Neuroscience 253 (2013) 221–234

PRIOR COLD WATER SWIM STRESS ALTERS IMMOBILITY IN THE FORCED SWIM TEST AND ASSOCIATED ACTIVATION OF SEROTONERGIC NEURONS IN THE RAT DORSAL RAPHE NUCLEUS

R. C. DRUGAN,[a] P. T. HIBL,[b] K. J. KELLY,[b] K. F. DADY,[b] M. W. HALE[c] AND C. A. LOWRY[b]*

[a] *Department of Psychology, University of New Hampshire, Durham, NH 03824-3567, USA*

[b] *Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO 80309-0354, USA*

[c] *School of Psychological Science, La Trobe University, Melbourne, Victoria 3086, Australia*

Abstract—Prior adverse experience alters behavioral responses to subsequent stressors. For example, exposure to a brief swim increases immobility in a subsequent swim test 24 h later. In order to determine if qualitative differences (e.g. 19 °C versus 25 °C) in an initial stressor (15-min swim) impact behavioral, physiological, and associated neural responses in a 5-min, 25 °C swim test 24 h later, rats were surgically implanted with biotelemetry devices 1 week prior to experimentation then randomly assigned to one of six conditions (*Day 1* (15 min)/*Day 2* (5 min)): (1) home cage (HC)/HC, (2) HC/25 °C swim, (3) 19 °C swim/HC, (4) 19 °C swim/25 °C swim, (5) 25 °C swim/HC, (6) 25 °C swim/25 °C swim. Core body temperature (T_b) was measured on *Days 1* and *2* using biotelemetry; behavior was measured on *Day 2*. Rats were transcardially perfused with fixative 2 h following the onset of the swim on *Day 2* for analysis of c-Fos expression in midbrain serotonergic neurons. Cold water (19 °C) swim on *Day 1* reduced T_b, compared to both 25 °C swim and HC groups on *Day 1*, and, relative to rats exposed to HC conditions on *Day 1*, reduced the hypothermic response to the 25 °C swim on *Day 2*. The 19 °C swim on *Day 1*, relative to HC exposure on *Day 1*, increased immobility during the 5-min swim on *Day 2*. Also, 19 °C swim, relative to HC conditions, on *Day 1* reduced swim (25 °C)-induced increases in c-Fos expression in serotonergic neurons within the dorsal and interfascicular parts of the dorsal raphe nucleus. These results suggest that exposure to a 5-min 19 °C cold water swim, but not exposure to a 5-min 25 °C swim alters physiological, behavioral and serotonergic responses to a subsequent stressor. Published by Elsevier Ltd. on behalf of IBRO.

Key words: swim stress, forced swim test, core body temperature, depression, serotonin, raphe.

INTRODUCTION

The stress–diathesis model of depression proposes that vulnerability to depression is a combination of genetic vulnerability and an additional trigger by an environmental event, such as stress, to unveil depressive symptomology (Caspi et al., 2003; Munafo et al., 2009). Several animal models of depression involve stress exposure that results in observable behavioral signs, including shifts from proactive to more reactive emotional coping styles, which have been proposed to represent behavioral depression. These models include inescapable shock/learned helplessness (Weiss et al., 1981; Henn et al., 1993; Vollmayr and Henn, 2001; Maier and Watkins, 2005), forced swimming (Porsolt et al., 1977; Detke et al., 1995; Weiss et al., 1998; Christianson and Drugan, 2005) chronic social stress (Rygula et al., 2006), and resident/intruder defeat (Krishnan et al., 2007; Paul et al., 2011).

The serotonergic system has been shown to be critically involved in the majority of the stress-induced behavioral responses in the models mentioned above (Detke et al., 1995; Maier and Watkins, 2005; Rygula et al., 2006) although certain models indicate significant noradrenergic involvement as well (Weiss et al., 1981; Christianson et al., 2008; Drugan et al., 2010). The full spectrum of stress reactivity is important to consider, such as proactive versus reactive emotional coping behavior, which is thought to be controlled in part by serotonergic systems (Chung et al., 1999, 2000; Koolhaas et al., 2007). For example, male mice that respond with high aggression in response to an intruder (proactive coping) respond with active swimming and climbing in the forced swim test (FST), whereas non-aggressive males with low aggression in response to an intruder (reactive coping) respond with predominantly immobility (Veenema et al., 2005). Mice with a proactive emotional coping style have higher 5-HT_{1A} receptor expression and binding capacity in forebrain limbic structures (Korte et al., 1996), and a higher sensitivity of postsynaptic 5-HT_{1A} receptors (van der Vegt et al.,

*Corresponding author. Tel: +1-303-492-6029; fax: +1-303-492-0811.
E-mail address: christopher.lowry@colorado.edu (C. A. Lowry).

Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; ANOVA, analysis of variance; dH_2O, distilled H_2O; DR, dorsal raphe nucleus; DRD, dorsal raphe nucleus, dorsal part; DRI, dorsal raphe nucleus, interfascicular part; DRVL/VLPAG, dorsal raphe nucleus, ventrolateral part/ventrolateral periaqueductal gray; FST, forced swim test; HC, home cage; H_2O_2, hydrogen peroxide; ir, immunoreactive; LSD, least significant difference; NaN_3, sodium azide; PBS, phosphate-buffered saline; PBST, PBS containing 0.1% Triton X-100; SS, swim stress; T_b, core body temperature (°C); Tph, tryptophan hydroxylase.

0306-4522/13 $36.00 Published by Elsevier Ltd. on behalf of IBRO.
http://dx.doi.org/10.1016/j.neuroscience.2013.08.038

2001). Activation of 5-HT_{1A} receptors in these mice with a proactive emotional coping style induces a shift toward a more reactive emotional coping style (Veenema et al., 2005). Although the anatomical origin of protective responses to aversive stimuli (e.g. active behavioral coping, behavioral immunization), may originate in areas of the brain such as the prefrontal cortex (Amat et al., 2006), their ultimate protection against subsequent stress effects has to do with their modulatory effect on midbrain serotonergic systems (Greenwood et al., 2003; Amat et al., 2006, 2010). Therefore, evaluation of midbrain serotonergic systems is important because they are important in all aspects of these models including stress, coping, and behavioral depression.

Continuous swim stress (SS) has been shown to recruit serotonergic activity in brainstem and hippocampal regions during an initial 15-min continuous swim in a water temperature and time-dependent manner (Linthorst et al., 2008; Kelly et al., 2011). For example, in the hippocampus, 25 °C or 35 °C swim results in an immediate elevation of extracellular serotonin (5-hydroxytryptamine; 5-HT), while a 19 °C swim results in a delayed increase of hippocampal 5-HT 1 h later (Linthorst et al., 2008). Prior research has shown that serotonergic neurons in the dorsal raphe nucleus (DR) are thermosensitive (Hale et al., 2011a). Indeed, Kelly et al. (2011) have reported that cold swim, relative to swimming at a warmer temperature, increases numbers of double immunostained c-Fos/tryptophan hydroxylase (Tph) neurons in subregions of the DR. Acute cold swim, relative to swim at higher temperatures, increased c-Fos expression in serotonergic neurons within the ventrolateral part of the dorsal raphe nucleus/ventrolateral periaqueductal gray (DRVL/VLPAG) as well as the interfascicular part of the dorsal raphe nucleus (DRI), subregions of the DR that are thought to be involved in thermoregulatory processes and stress-related behavioral coping mechanisms (Hale et al., 2011a; Lowry et al., 2007). Serotonergic neurons within the DRI project to the hippocampus (Pierce et al., 1976; Köhler and Steinbusch, 1982; McKenna and Vertes, 2001), and therefore this subset of serotonergic neurons may account for the effects of temperature on hippocampal serotonin release observed by Linthorst and colleagues (Linthorst et al., 2008). The midbrain 5-HT system appears to be implicated in the psychological (exteroceptive) stress – as well as the physiological (interoceptive) stress-induced alterations associated with forced swim in cold water. However, as noted by Kelly et al. (2011), since widespread activation in the DR was observed independent of water temperature, these DR serotonergic neurons are likely to be responding to both the psychological stress of forced swimming as well as the physiological stress of hypothermia.

The above findings provide direct support for the role of 5-HT in several brain regions that mediate the responses to either hyperthermia or hypothermia. However, no work to date has evaluated the impact of prior exposure to differing water temperatures on the subsequent reactivity of midbrain serotonergic systems to a subsequent forced swim exposure. This reactivity may be very different from the studies reported above, because now all groups of subjects are experiencing the same water temperature in the subsequent acute and brief (5 min) forced swim. The test situation is therefore identical for all subjects and would allow determination of the impact of prior swim exposure at cold or warm temperatures on this subsequent identical swim stress challenge for all groups.

Few studies have examined the impact of different temperatures in the swim stress-induced behavioral depression and serotonergic correlates in the FST (for exceptions see Linthorst et al., 2008; Kelly et al., 2011). Since forced swimming involves both psychological (anxiety, panic) and physiological (hypothermia) stress (Stone, 1970a,b; Abel, 1993), we sought to examine the behavioral, physiological, and cellular effects of different water temperatures on *Day 1* swim stress by measuring behavior, T_b, and c-Fos/Tph double immunostaining in response to a second swim exposure (25 °C water, 5 min) 24 h later.

EXPERIMENTAL PROCEDURES

Animals

Adult male Wistar rats (HSD-WI, Harlan Labs, Indianapolis, IN, USA; 250–275 g) were used throughout the course of the experiment. Eighty experimental rats were pair housed in transparent polycarbonate cages (26 cm W × 47.6 cm L × 20.3 cm H; Cat. No. RC88D-PC, Alternative Designs, Siloam Springs, AR, USA) using standard cage bedding (Teklad Laboratory Grade Aspen Bedding, Harlan, Madison, WI, USA). Both food (Cat No. 8640, Teklad22/5 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, with lights on at 0700 h. Rats were allowed to acclimate to housing conditions for 5 days prior to any experimental procedures. All experiments were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*, Eighth Edition (Institute for Laboratory Animal Research, The National Academies Press, Washington, DC, 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.

Surgical procedures

Telemetric transmitters were implanted into the peritoneal cavity for measurement of T_b in freely moving animals. Surgery was performed under inhaled isoflurane anesthesia (5% initial and 2% maintenance). Briefly, following anesthesia the rat was placed onto its back, the rat's 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