose of citalopram has previously been demonstrated to increase swimming behaviour in the FST in juvenile rats [18]. Injections were made in a volume of 2.0 ml/kg via the subcutaneous (s.c.) route. Drug or vehicle injections were made 23, 5 and 1 h prior to the second exposure to the FST. The timing and route of administration is consistent with our previous report [15] and was based on Porsolt's original description of the forced swim test [3] as well as Cryan and colleagues' review of forced swim test behaviour [19] and Detke and colleagues' [20] comprehensive characterization of the effects of serotonergic and noradrenergic antidepressants on behavior in the forced swim test. It is important to note the clinical benefits of serotonin reuptake inhibitors in humans are not apparent until 2–3 weeks following treatment [21], and acute dosing of these drugs may even exacerbate symptoms [22]. This administration protocol has been proposed to mimic a subchronic drug exposure [23]; however, a number of studies have indicated that chronic exposure to antidepressant drugs has similar effects on behavior in the forced swim test [for review, see 23].

2.5. Acute whole-body heating (WBH)

Exposure to acute WBH was conducted as previously described [24]. Briefly, for the habituation stage, rats were transferred from their home cages, which were housed in a separate colony room, into smaller cages (29.2 cm L × 19.1 cm W × 12.7 cm H; fresh bedding, 2 cm deep, Teklad Laboratory Grade Aspen Bedding, Cat No. 7088, Harlan, Madison, WI, USA) in an experimental room and placed into the incubation chamber (Binder APT.Line™ BD [E2] Incubator with gravity [natural] convection, Tuttlingen, Germany) at room temperature (23 °C) for 15 min each day for 7 days (days 1–7) prior to the test day (day 8). Following exposure to the incubation chamber, rats were returned to their home cage. On the test day, rats were again transferred from their home cage into smaller cages and placed in the incubation chamber at either 23 °C or 37 °C for 85 min.

2.6. Forced swim test

On day 7, immediately following habituation to the chamber at 23 °C, rats were exposed to day 1 of the FST procedure [20,25]. Rats were placed individually for 15 min in glass cylinders (45.7 cm H × 30.5 cm internal diameter; Cat No. 36360-201, VWR, West Chester, PA, USA), filled with water (25 °C) to a depth of 30 cm, which were

located in the same experimental room as the incubation chambers. Body temperature was measured throughout the habituation (see above) and FST. After the 15-min forced swim exposure, rats were dried and returned to their home cages in the colony room. The FST chambers were drained, cleaned, and refilled between testing each rat. One hour following the onset of the 15-min forced swim exposure, rats received injections of either vehicle or citalopram and were returned to their home cage. The following day, five hours prior to the second exposure to the FST on day 8, rats received the second injection of either vehicle or citalopram and were returned to their home cage. Ninety-five min prior to the second FST, rats were transferred to the small cages and T_b was recorded for 10 min to establish a baseline T_b. Following this, rats were placed in the incubation chamber at either 23 °C or 37 °C. Twenty-five min following the onset of exposure to the incubation chamber, the rats were removed and received the final injection of either vehicle or citalopram and immediately replaced into the chamber for a further 60 min. Rats were then removed from the chamber and immediately exposed to the second FST. Rats were placed individually for 5 min in the same glass cylinders filled with water (25 °C) to a depth of 30 cm. In *Experiment 1*, T_b was recorded throughout the 85 min exposure to acute WBH or 23 °C control conditions, during the 5-min FST, and throughout the 25-min home cage recovery period. In *Experiment 2*, rats were killed via rapid decapitation immediately following exposure to acute WBH or control conditions on the test day (day 8).

2.7. Behavioural testing and analysis

For the 15-min pretest on day 7 and the 5-min test on day 8, behaviour was recorded using CCD cameras and a DVR multiplexer (Q-See, QSD2308C8-320, Digital Peripheral Solutions, Anaheim, CA, USA). Cameras were positioned above each cylinder. Behaviour was scored according to Cryan et al [25] as previously applied in our laboratory [15,17,26]. The behaviours selected for analysis were: 1) climbing, defined as upward movements of the forepaws, usually directed toward the side of the swim chamber; 2) swimming behaviour, defined as movement throughout the swim chamber, which included crossing across quadrants of the cylinder; 3) immobility, measured when no additional activity was observed other than that required to keep the rat's head above water; and 4) diving behaviour, classified as an event and defined as when the rat's entire body was submerged [20]. The duration, calculated as the percentage of time spent engaged in swimming, immobility and climbing, and the frequency of dives were scored. Inter-rater reliability analysis was conducted based on behavioural scoring by two investigators blind to treatment group. The analysis was conducted for individual behaviours, using Pearson correlation with all observations. Analysis revealed high inter-rater reliability for all behaviors: 1) climbing ($r = 0.853$; $p < 0.001$); 2) swimming ($r = 0.734$; $p < 0.001$); 3) immobility ($r = 0.438$; $p < 0.05$); 4) diving ($r = 0.929$; $p < 0.001$). Based on the high inter-rater reliability of behavioural scoring, only behavioural scores conducted by a single investigator (JLL) blind to treatment group are presented.

2.8. High performance liquid chromatography (HPLC) with electrochemical detection measurement of tissue concentrations of 5-HT and 5-HIAA

In *Experiment 2*, tissue concentrations of 5-HIAA and 5-HT were measured using a brain microdissection technique combined with HPLC with electrochemical detection that has been described previously, but with slight modifications [27,28]. Brain regions selected for microdissection were based on the criteria of being innervated by DRI and involvement in control of cognitive and affective function, and included the infralimbic (IL) and prelimbic (PrL) cortices [forreviews, see 29,30]. The right and left hemispheres were microdissected separately at one rostrocaudal level for both the PrL (3.00 mm from bregma) and IL (2.70 mm from bregma). Briefly, coronal brain sections (300 µm) were taken at −10 °C using a Leica CM1950 cryostat (North Central Instruments, Plymouth, MN, USA), thaw-mounted onto glass microscope slides (Cat. No. 16004-368; VWR, Radnor, PA, USA), and stored at −80 °C for later microdissection. Next, frozen brain sections were kept at −10 °C on a cold plate (Thermoelectric Cold Plate TCP-2; Cat. No. Z176664; Sigma-Aldrich, St. Louis, MO, USA) under a stereomicroscope (Nikon, Tokyo, Japan) and microdissections were performed using a stainless-steel microdissection needle (1000 µm inner diameter; Cat. No. 18035-01; Fine Science Tools, Foster City, CA, USA). Microdissected pellets from each subject were placed in individual microcentrifuge tubes (Cat. No. 02-682-550; Lot No. 11,320,360; Fisher Scientific, Rockford, IL, USA) containing 100 µL of acetate buffer (3.0 g/L sodium acetate (Cat. No. 71185-50 G; Lot No. 0,001,440,360; Fluka Analytical, St. Louis, MO, USA), 4.3 mL/L glacial acetic acid (Cat. No. A35-500; Lot No. 067,970; Fisher Scientific); pH adjusted to 5.0 with NaOH pellets (Cat. No. S8045-500 G; Lot No. 126K0695; Sigma-Aldrich), which were immediately frozen on dry ice and stored at −80 °C. Samples were later thawed on ice and then centrifuged at 4 °C and 13,000 rpm (~12,000 *g*; Beckman Microfuge 22R with refrigeration; Cat. No. MPBK368830; VWR) for 3 min. The supernatant was aspirated and the remaining pellet reconstituted with 175 µL of 0.2 M NaOH to later assay for protein content (Micro BCA protein assay kit; Cat. No. 23,235; Lot No. MF158759; Pierce Protein Research Products, Rockford, IL, USA; see manufacturer protocol). Then, 35 µL of the supernatant from each sample was dispensed into polypropylene vials (Cat. No. 200 046; Lot No. 00,140,091; SUN-SRi, Rockwood, TN, USA) sealed airtight with polyethylene snap caps (Cat. No. 502 080; Lot No. 71,189,211,013; SUN-SRi) that were placed into an ESA model 542 autosampler (Cat. No. 70-4151 A; ESA, Chelmsford, MA, USA) with the tray kept at 4 °C; the autosampler was programmed to inject 20 µL of each sample into the HPLC chromatographic system. The HPLC system also consisted of an ESA Model 582 Solvent Delivery Module (Cat. No. 70-4050 A; ESA) to pump the mobile phase (9.53 *g*/L potassium dihydrogen orthophosphate dihydrate (Cat. No. P286-1; Lot No. 0,444,037; Fisher Scientific), 300 mg/L octanesulphonic acid (Cat. No. 0/0029/46; Lot No. 0,114,435; Fisher Scientific), 35 mg/L EDTA (Cat. No. S311-500; Lot No. 070,719; Fisher Scientific), 920 mL/L HPLC-grade H_2O (Cat. No. W5-4, Lot No. 112,789; Fisher Scientific), and 80 mL/L HPLC-grade methanol (Cat. No. 34885-2L-R; Lot No. 57196MMV; Sigma-Aldrich) with pH adjusted to 3.4 using orthophosphoric acid (Cat. No. 79,606; Lot No. 1,298,236; Fluka Analytical) through the chromatographic system. The stationary phase where chromatographic separation occurred consisted of an integrated precolumn (Ultrasphere-XL 3 µm Octyl Guard Cartridge, 5 × 4.6 mm; Cat. No. 237,521; MAC-MOD Analytical, Chadds Ford, PA, USA)/column (Ultrasphere-XL 3 µm Octyl Cartridge Column, 70 × 4.6 mm; Cat. No. 237,501; Lot No. S507058; MAC-MOD Analytical) setup maintained in an oven with the temperature set at 29 °C. Electrochemical detection was accomplished using an ESA Model 5200 A Coulochem II detector with dual potentiostats (Cat. No. 70-0650B; ESA) connected to an ESA 5021 Conditioning Cell (Cat. No. 55-0450; ESA) and an ESA 5014B Microdialysis Cell (Cat. No. 70-0520B; ESA), with the respective electrode potentials set at 100 mV and 250 mV. For each run, the average peak height of two standards prepared fresh each day and containing a known concentration of 5-HIAA and 5-HT was determined manually using chromatography analysis software (EZChrom Elite for Windows, Version 2.8; Agilent Technologies, New Castle, DE, USA); the concentration of the standards was used to calculate the concentration of the unknown samples by comparing the peak heights of the unknowns to the standards. Tissue concentrations of 5-HT and 5-HIAA were standardized to the amount of protein in each pellet and final values were expressed as pg/µg protein. The mean value of the left and the right hemispheres were calculated and used in the statistical analysis.

2.9. Statistical analysis

Data were analyzed using multifactor analysis of variance (ANOVA)

with repeated measures (body weight, T_b) or two-factor ANOVA (behavior, neurochemistry) or Mann-Whitney U-tests when the assumption of homogeneity of variance was violated (*Experiment 1*, FST diving behaviour). When required, a lower bound (for body weight data) or Greenhouse-Geisser (for T_b data) correction epsilon (ε) was used for repeated measures analysis to correct for violation of the sphericity assumption [31]; these corrections multiply both the numerator and the denominator degrees of freedom by epsilon and the significance of the *F*-ratio is evaluated with the new degrees of freedom, resulting in more conservative statistical tests. Correlations between T_b and behaviour or neurochemistry data were determined using Pearson's correlation coefficient. Significant main effects or interactions based on ANOVA analysis were followed, when appropriate, by *post hoc* analysis using Fisher's least significant difference (LSD) comparisons. Paired samples *t*-test comparisons were used to explore stress-induced hyperthermia following exposure to the incubator or s.c. injections. Statistical outliers were identified using Grubbs' test [32] and excluded (*Experiment 1*; body weight, 0.0% of total data; T_b, 1.1% of total data; FST behavior, 1.6% of total data; *Experiment 2*: body weight 0.0% of total data; T_b, 0.0% of total data; neurochemistry, 2.2% of total data). There were also a number of missing data points in the T_b telemetry data (for example, when the telemetry receiver failed to detect a signal; *Experiment 1*, 3.3%; *Experiment 2*, 1.0%). For the repeated measures analyses of variance, replacement values were calculated using the Petersen method [33]. Replacement values were not included in *post hoc* tests or in any graphical representations of the data.

3. Results

3.1. Experiment 1. Effects of WBH and citalopram on core body temperature and behaviour

3.1.1. Body weight

All rats gained weight over the seven-day habituation period (day main effect, $F_{(}128_{)} = 190.4$, $p < 0.001$, $\varepsilon = 0.167$; Fig. S1). There were no differences in body weight among the different treatment conditions during the habituation procedure, or prior to the beginning of the drug administration and FST procedure.

3.1.2. Body temperature during the final habituation and 15-min FST

Habituation to the incubation chamber for 15 min on day 7 and exposure to the first day of the FST altered core T_b (time main effect, $F_{(30,840)} = 3352.5$, $p < 0.001$; Fig. 2). None of the rats had received the drug (vehicle or citalopram) or WBH (23 °C or 37 °C) treatment at this time; as expected, there were no difference in the T_b responses among the rats that were eventually assigned to the different treatment conditions. *Post hoc* two-sided Dunnett's tests, using the baseline as the control, indicated that T_b increased during the 15-min habituation period; the average temperature increase from baseline to the final T_b measurement during the habituation was 0.62 ± 0.05 °C (mean ± SEM). Body temperature remained elevated during the first min of the FST compared with baseline, but decreased precipitously after that time point. Consistent with our previous reports [17], the average temperature decrease from baseline to the final T_b measurement during the FST procedure was –9.3 ± 0.16 °C (mean ± SEM).

3.1.3. Citalopram effects on body temperature at baseline

To determine if repeated administration of a suprathreshold dose of citalopram (20 mg/kg, s.c.) alters T_b prior to exposure to the WBH procedure, baseline T_b measurements, consisting of the mean T_b for the 10-min period prior to the habituation session on day 7 (i.e., prior to any drug treatment) and the mean T_b for the 10-min period prior to the 85-min WBH or control condition on day 8 (i.e., following the first 2 injections of either citalopram or vehicle) were analyzed. Citalopram (injected 22.5 h (injection 1) and 4.5 h (injection 2) prior to the baseline measurement), increased T_b at baseline prior to exposure to acute whole-body heating on day 8 (*day* x *citalopram* interaction, $F_{(}1,30_{)} = 7.0$, $p = 0.013$; *day* main effect, $F_{(}1,30_{)} = 8.7$, $p = 0.006$; *citalopram* main effect, $F_{(}1,30_{)} = 4.9$, $p = 0.035$; Fig. 3A). Prior to any drug treatment on day 7 there were no differences in T_b among rats; however, at baseline on day 8, i.e., 21 h 35 min after the 1 st injection and 3 h 35 min following the 2nd injection, and prior to placement in the incubator, citalopram increased T_b compared with day 7 baseline and day 8 vehicle-injected rats.

3.1.4. Citalopram and WBH effects on body temperature

To determine if repeated administration to a suprathreshold dose of citalopram and exposure to WBH alter T_b during the WBH procedure, telemetric receivers were placed inside the incubation chambers and T_b was measured throughout the WBH procedure. Baseline T_b was measured as the mean T_b for the 10-min period prior to exposure to the WBH or 23 °C control conditions on day 8; mean T_b was then measured during 5-min periods for the duration of the 85-min WBH session. Note that the baseline T_b on Day 8 shown in Fig. 3A is the same as the baseline T_b on Day 8 shown in Fig. 3B, except that in the latter case the means and SEMs are shown for each of the four treatment groups separately. Citalopram and exposure to acute whole-body heating interacted to increase T_b in a time-dependent manner (*citalopram* x *WBH* x *time* interaction, $F_{(3.754,105.1)} = 4.0$, $p = 0.005$, $\varepsilon = 0.221$; *WBH* x *time* interaction, $F_{(3.754,\ 105.115)} = 11.5$, $p < 0.001$, $\varepsilon = 0.221$; *time* main effect, $F_{(3.752,\ 105.115)} = 10.5$, $p < 0.001$, $\varepsilon = 0.221$; *WBH* main effect, $F_{(}1,28_{)} = 8.0$, $p = 0.009$; *WBH* main effect, $F_{(}1,28_{)} = 4.2$, $p = 0.050$;

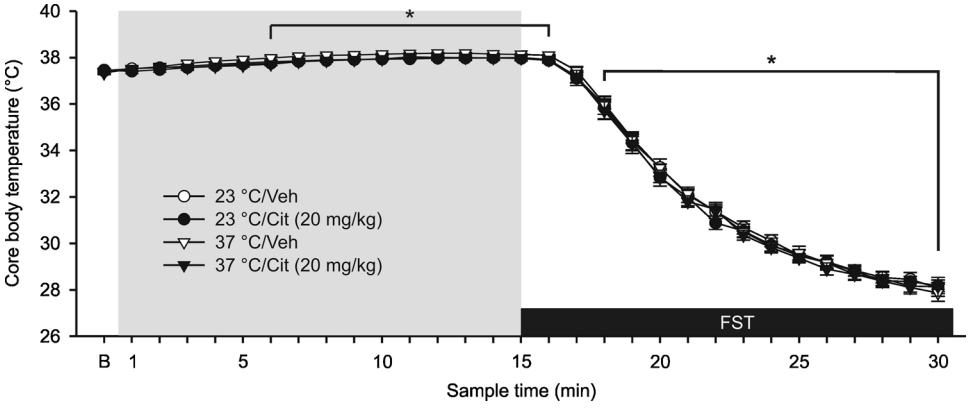

Fig. 2. Core body temperature (T_b) responses during habituation to the incubation chamber for 15 min on day 7, and during exposure to the 15-min FST training session. Compared with baseline, T_b increased during the habituation session from 6 min post-incubation onset, and decreased T_b from the second min post-FST onset. The gray box shows the period of time that the rats were in the incubation chamber at 23 °C. The black bar on the x-axis shows the period of time that the rats were in the FST with water temperature at 25 °C. The bracketed areas show the times where T_b was greater than, or less than, the baseline T_b measurement (consisting of the mean T_b for the 10-min period prior to the habituation session on day 7 (i.e., prior to any drug treatment; $p < 0.05$, two-sided post hoc Dunnett's tests using baseline T_b as the control. The sample size for each time point varied for each group, for example, when the telemetry receiver failed to detect signal, however the median sample sizes for all time points in each condition were; 23 °C/Veh, $n = 8$; 23 °C/Citalopram, $n = 8$; 37 °C/Veh, $n = 8$; 37 °C/ Citalopram, $n = 8$).

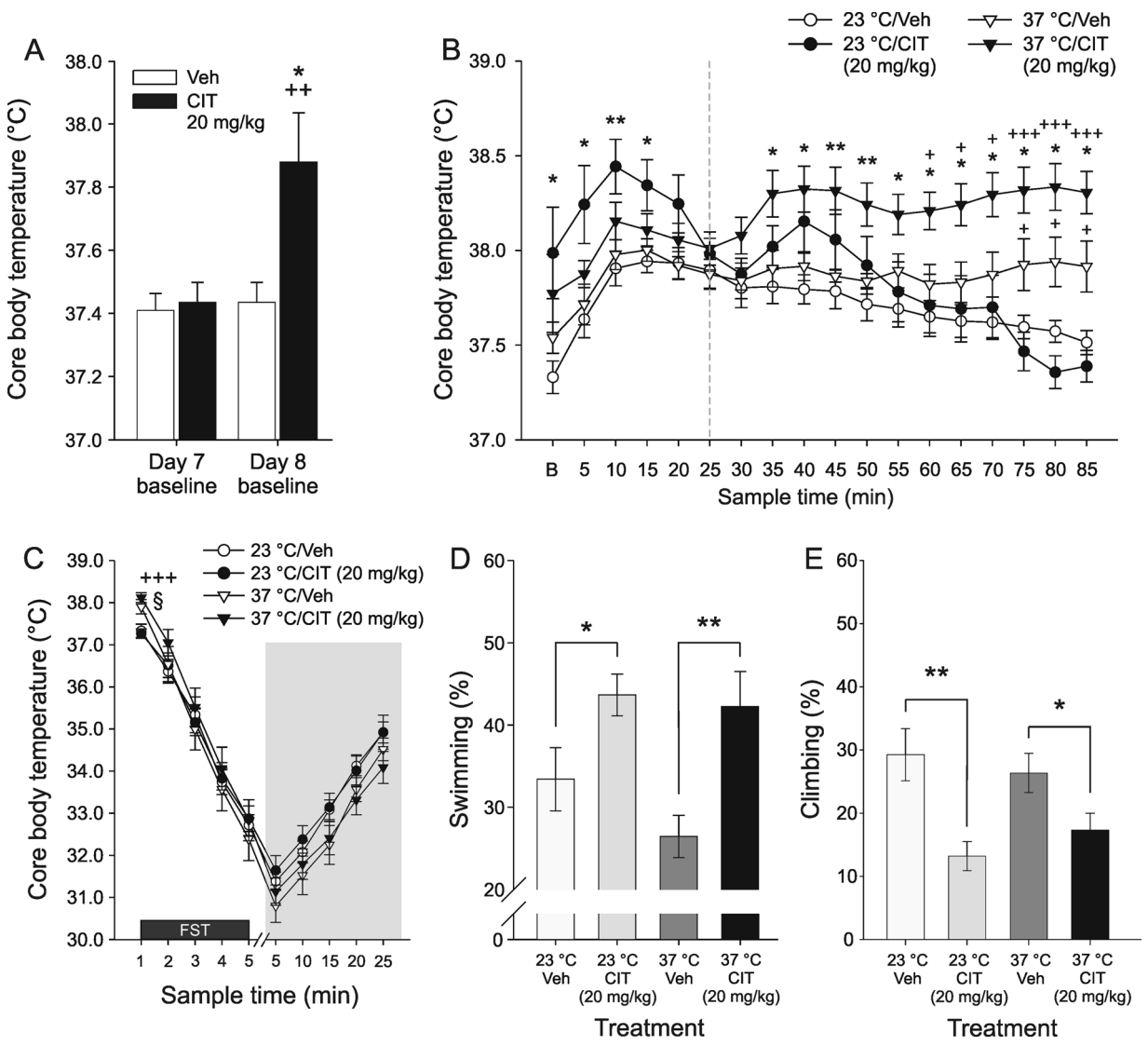

Fig. 3. Core body temperature and behavioural responses to citalopram and whole-body heating in *Experiment 1*. A) Bar graph showing baseline core body temperature (T_b) calculated as a mean of the 10-min period prior to habituation to the chamber on day 7 (i.e., before any drug treatment) and the 10-min period prior to exposure to the incubation chamber on day 8 (i.e., 21 h 35 min after the first injection of citalopram (CIT) or vehicle (Veh) and 3 h 35 min following the second injection of CIT or Veh). $^{++}p < 0.01$ versus day 7 CIT, $^{*}p < 0.05$ versus day 8 Veh; sample size: Veh, $n = 16$; CIT, $n = 16$. B) Line graph showing T_b during the whole-body heating (WBH) procedure. The gray dashed line indicates the time of the third and final CIT or Veh injection, which was followed by an enhanced stress-induced hyperthermia in CIT-injected rats. $^{*}p < 0.05$, $^{**}p < 0.01$, versus Veh-injected controls within each WBH treatment condition (i.e., 23 ºC or 37 ºC); $^{+}p < 0.05$, $^{+++}p < 0.001$, versus rats exposed to the 23 °C control conditions within each drug treatment group (i.e., Veh or CIT); sample size 23 ºC/Veh, $n = 8$; 23 °C/Citalopram, $n = 7$–8; 37 °C/Veh, $n = 7$–8; 37 °C/ Citalopram, $n = 7$-8. C) Line graph showing T_b during the 5-min FST and home cage recovery period. Both Veh-treated and CIT-treated rats exposed to WBH had higher T_b compared with rats exposed to control conditions in the first min of the FST. There were no differences in T_b among treatment groups in the remaining 4 min of the FST or during the home cage recovery period. The black bar on the x-axis indicates the FST period while the gray shading indicates the home cage recovery period. $^{+++}p < 0.001$, 37 °C/CIT versus 23 °C/CIT; $^{\S}p < 0.05$, 37 °C/Veh versus 23 °C/Veh; sample size 23 ºC/Veh, $n = 7$–8; 23 °C/Citalopram, $n = 7$–8; 37 °C/Veh, $n = 7$–8; 37 °C/ Citalopram, $n = 7$-8. D) Bar graph showing the percentage of time spent swimming in the FST. $^{*}p < 0.05$; $^{**}p < 0.01$, *post hoc* Fisher's LSD tests; sample size, $n = 8$ for all groups. E) Bar graph showing the percentage of time spent climbing in the FST. $^{*}p < 0.05$; $^{**}p < 0.01$, *post hoc* Fisher's LSD tests; sample size, $n = 8$ for all groups.

Fig. 3B). *Post hoc* tests indicated that, among vehicle-injected rats, whole-body heating increased T_b, relative to 23 °C controls, from 75 to 85 min following exposure to the incubation chamber. Meanwhile, among rats injected with citalopram, exposure to WBH increased T_b, relative to 23 °C controls, from 60 to 85 min following the onset of exposure to the incubation chamber. Among rats exposed to 23 °C conditions, citalopram increased T_b from baseline to 15 min following the onset of the exposure to the incubation chamber. Furthermore, among rats exposed to acute whole-body heating (37 °C), citalopram increased T_b, relative to 23 °C controls, from 35 to 85 min following the onset of exposure to the incubation chamber. Thus, citalopram potentiated the effects of whole-body heating on T_b.

To examine the stress-induced hyperthermia related to placing the rats in the chamber [34], paired samples *t*-tests were calculated using T_b data from baseline to 10 min post-incubation onset in 23 °C/Veh and 37 °C groups. Both groups showed a stress-induced hyperthermia after being placed into the incubation chamber (23 °C/Veh; 10 min, $t_{(7)} = 5.9$, $p = 0.001$; 37 ºC/Veh; 10 min, $t_{(7)} = 4.4$, $p = 0.003$; Fig. 3B). Citalopram-treated rats showed similar increases in body temperature in the first 10 min following the onset of the WBH procedure (23 °C/Citalopram; 10 min, $t_{(7)} = 4.6$, $p = 0.003$; 37 ºC/Citalopram; 10 min, $t_{(7)} = 2.7$, $p = 0.03$; Fig. 3B)

3.1.5. Body temperature during the 5-min FST and home cage recovery period

To determine if repeated administration of a suprathreshold dose of citalopram and exposure to WBH affected T_b during the 5-min FST procedure, telemetry receivers were placed below the FST cylinder during the test itself, and then placed below the rat's home cage to record T_b changes following the test. During the FST, T_b fell rapidly and partially recovered during the 25-min home cage recovery period (*WBH* x *time* interaction, $F_{(2.603,\ 72.881)} = 6.4$, $p = 0.001$, $\varepsilon = 0.289$; Fig. 3C). Both vehicle-treated and citalopram-treated rats exposed to WBH had increased T_b compared with rats exposed to control conditions in the first min of the FST; however, there were no differences among treatment groups in the remaining 4 min of the FST or during the home cage recovery period.

3.1.6. Forced swim test behaviour

Citalopram increased the percentage of time spent swimming in rats exposed to WBH and 23 °C control conditions (*citalopram* main effect, $F(1,28) = 14.7$, $p = 0.001$; Fig. 3D); however, there were no effects of WBH on swimming in the FST, either in vehicle- or citalopram-treated rats. There were no citalopram or WBH effects on the percentage of time spent immobile (data not shown); however, analysis of the percentage of time spent climbing indicated that citalopram decreased climbing behaviour in rats exposed to WBH and control conditions (*citalopram* main effect, $F(1,28) = 16.1$, $p < 0.001$; Fig. 3E). There were no effects of WBH on climbing behaviour in the FST. Citalopram prevented diving behaviour, calculated as the number of dives, in the FST ($U = 45$, $p = 0.004$), in fact aside from one outlier, no rats that received citalopram were observed to dive during the 5-min FST, whereas 9 out of 15 rats exposed to vehicle dived at least once during the 5-min test (mean ± SEM; Veh, 1.40 ± 0.39; citalopram, 0.00 ± .00). Exposure to WBH was without effect on the frequency of dives in the 5-min FST ($U = 103.5$, $p = 0.728$; mean ± SEM; Veh/23 ºC, 1.38 ± 0.6; Veh/37 ºC, 1.43 ± 0.5).

3.1.7. Correlations

Pearson's correlations were used to determine the extent to which T_b, measured immediately following exposure to WBH or control conditions, was associated with measures of antidepressant-like behaviour in the subsequent FST. These analyses indicated that T_b was not associated with behaviour in the FST.

3.2. Experiment 2. Effects of whole-body heating and citalopram on core body temperature and neurochemistry

3.2.1. Body weight

All rats gained weight over the seven-day habituation period (*day* main effect, $F_{(1.00,19.00)} = 81.8$, $p < 0.001$, $\varepsilon = 0.167$; Fig. S2). There were no differences in body weight among the different treatment conditions during the habituation procedure or prior to the beginning of the drug administration and WBH procedure.

3.2.2. Effects of citalopram on core body temperature

To determine the effects of administration of the first and second dose of citalopram on T_b prior to exposure to the WBH procedure, baseline T_b was measured for a 10-min period prior to each injection, and then measured for a 60-min period after each injection (*N.B.*, all baseline, habituation and WBH procedures were conducted during the light, inactive, phase). Immediately following the first injection, vehicle-treated- rats showed a stress-induced hyperthermia, as evidenced by an increase in core T_b (*drug* x *time* interaction, $F_{(3.182,\ 66.815)} = 3.8$, $p = 0.012$, $\varepsilon = 0.265$; *time* main effect, $F_{(3.182,\ 66.815)} = 9.9$, $p < 0.001$, $\varepsilon = 0.026$; Fig. 4A$_1$). While the stress-induced hyperthermia in rats that had received vehicle was transient, returning to baseline within 25 min, the hyperthermia of rats that had received citalopram was both exaggerated in amplitude and prolonged compared with vehicle-treated rats. These effects were evident from 10 to 60 min following the injection (*drug* main effect, $F(1,21) = 12.8$, $p = 0.002$). Following the second injection, which was given 18 h after the first injection, all rats showed an increased T_b (*time* main effect, $F_{(3.833,\ 65.155)} = 15.5$, $p < 0.001$, $\varepsilon = 0.319$; Fig. 4A$_2$); however, the main effect for *citalopram* and the *citalopram* x *time* interaction effect were not statistically significant, indicating that for the second injection, the transient increase in T_b was independent of the effects of citalopram.

3.2.3. Effects of citalopram and WBH on body temperature in the absence of exposure to the forced swim test

To determine if exposure to a suprathreshold dose of citalopram and exposure to WBH alter T_b during the WBH procedure in the absence of prior exposure to the 15-min FST training session, telemetric receivers were placed inside the incubation chambers and T_b was measured throughout the WBH procedure. Exposure to WBH increased T_b in a time-dependent manner (*WBH* x *time* interaction, $F_{(3.606,68.516)} = 10.2$, $p < 0.001$, $\varepsilon = 0.212$; Fig. 4B). *Post hoc* tests indicated that, among vehicle-treated rats, exposure to acute whole-body heating increased T_b, relative to 23 °C controls, from 55 min to 85 min following the onset of exposure to the incubation chamber. Meanwhile, among citalopram-treated rats, whole-body heating increased T_b, relative to 23 °C controls, from 40 to 85 min following exposure to the chamber.

As in *Experiment 1*, we explored possible stress-induced hyperthermia responses in 23 °C/Veh and 37 °C/Veh rats using paired samples *t*-tests comparing baseline to 10 min following placement of rats in the chamber [34]. Consistent with *Experiment 1,* both groups showed a stress-induced hyperthermia after being placed into the incubation chamber (23 °C/Veh, $t_{(5)} = 4.8$, $p = 0.005$; 37 ºC/Veh, $t_{(4)} = 6.1$, $p = 0.004$; Fig. 4B). Citalopram-treated rats exposed to the WBH condition showed a similar increase in temperature in the first 10 min of the procedure (37 °C/Citalopram, $t_{(5)} = 3.4$, $p = 0.02$; Fig. 4B). In citalopram-treated rats exposed to control conditions, the increase in body temperature in the first 10 min of the procedure approached statistical significance (23 °C/Citalopram, $t_{(5)} = 2.4$, $p = 0.06$; Fig. 4B).

3.2.4. Effects of citalopram and WBH on neurochemistry

Citalopram decreased tissue concentrations of 5-HIAA in the PrL and IL cortices (*citalopram* main effect, $F(1,19) = 345.5$, $p < 0.001$; Fig. 4C–E). Although there was a main effect for brain region ($F(1,19) = 10.6$, $p = 0.004$; Fig. 4C–E) indicating a difference between tissue concentrations of 5-HIAA in the PrL compared with the IL, there were no effects of whole-body heating on 5-HIAA concentrations.

Citalopram and exposure to WBH decreased tissue concentrations of 5-HT in the IL (*citalopram* x *WBH* x *brain region* interaction effect, $F(1,19) = 6.5$, $p = 0.019$; *brain region* main effect, $F(1,19) = 87.2$, $p < 0.001$; *citalopram* x *WBH* interaction effect, $F(1,19) = 5.3$, $p = 0.033$; Fig. 4C–G). *Post hoc* tests indicated that, among rats exposed to the 23 °C control condition, citalopram decreased tissue concentrations of serotonin in the IL compared with vehicle. Furthermore, among rats treated with vehicle, exposure to whole-body heating decreased tissue concentrations of serotonin in the IL compared with control conditions. There were no differences in tissue concentrations of serotonin in the IL between citalopram-treated rats exposed to WBH and citalopram-treated rats exposed to 23 °C conditions or vehicle-treated rats exposed to WBH. Also, there were no treatment effects on tissue concentrations of serotonin in the PrL.

Citalopram and exposure to WBH altered the 5-HIAA/5-HT ratio in the PrL and IL (*brain region* x *citalopram* interaction effect, $F(1,19) = 9.9$, $p = 0.005$; *brain region* main effect, $F(1,19) = 181.2$, $p < 0.001$; *WBH* main effect, $F(1,19) = 4.9$, $p = 0.039$; Fig. 4C,H,I). *Post hoc* tests indicated that citalopram decreased the 5-HIAA/5-HT ratio compared with vehicle-treated rats in the PrL and IL under both 23 °C and 37 °C conditions. Among vehicle-treated rats, exposure to whole-body heating increased the ratio of 5-HIAA/5-HT in the IL.

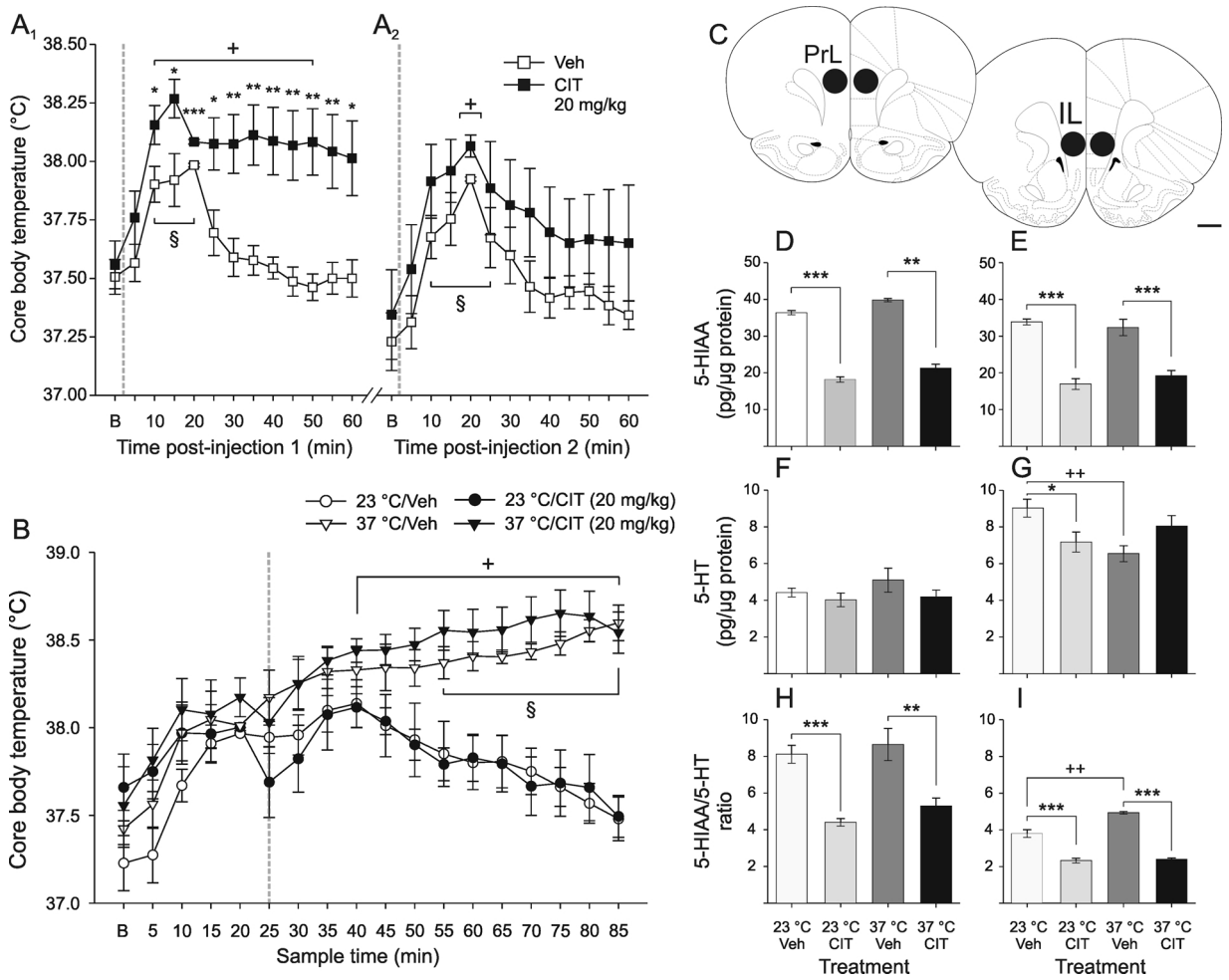

Fig. 4. Core body temperature and neurochemical responses to citalopram and whole-body heating in *Experiment 2*.A_1, A_2) Line graphs illustrating the effects of the first two injections, given 24 h apart, of repeated administration of a suprathreshold dose of citalopram (CIT; 20 mg/kg, s.c.; A_1, $n = 12$; A_2, $n = 10$) or vehicle (Veh; A_1, $n = 11$; A_2, $n = 9$) treatment, on core body temperature (T_b). Baseline data point (labeled B in the x-axis) represents the mean $T_b \pm$ SEM during the 10-min period prior to injection. All other data represent mean $T_b \pm$ SEM during 5-min periods. Dashed lines indicate time of injection of either Veh or CIT. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ versus Veh, *post hoc* Fisher's LSD tests. The data within the upper brackets of A_1 and A_2 (+) indicate the range of time points at which CIT-treated rats differed from baseline ($p < 0.05$). The data within the lower bracket of A_1 and A_2 (§) indicate the range of time points at which Veh-treated rats differed from baseline ($p < 0.05$). B) Line graph showing T_b during the whole-body heating (WBH) procedure. The gray dashed line indicates the time of the third and final CIT or Veh injection. The data within the upper bracket (+) indicate the range of time points at which CIT-treated rats exposed to WBH differed from CIT-treated rats exposed to 23 °C control conditions ($p < 0.05$). The data within the lower bracket (§) indicate the range of time points at which Veh-treated rats exposed to WBH differed from Veh-treated rats exposed to 23 °C control conditions ($p < 0.05$); sample size 23 ºC/Veh, $n = 6$; 23 °C/Citalopram, $n = 6$; 37 °C/Veh, $n = 5$; 37 °C/ Citalopram, $n = 6$. C) Line drawings illustrating coronal rat brain sections showing the location and diameter of microdissected brain regions: prelimbic cortex (PrL; 3.00 mm bregma) and infralimbic cortex (IL; 2.70 mm bregma). Scale bar, 1 mm. D,E) Bar graphs showing (D) tissue concentrations of 5-hydroxyindoleacetic acid (5-HIAA, pg/µg protein) in the PrL and (E) IL. $**p < 0.01$; $***p < 0.001$,*post hoc* Fisher's LSD tests. F–G) Bar graphs showing tissue concentrations of serotonin (5-HT, pg/µg protein) in the (F) PrL and (G) IL. $*p < 0.05$; $++p < 0.01$, post hoc Fisher's LSD tests. H–I) Bar graphs showing the ratios of 5-HIAA/5-HT in the PrL (H) and IL (I). $**p < 0.01$; $***p < 0.001$; $++p < 0.01$, post hoc Fisher's LSD tests; neurochemistry sample size, IL: 23 ºC/Veh, $n = 6$; 23 °C/Citalopram, $n = 6$; 37 °C/Veh, $n = 5$; 37 °C/ Citalopram, $n = 6$; PrL: 23 °C/Veh, $n = 5$; 23 °C/Citalopram, $n = 6$; 37 °C/Veh, $n = 4$–5; 37 °C/ Citalopram, $n = 6$.

3.2.5. Correlations

Pearson's correlations were used to determine the extent to which T_b, measured during the final five-min period of exposure to WBH or control conditions, was associated with tissue concentrations of 5-HIAA and 5-HT and the 5-HIAA/5-HT ratio in the PrL and IL. These analyses indicated that T_b was negatively correlated with the tissue concentrations of serotonin in the IL with a medium to large effect size [35] ($r = -4.467$, $p = 0.025$; Fig. 5). However, when the alpha was adjusted for multiple comparisons, this correlation was not statistically significant.

4. Discussion

Whole-body hyperthermia has shown some promise as an antidepressant therapy [5,6] and our previous preclinical research has demonstrated that WBH synergises with subthreshold treatment with the SSRI citalopram to produce antidepressant-like behavioural effects in adolescent rats [15]. In this experiment, we investigated if WBH could synergize with a suprathreshold, behaviourally active dose of citalopram (20 mg/kg, s.c.), to potentiate citalopram-induced antidepressant-like behavioural effects. If WBH and citalopram elicit antidepressant behavioural effects through convergent mechanisms, we would expect that, in the presence of a maximally effective dose of citalopram, WBH would have no additional effect. Here, we show that, as expected, repeated administration of citalopram had antidepressant-like behavioural effects, as measured by increased swimming in the FST, in adolescent male rats. Also as expected, citalopram altered 5-HT and serotonin metabolite concentrations within the medial prefrontal cortex, a common feature of many antidepressant drugs. Interestingly,

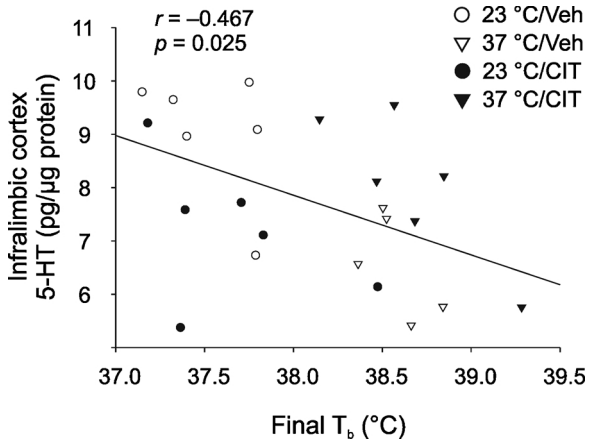

Fig. 5. Scatter plot showing a negative correlation between the final body temperature (T_b) measurement, following vehicle (Veh) or citalopram (CIT) treatment and an 85-min exposure to 23 °C or 37 °C conditions, and tissue concentrations of 5-hydroxytryptamine (5-HT, serotonin) in the infralimbic cortex (IL) in *Experiment 2*.

citalopram also increased T_b, measured immediately after the first injection, at baseline prior to exposure to WBH, and potentiated the effects of WBH on T_b. Whole-body heating, by itself, decreased 5-HT concentrations in the IL, to a level similar to that observed following treatment with citalopram, suggesting that these treatments have convergent effects on a mesolimbocortical system innervating the medial prefrontal cortex, an effect that was correlated with effects of treatment on T_b.

As expected, citalopram had antidepressant-like behavioural effects in adolescent rats. Rats treated with a 20 mg/kg dose of citalopram responded with increased swimming behaviour, considered an active coping strategy in the FST [36]. In contrast, rats treated with citalopram responded with decreased climbing behaviour; consequently, there was no overall effect of citalopram on immobility [36]. This is consistent with the previous observation in juvenile rats, demonstrating that 10 mg/kg of escitalopram, the more potent *S*-(+)-enantiomer of citalopram, is an effective dose to increase swimming in the FST, whereas 20 mg/kg of escitalopram is required to decrease immobility [37]. Furthermore, a selective effect of citalopram on swimming behaviour is consistent with previous studies suggesting that SSRI's selectively alter swimming behaviours, whereas antidepressant drugs that act through noradrenergic mechanisms selectively alter climbing behaviours [20]. Whole-body heating, either by itself, or in combination with the behaviourally active dose of citalopram, had no effects on behaviour in the FST.

Also as expected, citalopram altered 5-HT and serotonin metabolite concentrations within the prefrontal cortex, a common feature of many antidepressant drugs [38,39]. Citalopram decreased 5-HIAA concentrations in both the PrL and IL among rats exposed to either WBH or 23 °C control conditions, presumably because, by preventing reuptake of 5-HT into presynaptic serotonergic terminals, citalopram decreases metabolism of synaptically-released 5-HT by presynaptic monoamine oxidase B within serotonergic terminals [40]. Furthermore, citalopram decreased 5-HT concentrations in the IL, but not the PrL, but only in rats exposed to 23 °C control conditions. Interestingly, among vehicle-treated rats, WBH, by itself, decreased 5-HT concentrations in the IL, to a level that was similar to that observed following treatment with citalopram, suggesting that these treatments may have convergent effects on a mesolimbocortical serotonergic system innervating the medial prefrontal cortex, an effect that was correlated with T_b (measured during the final 5-min period of exposure to the WBH procedure or control conditions). The functional significance of WBH-induced decreases in 5-HT concentrations in the IL and its role in antidepressant-like or emotional behaviour is not yet known; however, four days of exposure to warm ambient temperature (31 °C) alters fear learning and is associated with decreased c-Fos expression in the IL [41].

The citalopram-induced decrease in serotonin metabolism in the mPFC in association with increased swimming behaviour in the forced swim test is consistent with previous reports of SSRI effects on serotonergic metabolism and FST behaviour. The SSRI sertraline decreases serotonin metabolism in the mPFC and increases swimming in Wistar rats [42]. Importantly, serotonin metabolism, as measured by the 5-HIAA/5-HT ratio, in the mPFC is negatively correlated with swimming behavior, suggesting a functional association between serotonin neurotransmission in the mPFC and active coping responses in the FST [42]. Consistent with this observation, 5,7-dihydroxytryptamine (DHT)-induced selective lesion of serotonin terminals in the medial prefrontal cortex reduces immobility in the FST [43]. Although the mechanisms through which SSRIs increase swimming behavior in the FST are not fully understood, serotonergic neurotransmission in the mPFC has been demonstrated to control behavioural coping responses though modulation of GABA release in the amygdala [43,44]. It is possible that SSRI drugs modulate this DR-mPFC-amygdala circuit to produce antidepressant-like behavioural effects.

Although there were no synergistic effects of WBH and citalopram on antidepressant-like behaviour in the FST, both WBH and citalopram had effects on body temperature. In particular, among vehicle-treated rats, WBH increased body temperature toward the end of the WBH session (75–85 min). Citalopram had a number of effects on body temperature, including 1) increased body temperature during the baseline period prior to exposure to the WBH procedure (i.e., after two injections of citalopram, 22.5 h and 4.5 min [injection 2] prior to the baseline, respectively); and 2) potentiated the effects of WBH to increase core body temperature during the WBH procedure. When body temperature was measured prior to exposure to the WBH procedure, rats treated with citalopram showed an elevated T_b (almost 0.5 °C higher) than vehicle-treated controls. Although clinical evidence has indicated SSRI-induced hyperthermic effects, both in the context of serotonin syndrome (for review, see [45]) and altered metabolic rate [46], evidence from preclinical studies mostly demonstrate hypothermic effects of SSRI drugs. For example, fluoxetine has been demonstrated to dose-dependently decrease T_{rec} at a range of ambient temperatures (from 8 °C to 22 °C). At an ambient temperature of 8 °C, fluoxetine-induced hypothermia was associated with decreased metabolic rate; however, at an ambient temperature of 22 °C, the fluoxetine-induced hypothermia was associated with increased tail and foot temperature [47], suggesting that fluoxetine may engage thermoregulatory cooling systems [48]. Continuous recording of T_b using biotelemetry has also demonstrated a hypothermic effect of fluoxetine (10 mg/kg), with a nadir at approximately –2 °C below baseline T_b, 1 h following i.p. injection [34]. Citalopram has also been demonstrated to have an acute hypothermic effect [49]. At a dose of 100 µmol/kg (40.5 mg/kg), which is approximately two times the behaviourally active (suprathreshold) dose of citalopram that was used in the present study (20 mg/kg), citalopram decreased T_{rec} at 20 and 60 min post injection. However, a lower dose 25 µmol/kg (10.1 mg/kg) of citalopram, which is more similar to the behaviourally inactive, subthreshold dose used in our previous report (5 mg/kg; [15]), showed a small hypothermic effect 20 min post-injection, which recovered by 60 min post-injection. Meanwhile, a lower dose of citalopram (6.5 µmol/kg or 2.6 mg/kg) showed no acute hypothermic effects [49]. The basis of the differences between the acute hypothermic effects of fluoxetine and citalopram noted above and the present results are not clear, however in *Experiment 1*, the citalopram-induced increase in T_b was observed immediately prior to exposure to the WBH procedure, which occurred 22.5 h after the first, and 4.5 h after the second injection of citalopram. Therefore, it is possible that this longer term hyperthemic effect may have occurred independently of an acute citalopram-induced hypothermic effect. Counter to this hypothesis, continuous recording of T_b following the first and second citalopram injections in *Experiment 2*, showed an

increase in T_b in citalopram-injected rats, compared with vehicle-injected rats lasting at least 60 min following the first injection of citalopram. The reason for these discrepant findings is not known, but the hypothermic effect of citalopram noted by Oerther & Ahlenius [49] was measured via rectal temperature, during the dark (active) phase of the light/dark cycle, whereas in the present study, the effects of citalopram on T_b were measured using in vivo biotelemetry, during the light (inactive) phase of the light/dark cycle. It is also possible that the seven days of habituation to the incubation chamber at 23 °C conditions, and associated handling, may have altered the temperature responses to citalopram. Interestingly, the site of action of SSRI drugs is likely to influence their effects on body temperature. For example, pharmacological evidence suggests that increased serotonin concentrations in the hypothalamus, induced by site-specific infusion of fluoxetine, produces hyperthermic effects [50].

As well as increasing T_b prior to the WBH procedure, citalopram potentiated the effects of WBH to increase T_b. Among rats exposed to WBH, citalopram increased T_b by approximately 0.4 °C compared to vehicle-treated controls. While this result is consistent with our previous report using a behaviourally subthreshold dose of citalopram [15], in *Experiment 2*, citalopram did not potentiate the effects of WBH on T_b. While the reason for this inconsistency is not known, in *Experiment 2*, rats were not exposed to the 15-min FST on day one, and it is possible that this thermoregulatory challenge induces adaptations that play an important role in the subsequent effects of citalopram on body temperature and, potentially, on behaviour. Consistent with a number of previous observations [17,26,51], the 15-min FST at a water temperature of 25 °C induced a pronounced hypothermic state in the rats. Compared with baseline, at the end of the 15-min FST procedure, on average rats showed a 9 °C decrease in body temperature. While previous research suggests that T_b recovers to baseline within about 60–100 min of the FST procedure [17,26], brain 5-HT and 5-HIAA concentrations remain elevated in the hippocampus for at least 90 min following the onset of the FST [51]; therefore, in *Experiment 1*, it is likely that rats received their first injection of citalopram in the context of both an altered thermoregulatory state as well as altered brain serotonin metabolism. It is possible that challenge to thermoregulation inherent in the 15-min swim stress on day 1 of the FST procedure plays an important role in the subsequent behavioural response to the 5-min swim stress on day 2 of the FST procedure [26], and may also be an important component of the antidepressant-like behavioural responses of the SSRI drugs. Further research is required to test this hypothesis.

Continuous recording of T_b following exposure to the incubation chamber in *Experiments 1* and *2* and following the citalopram injections in *Experiment 2* allowed for analysis of stress-induced hyperthermia, resulting from the handling and injection procedure. Stress-induced hyperthermia was observed in vehicle-treated rats in both the control (23 °C) and WBH conditions (37 °C). In *Experiment 1*, citalopram-treated rats exposed to control and WBH conditions showed similar increases in core body temperature after placement in the incubation temperature (albeit citalopram-treated rats started at a higher baseline temperature), suggesting that citalopram had little effect on the stress-induced hyperthermia that resulted from exposure to the incubation chamber. In *Experiment* 2, the injection procedure produced a stress-induced hyperthermia in vehicle-treated control rats. Following the first injection procedure, the stress-induced hyperthermia in rats that had received vehicle was transient, returning to baseline within 25 min; however, the hyperthermia of rats that had received citalopram was both exaggerated in amplitude and prolonged compared with vehicle-treated rats. While it is possible the interaction between stress-induced hyperthermia, arising from the injection procedure, and the SSRI treatment may be important for the antidepressant-like behavioural response, additional research would be required to test this hypothesis. However, some evidence suggests that chronic treatment with the SSRI fluoxetine attenuates stress-induced hyperthermia in both rats and mice [34].

As mentioned above, although WBH did not potentiate the effects of citalopram on antidepressant-like behavioural responses, it did potentiate the effects of citalopram on core body temperature, specifically when assessed after the final dose of citalopram in *Experiment 1*. During the same period, citalopram did not increase core body temperature among rats housed at room temperature, consistent with previous studies [18]. The potentiation of core body temperature by citalopram was evident at a number of different time points: 1) immediately post-injection following the initial injection of citalopram, at 23 °C, during and following the initial stress-induced hyperthermia phase (i.e., increase in core body temperature following initial handling and injection; *Experiment 2*); 2) at baseline, again at 23 °C, prior to initiation of the whole-body heating (*Experiment 1*); and 3) during the 55-min period prior to initiation of behavioural testing (*Experiment 1*). These findings suggest that whole-body heating and citalopram increase core body temperature through non-convergent mechanisms.

Serotonin is known to play an essential role in stress-induced hyperthermia [19]; 5-HT_{1A} receptor agonists in particular reduce the amplitude of stress-induced hyperthermia. Meanwhile, serotonin is essential for maintaining core body temperature; mice maintained at room temperature, 21–23 °C, with conditional inhibition of brain serotonergic neurons respond with a rapid and profound decrease in core body temperature [20]. An understanding of how citalopram increases core body temperature, and how that differs from mechanisms through which whole-body heating, by itself, increases core body temperature will require further study.

Exposure to whole-body heating and forced swimming at a water temperature of 25 °C represent two different thermal challenges. Therefore, the appraisal of the stressor (i.e. swim stress) may have differed among rats exposed to whole-body heating compared with ambient temperature-exposed controls. Consistent with this possibility, both vehicle and citalopram-treated rats exposed to the whole-body heating, relative to vehicle- and citalopram-treated rats exposed to 23 °C, maintained a higher core body temperature during the first minute of the five-minute forced swim procedure (Fig. 3C). While assessing stressor appraisal in preclinical models is challenging [52], altering the water temperature in the forced swim test may account for any differences in stressor appraisal. Swim stress at a water temperature of 35 °C results in a relatively small decrease in body temperature [17,51]. However, no differences in the amount of swimming or immobility were observed in rats exposed to 25 °C or 35 °C, suggesting that the perception of water temperature has little impact on behavioural responses, at least within the temperature range of 25 °C–35 °C. Assessing antidepressant-like behavioural effects using tests without a thermal challenge component, such as the sucrose preference test, may provide additional insight into the effects of whole-body heating on emotional behaviour.

Our previous observations that whole-body heating increases c-Fos expression in functionally-related subsets of serotonergic neurons [13] and synergises with a subthreshold dose of citalopram to induce antidepressant-like behavioural effects [15] used adolescent male rats only. To be consistent with these previous observations, we used adolescent male rats in the present study. We can't exclude the possibility that the effects of whole-body heating may differ among male and female rats. Several lines of evidence indicate sex differences in thermoregulatory processes [53]. For example, female Sprague Dawley rats have a higher body temperature (measure as colonic temperature) measured 1–2 h before dark onset compared with males [54], and, like humans [for review, see 55], show variation in body temperature across the reproductive cycle [54,56]. Following exposure to a warm ambient temperature (28 °C), rats in the estrus phase show an altered vasomotor response compared with rats in proestrus [56], suggesting that gonadal hormones may play a role in the thermoregulatory response to warm temperature exposure. Further research is required to understand the effects of whole-body heating on antidepressant-like behavioral responses in female rats, as well as rats at different developmental stages.

5. Conclusions

We have previously demonstrated that a behaviourally subthreshold dose of citalopram synergises with WBH to produce an antidepressant-like behavioural response. Furthermore, we previously have shown that body temperature, measured immediately after the WBH was highly correlated with the antidepressant-like behavioural response. Although there were no synergistic effects of a behaviourally active dose of citalopram with WBH to produce an antidepressant-like behavioural response in the present study, these results may nonetheless have some clinical relevance. In a small-scale open-label clinical trial, one session of whole-body hyperthermia decreased depression symptoms in depressed individuals during the five days following treatment. While most patients in the trial received no pharmacological intervention during the study period, three participants were being chronically treated with an SSRI with no change in dosage during the study period. Interestingly, when examined separately, whole-body hyperthermia had no effect in the three individuals receiving SSRI treatment [5]. Additional preclinical research, including both male and female rats at different developmental stages, is required to fully understand the neurochemical and functional neuroanatomical mechanisms that underlie the interaction between the subthreshold dose of citalopram and WBH.

Declaration of interest

Dr. Lowry reports serving on the Scientific Advisory Board of Immodulon Therapeutics Ltd. In the previous 12 months, Dr. Raison is director of Clinical and Translational Research for Usona Institute and serves as a consultant to Usona Institute, Emory Healthcare, Alkermes and Novartis. None of the investigators have a financial interest in the companies that manufacture the Heckel HT300 hyperthermia device used to demonstrate antidepressant effects of whole-body hyperthermia in MDD in previous studies. Drs. Hale, Lukkes, Paul, Smith, and Ms. Dady and Mr. Kelly reported no biomedical financial interests or potential conflicts of interest.

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