Progress in Neuropsychopharmacology & Biological Psychiatry 79 (2017) 162–168

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Whole-body hyperthermia and a subthreshold dose of citalopram act synergistically to induce antidepressant-like behavioral responses in adolescent rats

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ARTICLE INFO

Keywords:
Alternative
Antidepressant
Complementary
Integrative health care
Serotonin
Whole-body heating

ABSTRACT

Background: Open and randomized, double blind, placebo-controlled clinical trials have demonstrated clinical efficacy of infrared whole-body hyperthermia in treatment of major depressive disorder (MDD). Demonstration of antidepressant-like behavioral effects of whole-body hyperthermia in preclinical rodent models would provide further support for the clinical use of infrared whole-body hyperthermia for the treatment of MDD, and would provide additional opportunities to explore underlying mechanisms.

Methods: Adolescent male Wistar rats were habituated daily for 7 days to an incubator (23 °C, 15 min), then exposed, 24 h later, to an 85-min period of whole-body hyperthermia (37 °C) or control conditions (23 °C), with or without pretreatment with a subthreshold dose of the selective serotonin reuptake inhibitor, citalopram (5 mg/kg, s.c., 23 h, 5 h, and 1 h before behavioral testing in a 5-min forced swim test). Rectal temperature was monitored daily and immediately before and after the forced swim test to determine the relationship between body temperature and antidepressant-like behavioral responses.

Results: Whole-body hyperthermia and citalopram independently increased body temperature and acted synergistically to induce antidepressant-like behavioral responses, as measured by increased swimming and decreased immobility in the absence of any effect on climbing behaviors in the forced swim test, consistent with a serotonergic mechanism of action.

Conclusions: Preclinical data support use of infrared whole-body hyperthermia in the treatment of MDD.

1. Introduction

Major depressive disorder (MDD) is predicted to be the second leading cause of overall global disease burden by 2020 (Collins et al., 2011). Much of this burden derives from the fact that currently available pharmacologic interventions suffer from important shortcomings, including limited efficacy, delayed onset of action and significant side effects that impair quality of life and promote treatment non-adherence and/or discontinuation (Mathew et al., 2012; Papakostas and Fava, 2009; Li et al., 2012; Rush et al., 2006; Andrews

Abbreviations: CIT, citalopram; FST, forced swim test; MDD, major depressive disorder; Pre, pre-incubation; Post, post-incubation; s.c., subcutaneous; T_b, body temperature; T_{rec}, rectal temperature; TRPv4, transient receptor potential cation channel subfamily V member 4; Veh, vehicle (0.9% saline); WBH, whole-body hyperthermia

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http://dx.doi.org/10.1016/j.pnpbp.2017.06.006
Received 13 November 2016; Received in revised form 31 May 2017; Accepted 12 June 2017
Available online 12 June 2017
0278-5846/

et al., 2011; Lin et al., 1995; Freedman, 2010). These factors provide incentive to continue the search for novel antidepressant interventions that might maximize the benefits of currently-available modalities, while minimizing their limitations.

Recent open and randomized, double blind, placebo-controlled clinical trials have supported clinical efficacy of infrared whole-body hyperthermia in treatment of MDD (Hanusch et al., 2013; Janssen et al., 2016). Although a theoretical basis for antidepressant effects of whole-body hyperthermia, and potential mechanisms, have been proposed previously (Hale et al., 2013, 2012; Lowry et al., 2009; Raison et al., 2015), the potential of whole-body hyperthermia to induce antidepressant-like behavioral responses in preclinical models has not been tested. However, in previous studies using adolescent male rats, we have shown that exposure of rats to warm temperature (37 °C, relative to room temperature, 23 °C, control conditions), for 105 min, activates serotonergic neurons in the brainstem dorsal raphe nucleus (Hale et al., 2011). Of particular interest, whole-body heating increased c-Fos expression in serotonergic neurons within the interfascicular part of the dorsal raphe nucleus (DRI). Serotonergic neurons within the DRI are also activated during forced swimming in rats, suggesting that they may play a role in the behavioral responses during this task (Commons, 2008). Also of interest, DRI serotonergic neurons project to a distributed neural system implicated in the pathophysiology of MDD (Drevets, 1998; Steele et al., 2007), including the medial prefrontal cortex, dorsolateral prefrontal cortex, medial orbital cortex, inferior convexity, and anterior cingulate cortex, hippocampus, and mediodorsal thalamic nucleus (Lowry et al., 2009, 2008). In support of a potential role for thermosensitive DRI serotonergic neurons in the pathophysiology of MDD, and the actions of antidepressant drugs, we found that peripheral immune activation selectively induced c-Fos expression in DRI serotonergic neurons and increased serotonin concentrations and metabolism in the medial prefrontal cortex of mice (Lowry et al., 2007; Hollis et al., 2006). These effects on DRI serotonergic systems were associated with antidepressant-like behavioral effects in the forced swim test (Lowry et al., 2007). Together, these data suggest that thermosensitive DRI serotonergic neurons may play a role in the regulation of emotional behavior. Effects of whole-body heating on emotional behavior may involve activation of warm-sensitive afferent signaling pathways, including the spinoparabrachial (Nakamura and Morrison, 2010) or spinothalamic pathways (Raison et al., 2015; Hale et al., 2013).

In this experiment, we exposed rats to whole-body hyperthermia, either with or without treatment with a subthreshold dose of the selective serotonin reuptake inhibitor citalopram, and monitored body temperature and antidepressant-like behavioral responses in the forced swim test, a standard behavioral screen for antidepressants.

2. Methods and materials

2.1. Animals

As our previous studies showing that whole-body heating activates serotonergic neurons in the midbrain dorsal raphe nucleus were conducted using adolescent male rats (Hale et al., 2011), we used adolescent male rats in the present study. Adolescent male Wistar rats (N = 32; approximately 85 g, 28 days old, at the time of arrival, approximately 145 g, 35 days old at the time of behavioral 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], middle adolescence (periadolescent, pnd 34–46), and late adolescence (pnd 46–59) time periods (Spear, 2000); therefore, rats were tested during mid-adolescence. All 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 finding that whole-body hyperthermia increased c-Fos expression in a subset of serotonergic neurons that have been associated with antidepressant-like behavioral effects (Hale et al., 2011). Second, as the rats underwent the whole-body heating procedure individually (i.e., not in cages containing multiple conspecifics), it was important that they were habituated to individual housing. While is it is possible that these single housing conditions may have altered behavior in the FST (for review, see Bogdanova et al., 2013), all rats were exposed to the same housing conditions. Furthermore, previous research using longer periods of social isolation in male Sprague Dawley rats (Simpson et al., 2012) and male Wistar rats (Hall et al., 1998) have shown no difference in FST behavior compared with group-housed controls. 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 0500). All 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. Every effort has been made to conform to the proposed standards of animal research data presentation (Omary et al., 2016).

2.2. Drugs

Rats received three injections of either a subthreshold dose of citalopram hydrobromide (Sigma-Aldrich, Cat. No. C7861, St. Louis, MO, USA; 5.0 mg/kg in 0.9% sterile saline) or vehicle (0.9% sterile saline). Injections were made in a volume of 2.0 ml/kg via a subcutaneous (s.c.) route. A subthreshold dose of citalopram was given in order to maintain synaptic concentrations of serotonin, released during warm temperature exposure by warm-sensitive, serotonergic, cutaneous receptors in the periphery (Hale et al., 2013), even after placing the rats in 25 °C water for behavioral testing.

2.3. Apparatus

2.3.1. Acute whole-body heating

Exposure to acute whole-body heating (37 °C) or room temperature (23 °C) control conditions was conducted as previously described (Hale et al., 2011). Briefly, rats were transferred from their home cages 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) and placed into an incubation chamber (Binder APT.Line® BD E2 Incubator with natural convection, Tuttlingen, Germany).

2.3.2. Forced swim test

For the forced swim test (FST), rats were placed individually 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, as described previously (Drugan et al., 2013; Kelly et al., 2011). The FST was conducted during the light phase of the light: dark period, according to the protocol described by Detke et al. (1995), under standard animal facility house lighting conditions. Four rats were tested at a time, one from each treatment condition, in individual swim tanks that were separated by dividers to prevent visual contact between rats.

2.4. Experimental procedure

To avoid physiological responses to novelty, all rats were habituated to the incubation chamber 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 cages. In order to determine if exposure to whole-body heating could synergize with a subthreshold dose of citalopram to elicit

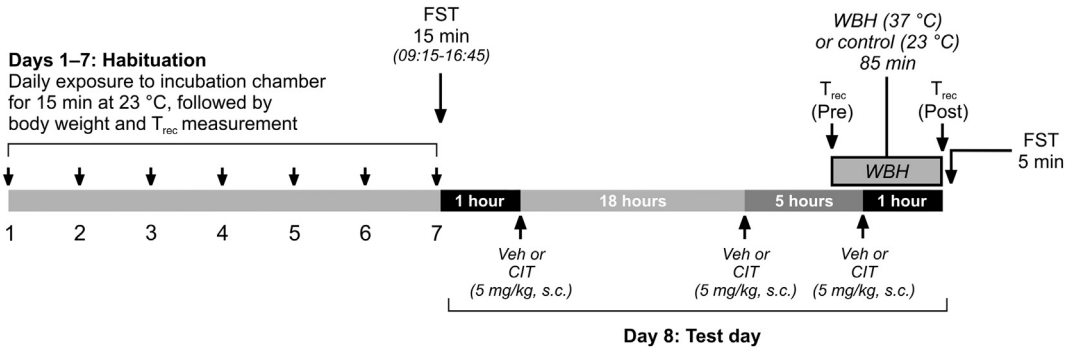

Fig. 1. Schematic illustration of the experimental timeline. Abbreviations: CIT, citalopram, 5 mg/kg, s.c.; FST, forced swim test; Pre, pre-incubation; Post, post-incubation; s.c., subcutaneous; T_{rec}, rectal temperature; Veh, vehicle (0.9% saline); WBH, whole-body heating.

antidepressant-like behavioral effects, rats received subchronic injections of citalopram or vehicle (5 mg/kg s.c., 23 h, 5 h, and 1 h prior to the onset of the 5-min FST, consistent with previously reported injection times and route of administration (Cryan et al., 2002; Detke et al., 1995; Reed et al., 2008) that have defined specific effects of SSRIs on swimming behaviors under these conditions), and exposed to acute whole-body heating (37 °C) or room temperature (23 °C) conditions for 85 min prior to the 5-min FST (for an illustration of the experimental timeline, see Fig. 1). Thus, this was a 2 (*vehicle*, *citalopram*) × 2 (23 °C, 37 °C) experimental design (n = 8 per group; N = 32).

Rectal temperature (T_{rec}) was measured daily immediately following the 15-min habituation sessions and, on the test day, immediately before and immediately after the 85-min exposure to acute whole-body hyperthermia or control conditions. Rectal temperature was measured using a digital thermometer (Ellab, Model No. DM852, Hilleroed, Denmark). Following lubrication with petroleum jelly, the temperature probe was inserted 3 cm beyond the rectal opening for approximately 30 s until a stable temperature was reached. Body weight was also measured on each of the habituation days (days 1–7), but not on the test day (day 8). On day 7, immediately following T_{rec} measurement, rats were exposed to the 15-min FST pre-test procedure (Cryan et al., 2005), then dried and returned to their home cages. The FST chambers were drained and cleaned between testing each rat. One hour following the onset of the 15-min pre-test, rats received injections of either vehicle or citalopram and were then returned to their home cages. The following day, 5 h 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 cages. Ninety-five min prior to the second FST, rats were transferred to the small cages and T_{rec} was recorded, in order to establish a baseline T_{rec}. 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, T_{rec} was measured, and rats were immediately exposed to the 5-min FST.

2.5. Behavioral testing and analysis

For the 5-min test on day 8, behavior 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. Behavior was scored according to Cryan et al. (2005). The behaviors selected for analysis were, 1) climbing, defined as upward movements of the forepaws usually directed toward the side of the swim chamber, 2) swimming behavior, 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 behavior, classified as an event and defined as when the rat's entire body was submerged (Detke et al., 1995). The percentage of time spent engaged in each behavior was scored by an investigator blind to treatment.

2.5.1. Statistical analysis

Data were analyzed using multifactor analysis of variance (ANOVA) with repeated measures (body weight, T_{rec}) or two-factor ANOVA (behavior). A Greenhouse-Geisser correction epsilon (ε) was used for repeated measures analysis to correct for potential violation of the sphericity assumption (Vasey and Thayer, 1987); this correction multiplies 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 a more conservative statistical test. Correlations between pre- or post-incubation T_{rec} and behavior 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 Tukey HSD comparisons. For the paired samples t-test comparisons of the pre- or post-incubation T_{rec}, the Holm's multistage method was used to test four hypotheses (i.e. pre- versus post-incubation T_{rec} within each treatment condition) that were ordered by the effect size (Cohen's d), in descending size, against error rates (α) ordered by the size of the adjustment, in ascending α (i.e., $\alpha/1$, $\alpha/2$, $\alpha/3$, and $\alpha/4$; (Holm, 1979). Statistical analyses were conducted using SPSS software (Version 22 for Windows, International Business Machines Corp. (IBM), Armonk, New York, USA). Statistical outliers were identified using Grubbs' test (Grubbs, 1969) and excluded (body weight, 0.4%; T_{rec} habituation period, 1.3%; T_{rec}, 1.6%; swimming behavior, 1.6%; immobility, 1.6%; climbing, 0%). For the repeated measures analyses of variance, replacement values were calculated using the Petersen method (Petersen, 1985). Replacement values were not included in post hoc tests or in any graphical representations of the data.

3. Results

3.1. Body weight

All groups gained weight over the 7-day habituation period (Fig. 2A). There were no baseline differences in body weight among the treatment groups prior to the beginning of the drug administration and FST procedure.

3.2. Rectal temperature during habituation

There were no baseline differences in T_{rec} among the treatment groups prior to the beginning of the drug administration and FST procedure (Fig. 2B).

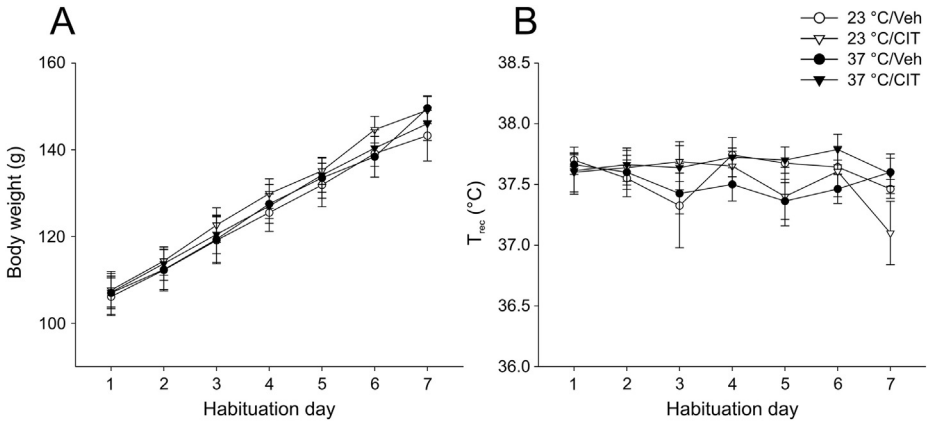

Fig. 2. Body weight gain and post-incubation rectal temperature during the habituation phase of the experiment (days 1–7). (A) Mean body weight (± SEM) across the seven day habituation procedure in each treatment condition (23 °C/Veh, n = 8; 23 °C/CIT, n = 8; 37 °C/Veh, n = 7 (Day 7)-8; 37 °C/CIT, n = 8). Body weight was measured immediately after the 15-min, 23 °C incubation chamber exposure. (B) Mean rectal temperature (T_{rec}; ± SEM) across the seven day habituation procedure in each treatment condition (23 °C/Veh, n = 7 (Day 4,6)-8; 23 °C/CIT, n = 8; 37 °C/Veh, n = 7 (Day 7)-8; 37 °C/CIT, n = 8). T_{rec} was measured following the weighing procedure.

3.3. Rectal temperature pre- and post-incubation

Citalopram treatment and acute whole-body hyperthermia for 85 min both had time-dependent effects to increase T_{rec} (whole-body hyperthermia × time interaction, $F_{(1,28)} = 7.995$, $p < 0.01$; $\varepsilon = 1.0$; citalopram × time interaction, $F_{(1,28)} = 16.681$, $p < 0.001$; $\varepsilon = 1.0$; Fig. 3A). There were no differences in T_{rec} among treatment groups at the pre-incubation time point. Citalopram increased T_{rec} in rats exposed to either 23 °C or 37 °C ambient temperature conditions compared to baseline T_{rec}. Post-incubation, citalopram increased T_{rec} compared with vehicle in rats exposed to either 23 °C or 37 °C conditions. Exposure to 37 °C, relative to 23 °C, increased T_{rec} in both citalopram- and vehicle-

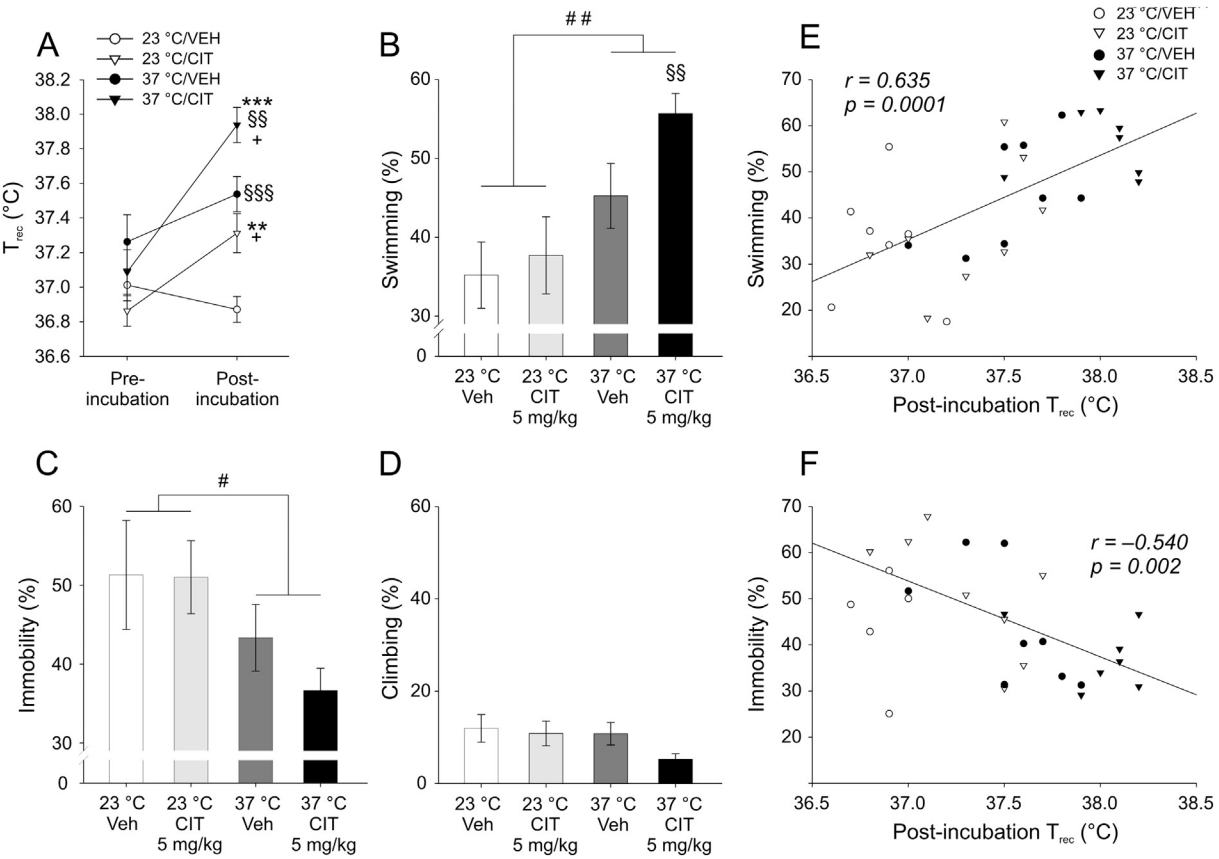

Fig. 3. Acute whole-body hyperthermia for 85 min and a subthreshold dose of the selective serotonin reuptake inhibitor citalopram (CIT; 5.0 mg/kg s.c., 23 h, 5 h, and 1 h before behavioral testing) increase rectal temperature (T_{rec}), while whole-body hyperthermia induced antidepressant-like behavioral responses in the forced swim test (FST). (A) Graph showing T_{rec} measured immediately prior to exposure to the incubation chamber (pre-incubation) and again immediately after exposure to the incubation chamber (post-incubation), immediately prior to exposure to the 5-min FST on day 8 (23 °C/Vehicle (Veh), n = 7–8; 23 °C/CIT, n = 8; 37 °C/Veh, n = 8; 37 °C/CIT, n = 8). §§$p < 0.01$; §§§$p < 0.001$ versus corresponding 23 °C control group, post hoc Tukey HSD tests. **$p < 0.01$; ***$p < 0.001$ versus corresponding pre-incubation time point, paired samples t-tests with Holm-Bonferroni adjusted error rates. +$p < 0.05$ versus corresponding Veh control group, post hoc Tukey HSD tests. (B) Bar graph illustrating the percent time spent swimming in the 5-min FST. Acute whole-body hyperthermia increased swimming behavior in the FST (23 °C/Veh, n = 8; 23 °C/CIT, n = 8; 37 °C/Veh, n = 8; 37 °C/CIT, n = 7). ##$p < 0.01$, main effect of whole-body hyperthermia, 2 × 2 univariate ANOVA, §§$p < 0.01$ vs 23 °C/CIT, post hoc Tukey HSD test (C) Bar graph illustrating the percent time spent immobile in the 5-min FST. Acute whole-body hyperthermia decreased immobility in the FST. #$p < 0.05$, main effect of whole body hyperthermia, 2 × 2 univariate ANOVA (23 °C/Veh, n = 8; 23 °C/CIT, n = 8; 37 °C/Veh, n = 8; 37 °C/CIT, n = 7). (D) Bar graph illustrating the percent time spent climbing in the 5-min FST. Acute whole-body hyperthermia in combination with CIT did not alter climbing in the FST (23 °C/Veh, n = 8; 23 °C/CIT, n = 8; 37 °C/Veh, n = 8; 37 °C/CIT, n = 8). (E) Scatter plot illustrating the correlation between the post-incubation T_{rec} and the percent time spent swimming during the 5-min forced swim test (FST). (F) Scatter plot illustrating the correlation between the post-incubation T_{rec} and the percent time spent immobile during the 5-min forced swim test (FST).

treated rats. Thus, both whole-body hyperthermia and citalopram increased T_{rec} and these effects was additive.

3.4. Forced swim test behavior

Acute whole-body hyperthermia had antidepressant-like behavioral effects in the FST. Whole-body hyperthermia had a main effect to increase the percent time spent swimming ($F_{(1,27)} = 11.56$, $p < 0.01$; Fig. 3B). Post hoc tests revealed that, among rats treated with citalopram, rats exposed to whole-body hyperthermia showed increased swimming behavior compared with rats exposed to 23 °C control conditions. There was a main effect for whole-body hyperthermia to decrease the percent time spent immobile ($F_{(1,27)} = 4.993$, $p < 0.05$; Fig. 3C). Post hoc tests revealed that, among citalopram-treated rats, whole-body hyperthermia decreased immobility. Neither citalopram treatment nor whole-body hyperthermia altered climbing behavior in the FST (Fig. 3D).

3.5. Correlations

Body temperature was correlated with antidepressant-like behavioral responses in the FST. Post-incubation T_{rec} was positively correlated with the percent time spent swimming ($r = 0.635$, $p < 0.001$; Fig. 3E) and negatively correlated with immobility ($r = -0.540$, $p < 0.01$; Fig. 3F).

4. Discussion

Whole-body hyperthermia potentiates the effects of treatment with a subthreshold dose of citalopram on antidepressant-like behavioral effects in rats. These behavioral effects of whole-body hyperthermia and a subthreshold dose of citalopram were positively correlated with increases in body temperature; although whole-body hyperthermia and citalopram independently increased body temperature, the effects of whole-body hyperthermia and citalopram were additive such that the highest temperatures were observed in rats subjected to combined treatment. Body temperature post-incubation, immediately prior to behavioral testing was positively correlated with the percent time spent swimming and negatively correlated with the percent time spent immobile in the FST.

Whole-body hyperthermia had antidepressant-like behavioral effects in rats, as measured, among citalopram-treated rats, by an increase in swimming behavior and a decrease in immobility in the FST. These effects were dependent on concurrent treatment with a subthreshold dose of citalopram. In other words, whole-body hyperthermia increased the percent time spent swimming in the FST, but only in rats pre-treated with citalopram, given at a dose that had no effect by itself. In contrast, whole-body hyperthermia had no effect on climbing behavior in the FST. A selective effect of whole-body hyperthermia on swimming, with no effect on climbing, is consistent with the hypothesis that the whole-body hyperthermia effect is mediated by serotonin, based on previous studies showing that 1) the same whole-body hyperthermia procedure activates brainstem serotonergic neurons in rats (Hale et al., 2011), 2) selective serotonin reuptake inhibitors increase swimming, but not climbing (Detke et al., 1995), and 3) noradrenergic reuptake inhibitors increase climbing, but not swimming (Detke et al., 1995). Whole-body heating was effective at increasing body temperature in both vehicle- and citalopram-treated rats, relative to baseline. Interestingly, citalopram increased body temperature from the pre-incubation to post-incubation time point in rats exposed to 23 °C. At the post-incubation time point, citalopram, relative to vehicle, increased body temperature under both 23 °C and 37 °C conditions, and the highest T_{rec} was observed in rats that both were treated with citalopram and exposed to 37 °C, relative to all other conditions. The close association between body temperature and antidepressant-like behavioral responses was evident from correlational analysis, in that the post-incubation T_{rec} (measured immediately before behavioral testing) was positively correlated with percent time spent swimming in the FST, and negatively correlated with time spent immobile. Together, these data suggest that thermoregulatory function and antidepressant-like behavioral responses are intimately linked.

The mechanisms underlying the behavioral effects of whole-body hyperthermia are not clear, but may involve effects on afferent signaling pathways originating in the periphery. Serotonin transporter is expressed not only in the brain but also in peripheral tissues, including the cutaneous membrane (Nordlind et al., 2008). Low dose citalopram is likely to have a high occupancy of serotonin transporter binding sites at the site of injection and would be predicted to facilitate peripheral serotonergic signaling. It is thought that some peripheral sensory cells are serotonergic and also temperature-sensitive. For example, Merkel cells, which are associated with the sense of light touch, necessary for discrimination of shapes and textures, are serotonergic (Nordlind et al., 2008; Slominski et al., 2005; Maksimovic et al., 2013). These sensory receptors express tryptophan hydroxylase, the rate-limiting enzyme in the biosynthesis of serotonin, and also express the serotonin transporter (Nordlind et al., 2008). Furthermore, Merkel cells are believed to express the thermosensitive ion channel, TRPv4 (Liedtke et al., 2000), suggesting that these sensory receptors transduce both mechanosensory and thermal signals. Consequently, as proposed previously (Hale et al., 2013; Lowry et al., 2009; Raison et al., 2015), exposure to warm temperature would be predicted to induce synaptic serotonin release at sensory synapses, which in turn would be predicted to increase signaling via the spinoparabrachial and spinothalamic sensory pathways (Hale et al., 2013; Lowry et al., 2009; Raison et al., 2015; Nakamura and Morrison, 2010). Activation of these warm sensitive signaling pathways in turn has been associated with positive affective responses, presumably through activation of cortical affective circuits including the pregenual anterior cingulate cortex, dorsal anterior cingulate cortex, and medial orbitofrontal cortex (Raison et al., 2010; Rolls et al., 2008). Cutaneous heating in humans is associated with activation of the pregenual cingulate cortex, mid-orbitofrontal cortex, ventral striatum, and insular cortex; activity within the pregenual anterior cingulate cortex, mid-orbitofrontal cortex, and ventral striatum is positively correlated with positive affective ratings while activation of the insular cortex is related to ratings of the intensity of the stimulus, but not the affective rating (Rolls et al., 2008). This pattern of activation suggests that the signaling of positive affective signals by whole-body hyperthermia may be relayed by the medial spinothalamic pathway, synapsing in the mediodorsal thalamus and relaying signals to the anterior cingulate cortex (Craig, 2015), which in turn projects to the medial orbitofrontal cortex (Cavada et al., 2000; Morecraft and Van Hoesen, 1993). Together, these structures are recognized as a distributed hedonic circuit in the brain (Pecina et al., 2006). Recent studies demonstrate that the functional connectivity of the medial orbitofrontal cortex is decreased in major depressive disorder (Cheng et al., 2016), and, therefore, whole-body hyperthermia may mediate its antidepressant effects through increased functional connectivity of medial orbitofrontal cortex.

Concurrent treatment with citalopram and warm temperature would be predicted to enhance signaling to these thermosensitive, affective circuits. Importantly, in the presence of a selective serotonin reuptake inhibitor, serotonin-dependent signaling by thermal afferents would be maintained during exposure to the cold temperature of the FST (25 °C), despite rapid decreases in skin and body temperature, due to prior warm temperature-induced serotonin release, and citalopram-dependent blockade of serotonin reuptake. Although there are many alternative hypotheses that could explain the effects of warm temperature and citalopram observed in our studies, enhancement of afferent thermal signaling provides one clear hypothetical framework that can be tested in future studies.

The behavioral effects of whole-body hyperthermia in the presence of a subthreshold dose of citalopram were positively correlated with

increases in body temperature; although whole-body hyperthermia and citalopram independently increased body temperature, the effects of whole-body hyperthermia and citalopram were additive such that the highest temperatures were observed in rats subjected to both treatments. The mechanisms underlying citalopram-induced increases in core body temperature are not clear. Serotonergic systems do play a role in stress-induced thermogenesis, as demonstrated in stress-induced hyperthermia models (Vinkers et al., 2016). In addition, serotonergic systems are thought to play a role in stress-induced thermogenesis at the level of the raphe pallidus, a serotonergic medullary sympathetic control system (Zaretsky et al., 2003; Ray et al., 2011; Lkhagvasuren et al., 2011), and at the level of the spinal cord, where serotonergic systems promote brown fat thermogenesis, and cutaneous vasoconstriction (Beig et al., 2009; Ootsuka et al., 2004; Madden and Morrison, 2010).

Interestingly, we have previously shown that, like exposure to whole body hyperthermia, exposure to swim stress in 19 °C water, which induces hypothermia, also increases c-Fos expression in serotonergic neurons in the DRI (Kelly et al., 2011). We have argued that activation of DRI serotonergic neurons may be a key feature of antidepressant action (Hale and Lowry, 2011; Hale et al., 2013, 2012; Raison et al., 2015), and therefore, both hyperthermia and hypothermia may activate afferent signals that have antidepressant effects, via activation of thermosensitive afferent spinoparabrachial or spinothalamic pathways and relevant central nervous system circuits. Additional research is necessary to fully explore the relationship between thermosensation/thermoregulation and antidepressant-like responses.

We have previously reported that whole-body hyperthermia increases c-Fos expression in a subset of serotonergic neurons that have been associated with antidepressant-like behavioral effects (Hale et al., 2011). As this observation was made in adolescent male Wistar rats, we used similarly aged rats in the present study. However, it is possible that the synergistic effects of whole-body hyperthermia and subthreshold antidepressant treatment may differ between young and older rats. While additional research is required to conclusively address this question, previous research using escitalopram, the more potent *S*-(+)-enantiomer of citalopram (Sanchez et al., 2003), has demonstrated that 21-day-old rats showed dose-dependent changes in antidepressant behavioral effects that are similar to those seen in adult rats (Reed et al., 2008). It is also possible that the increased swimming behavior, and decreased immobility, observed in rats exposed to whole-body heating and citalopram represents an effect on hyperlocomotion rather than an antidepressant-like effect *per se*. Few studies have examined hyperlocomotive effects of citalopram in rats; however, in mice, citalopram has been observed to increase locomotor activity in a dose-dependent manner (Brocco et al., 2002). Importantly, this citalopram-induced hyperlocomotion effect was only observed when mice were placed into a novel environment; when the mice were pre-exposed to the test environment, citalopram had no effect to increase locomotor activity (Brocco et al., 2002). In this study, rats were pre-exposed to the forced swim test 24 h prior to the whole-body heating and citalopram treatment; therefore, it is less likely that the increased swimming noted in this study is a drug-induced hyperlocomotive effect. However, additional research using tests of locomotor activity, such as the open-field test, may provide insight into any potential effects of citalopram and whole-body hyperthermia on non-specific locomotor activation. Furthermore, additional research using other antidepressant compounds, or other tests of antidepressant-like behavioral effects, for example, the sucrose preference test, may provide additional insight into the effects of whole-body heating on emotional behavior.

5. Conclusions

These data provide further justification for clinical trials using infrared whole-body hyperthermia in the treatment of MDD, or other conditions that are clinically responsive to selective serotonin reuptake inhibitors, such as posttraumatic stress disorder.

Author contributions

M.W.H., C.L.R., and C.A.L. designed research; M.W.H., J.L.L., K.F.D., K.J.K., and E.D.P. performed research; M.W.H. and D.G.S. analyzed data; and M.W.H. and C.A.L. wrote the paper; J.L.L., E.D.P., and C.L.R. provided critical evaluation of the manuscript.

Role of the funding source

This work was supported by the National Science Foundation (NSF CAREER Award; NSF-IOS #0845550) to CAL. Dr. Christopher A. Lowry is currently supported by the Department of the Navy, Office of Naval Research Multidisciplinary University Research Initiative (MURI) Award (grant number N00014-15-1-2809), National Institutes of Health (grant number R01 DA019921), Department of Veterans Affairs Office of Research and Development (grant number VA-ORD; 1 I21 RX002232-01), Colorado Clinical and Translational Sciences Institute (CCTSI) Center for Neuroscience (grant number CNSTT-15-145), the Colorado Department of Public Health and Environment (CDPHE; grant number DCEED-3510), and the Alfred P. Sloan Foundation (grant number G-2015-14165). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or other funding agencies.

Declaration of interest

Dr. Lowry reports serving on the Scientific Advisory Board of Immodulon Therapeutics Ltd. In the previous 12 months, Dr. Raison served on the speakers' bureau for Merck. 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.

Acknowledgments

We gratefully acknowledge the technical support of the Office of Animal Research technical staff at the University of Colorado Boulder.

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