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Impact of water temperature and stressor controllability on swim stress-induced changes in body temperature, serum corticosterone, and immobility in rats

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Abstract

The present study compared the effects of three different water temperatures (20, 25, and 30 °C) and stressor controllability on several physiological and behavioral endpoints in an intermittent swim stress paradigm. The escape latency of rats in the 20 and 25 °C water was less than that observed for the 30 °C group. Both escape and yoked groups at 20 and 25 °C exhibited moderate to severe hypothermia following the swim stress session that returned to prestress levels 30–40 min post-stress. At 30 °C core body temperature (T_b) only decreased by 1 °C for either swim group. Following swim, serum corticosterone (CORT) levels were significantly elevated in both escape and yoked groups in comparison to confined and home cage controls. The confined control group showed a significant elevation that was approximately halfway between the home cage control and the swim stress groups. At 30 °C, there was still a significant elevation of serum CORT in both swim groups in comparison to confined and home cage controls. Therefore, 30 °C appears to be the optimal water temperature to evaluate stress controllability effects in the current paradigm. In a final experiment, swim stressor controllability effects were examined in a 5 min forced swim test (FST) 24 h following the initial stress exposure. Rats exposed to yoked-inescapable swim stress at 30 °C exhibited more immobility than their escapable swim stress and confined counterparts, while the escape and confined controls did not differ. These results demonstrate that the behavioral deficits observed in the FST are attributable to the stress of inescapable swim and not swim stress per se.

Keywords: Body temperature; Controllability; Corticosterone; Hypothermia; Stress; Immobility

1. Introduction

We recently reported a new intermittent swim stress (ISS) paradigm designed to test the generality of intermittent, inescapable shock stress-induced deficits in behavioral and physiological functioning in the rat (Brown et al., 2001). The results from this initial report indicate some similarities, but predominantly differential behavioral and physiological responses when compared to the traditional tailshock stress model. For example, 24 h following the ISS, a small, but significant shuttlebox escape deficit was observed in yoked versus escape groups. However, contrary to the shock stress model, no stress-induced analgesia was observed immediately following the ISS session (Brown et al., 2001; Drugan et al., 1985). In addition, the yoked rats in the ISS paradigm exhibit a reduction in alcohol-induced motor incoordination in comparison to the enhanced motor ataxia observed following intermittent shock stress (Brown et al., 2001; Drugan et al., 1996).

Shock stress induces significant hyperthermia (Deak et al., 1997) while forced swim stress results in marked hypothermia (Arai et al., 2000; Porsolt et al., 1979), therefore, a direct comparison of these 2 paradigms is problematic. Rather than comparing our procedure to the shock model, we sought to create a stress paradigm using swim stress that represents a hybrid employing the strengths of the shock and continuous forced swim models of depression. The water temperature (T_w) used in the initial Brown et al. (2001) study was 23 °C,

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however, other reports in the literature demonstrate that even brief continuous ambient water swims induced significant hypothermia and elevations in CORT (Abel, 1993; Arai et al., 2000; Taltavull et al., 2003). The current study evaluated the role of T_w and stressor controllability on serum CORT, T_b, and behavioral despair. The goal was to identify the optimal procedure for ISS that minimizes such extraneous variables as hypothermia so that stressor controllability is more effectively evaluated on physiological and behavioral endpoints.

Basal and stress-induced changes in serum corticosterone (CORT) were evaluated over time. Blood levels of CORT are considered by many researchers to be the endocrine marker of stress (Maier et al., 1986; Mormede et al., 1988; Prince and Anisman, 1990; Swenson and Vogel, 1983). Basal and stress-induced changes in T_b were monitored so as to evaluate the severity of hypothermia across the different water temperatures. Once a T_w was identified that minimized the stress-induced hypothermia, a final experiment examined the effects of swim stress controllability on subsequent behaviors in the FST.

2. Methods

2.1. Subjects

Male Sprague Dawley rats acquired from Animal Resources Centre (Canning Vale, WA, Australia) weighing 180–200 g upon arrival served as subjects for the T_b and CORT studies. Rats were individually housed in plastic basin cages and allowed free access to food and water. The vivarium was maintained at 22±1 °C on a normal 12 h light/dark cycle (lights on at 0800). All behavioral procedures were conducted during 0800–1200 hours.

Male Sprague Dawley rats acquired from Charles River Laboratories (Stoneridge, NY) weighing 180–200 g upon arrival served as subjects for the FST. Rats were group housed 4/cage in Polyethylene tub cages and had identical food, water, and lighting conditions as described above. All behavioral and surgical procedures were reviewed and approved by the La Trobe University Animal Ethics Committee and the University of New Hampshire Institutional Animal Care and Use Committee.

2.2. Surgical procedure

For the rats involved in the assessment of T_b, surgical implantation of electronic devices in the abdominal cavity occurred 5 to 7 days after arrival in the vivarium and at least one week prior to experimentation. Rats received an intraperitoneal (i.p.) injection of anesthetic (61 mg/kg ketamine and 9 mg/kg xylazine) and biotelemetry devices (E-4000, Minimitter, Bend, OR) were placed into the peritoneal cavity. This procedure involved making a small incision (approximately 1 cm) in both the skin and peritoneal muscle wall. The site was first shaved then wiped with 70% ethanol. Following insertion of the device the incision in the muscle was closed with absorbable suture (Ethicon PDS), and a non-absorbable suture material (Supramid) was used to close the skin incision. Carprofen (9 mg/kg) was injected subcutaneously, and the incision was swabbed with betadine and Emla cream (Lidocaine). Immediately following surgery, rats were placed in individual cages on heating pads to maintain the T_b at 37 °C and were monitored until they regained consciousness (i.e., regain righting reflex) and placed back in the vivarium for a 1 week recovery period. The rats in the final experiment evaluating forced swim behaviors were not implanted with biotelemetry devices nor did they have blood drawn for CORT measures.

2.3. Stress procedure

The stress paradigm employed in the present study was identical to that described by Brown et al. (2001). Briefly, the escape/yoked/confined swim device consists of three Plexiglas cylinders (see Brown et al., 2001, for details). The shorter cylinder, housing the confined control, has a Plexiglas lid with several holes drilled in it to allow for air flow. All three cylinders are attached to a motor pulley system and suspended above a black rectangular basin filled to a height of 32 cm with 20, 25 or 30±1 °C water.

The cylinders can be lowered and raised out of the water simultaneously. Because the middle cylinder (confined control; CC) is shorter than the other two, it never enters the water. In the cylinder of the escape rat is an omnidirectional lever (Med Associates, Georgia, VT) coated with a thin film of petroleum jelly. The petroleum jelly prevents the rat from climbing out of the chamber via the lever. The movement of the lever in any direction by the escape rat activates a computer program and motor that lifts the two cylinders out of the water. The yoked rat did not have an omnidirectional lever in the chamber. Above each of the cylinders is a space heater that blows warm (36 °C at a distance of 6 in.) air into the large cylinders and unheated air into the small cylinder during the inter-trial interval (ITI) for both the 20 and 25 °C T_w studies. This continuous flow of warm air during the experiment is used to minimize the loss of T_b for the two swim stressed subjects, while the unheated air over the small cylinder controls for forced air exposure. Since the 30 °C swim was thought not to cause a significant reduction in T_b, unheated air was blown on all 3 groups of rats in this experimental condition. This controlled for the forced air exposure experienced at the other temperatures, but minimized the increase in ambient temperature and consequent heat stress for the CC rat. The water in the basin was changed after each group of animals and the basin was cleaned with antibacterial spray.

On the day of experimentation, rats were randomly assigned to one of four groups: escape (ESC), yoked (YOK), confined control (CC) and home cage control (HCC). Rats were then weighed and either placed into the swim stress apparatus (ESC, YOK, and CC) or returned to their home cage (HCC). The ESC and YOK rats were placed in the large cylinders, while the CC group was placed in the shorter cylinder. Fig. 1 provides an illustration of the swim stress controllability device.

Fig. 1. A picture of the swim stress controllability apparatus. There is an escape rat (left cylinder) that can terminate the forced swim by pressing an omnidirectional lever, a yoked-inescapable stress rat (right cylinder) that has no control over the duration of the forced swim and a confined rat (middle cylinder). The confined rat remains in the apparatus for the same period of time as the 2 swim stressed rats, is exposed to the same handling and movement, but is not exposed to the water. A fourth rat remains in the vivarium (home cage) during the stress session.

Rats were then exposed to 80, unsignalled forced swim trials with an average ITI of 60 s. During a typical trial, the two rats were lowered into the water and removed from the water when the ESC rat achieved the response requirement on the omnidirectional lever. If the response requirement was not met within 15 s, the trial was automatically terminated and the computer program activated the motor and the rats were removed from the water. For the first 20 trials, the response requirement was one press of the lever (FR-1). Starting on trial 21, if the escape rat performed the requirement in less than 10 s on four of the five previous trials, the response requirement increased to FR-2. The response requirement was increased to FR-3 on trial 51. However, in the event that the ESC rat performed the response in less than 3 s in four of the previous five trials, the FR requirements were increased up to a maximum of FR-4 to ensure that both rats were being exposed to a minimum of 2 s of swim stress on each trial. Each ESC rat's performance was recorded on a computer throughout the session and the acquisition curve for all rats at each T_w was determined. Rats were removed from the experiment if they did not exhibit a systematic reduction in latency to press the omnidirectional lever by trial 40 (i.e., 10 trials at 15 s between trials 20 to 40). This criterion was necessary due to the increased exposure to the cold water and subsequent hypothermia that prevented escape responses. This is similar to the criterion used for the wheel-turn response in the tailshock stress controllability model (Drugan et al., 1996). With this criterion, a total of 5 rats were removed from the experiment due to failure to learn the escape response (19% of the total tested). These failures were distributed across the T_ws in the following fashion: 3 failures at 25 °C and 2 failures at 30 °C.

Immediately following the 80 trial ISS session, rats were towel dried and placed under an incandescent lamp to ensure that they were dry before being placed back in the vivarium.

2.4. Serum corticosterone assay

Immediately following the swim stress session, rats were placed into restraining tubes and their tail placed into warm (46–48 °C) water for 1 min to promote vasodilatation. A # 10 scalpel blade was used to produce a small tail nick and a blood sample (approx. 100 μl) was collected in an Eppendorf tube for CORT determination. Once the blood was collected, Vetbond (3M, St. Paul, MN) was placed on the nick to arrest bleeding. The rat was placed back onto their biotelemetry receiver in the vivarium for T_b measurement. Successive tail nicks were taken at 60 and 120 min post-stress.

Serum CORT levels were determined by an OCTEIA corticosterone enzyme immunoassay (EIA) kit (Immunodiagnosis Systems Limited, Boldon, UK). Blood was collected in Eppendorf tubes and remained at room temperature for 15 min to allow clotting. Samples were centrifuged for 10 min at 3000 rpm to remove blood cells and the serum was stored at −20 °C until assayed. The EIA was carried out according to the manufacturer's instructions. The absorbance of the resultant mixtures was measured within 5 min at 450 nm (Benchmark Microplate Reader, BIO-RAD). CORT concentrations were calculated from the measured absorbance values using AssayZap software (BIOSOFT, Cambridge, UK). The inter- and intra-assay coefficients of variation ranged between 2% and 8%.

2.5. T_b measurement

At least 4 days prior to the ISS session, rats implanted with the biotelemetry devices were placed onto receivers to record T_b. Once the experiment began, minute by minute records of T_b were continuously recorded except for when the rats were in the ISS apparatus.

2.6. Forced swim test

The FST was conducted in Plexiglas cylinders (height: 36.4 cm, diameter: 19.5 cm) filled with 29 cm of 23 °C water. Twenty-four hours following the ISS, all groups of rats were evaluated for immobility during a 5-min FST. Rats were taken to a separate room from the ISS pretreatment and tested one at a time. The experimenter scoring the rat was blind to group membership. During the 5-min FST, immobility was defined as the rat making only those movements necessary to keep its head above the water (i.e., absence of vigorous activity such that the forepaws did not break the surface of the water). This procedure is similar to that previously described (Porsolt et al., 1977, Drugan et al., 1989, and Brown et al., 2001). At the end of the 5-min forced swim, rats were towel dried and placed under incandescent lamps for 30 min.

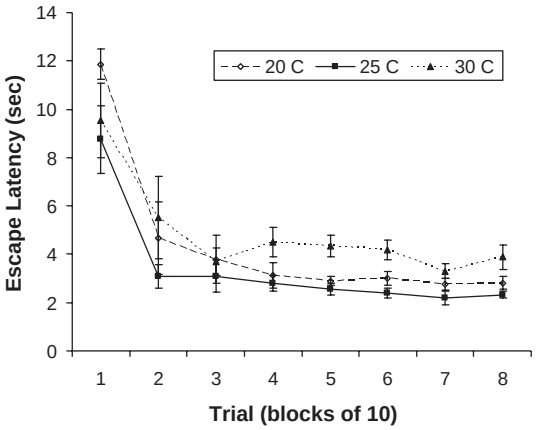

Fig. 2. Mean latency to escape (±SEM) the forced swim for rats exposed to either 20, 25 or 30 °C temperature water across the 80 trial ISS session.

2.7. Statistical analysis

All data were analyzed by between and within subject analysis of variance (ANOVA). The assumption of sphericity was tested using Mauchly's test of Sphericity. Where it was violated the within group data analysis was subjected to Huynh–Feldt correction. Escape latency was tested with a 3 (T_w) by 8 (mean of 10 trial blocks) repeated measures ANOVA. Changes in T_b post-ISS were determined with a 3 (T_w) by 4 (treatments) by 8 (T_b every 5th minute) repeated measures ANOVA. Serum CORT was analysed with a 3 (T_w) by 4 (treatments) by 3 (time points) repeated measures ANOVA. In most cases, simple main effects were determined and post hoc analyses were evaluated by Tukey's Honestly Significant Difference test with an alpha value of 0.05. Immobility scores were analyzed using a one-way ANOVA and post hoc analysis was conducted using the Newman–Keuls test with an alpha level of 0.05 (Kirk, 1995).

3. Results

3.1. Escape acquisition

ESC rats at all three different T_ws acquired the task very quickly (Fig. 2). The two lower T_ws appear to result in a

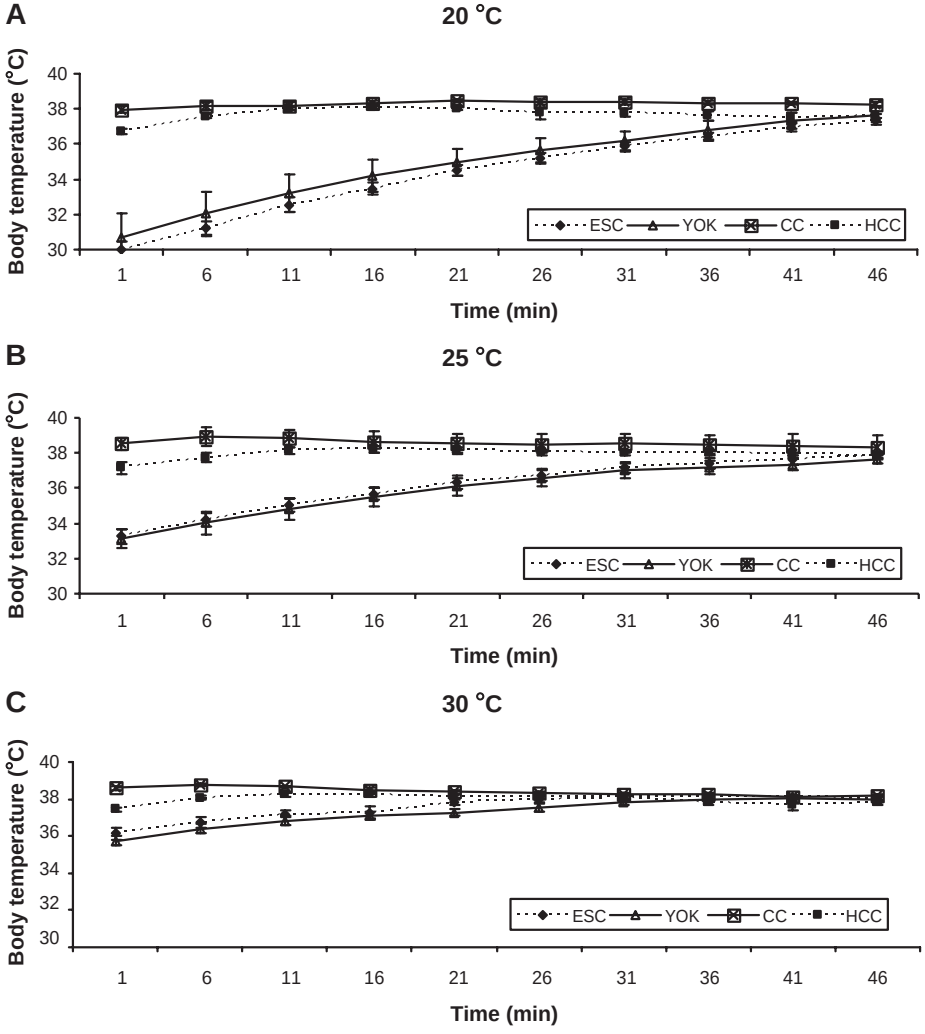

Fig. 3. Mean core body temperature (T_b) (±SEM) for all four groups of rats (escape, yoke, confine and home cage) for 46 min immediately following the 80 trial ISS session at various water temperatures including 20 (Panel A), 25 (Panel B) or 30 °C (Panel C).

slightly more consistent and rapid escape response throughout the session. ANOVA indicated a non significant main effect of T_w [$F(2,15)=2.55$], a significant effect of trial blocks [$F(3.51,52.65)=42.87$, $p<.001$], and a non significant temperature × trial block interaction [$F(7.02,52.65)=1.16$]. Analysis of simple main effects found a significant effect of T_w for trials 41–80 ($p<.002$ to .042). At each of these blocks, latency was greater for 30 than 25 °C ($p<.002$ to .033 with Tukey's HSD). Latency was also greater than 20 °C in blocks 41–60 ($p=.011$ and .045) Transition time in and out of the water is not included in the escape latency for each trial. The value on the graph represents the total time spent immersed in the water. Therefore, the total water exposure on each trial would equal the graphed value plus an additional 3 s.

3.2. Swim stress-induced hypothermia

It is clear from Fig. 3 that there is a systematic influence of T_w on core T_b. As the T_w is reduced, the core T_b is lower upon removal from the swim stress apparatus. Due to the very large main effect of T_w, as well as its interactions in the omnibus F test, data from each temperature were analysed separately.

At the 20 °C temperature, both ESC and YOK groups were severely hypothermic coming out of the water. Core T_b was 30.02±.62 and 30.72±.68 °C, respectively, and returned to prestress levels (37 °C) by 40 min post-stress. At the 25 °C temperature, the core T_b of the stress groups on termination of the ISS session was 33.26±.41 and 33.13±.41 °C and return to 37 °C by 30 min post-stress. Finally, following 30 °C swim both stress groups show a core T_b of 36.15± .23 and 35.76±.23 °C and return to 37 °C by 10–15 min post-stress. Repeated measures ANOVA at each T_w indicated significant main effects for group and time, as well as their interaction ($p<.001$ in all cases). Post hoc analyses with Tukey's HSD found that there were no differences between the T_b of ESC and YOK rats, but that their T_bs were significantly lower than either the CC or HCC groups ($p<.001$ to .018). The sole exception was at 30 °C where only the YOK rats had lower T_bs when compared to HCC rats.

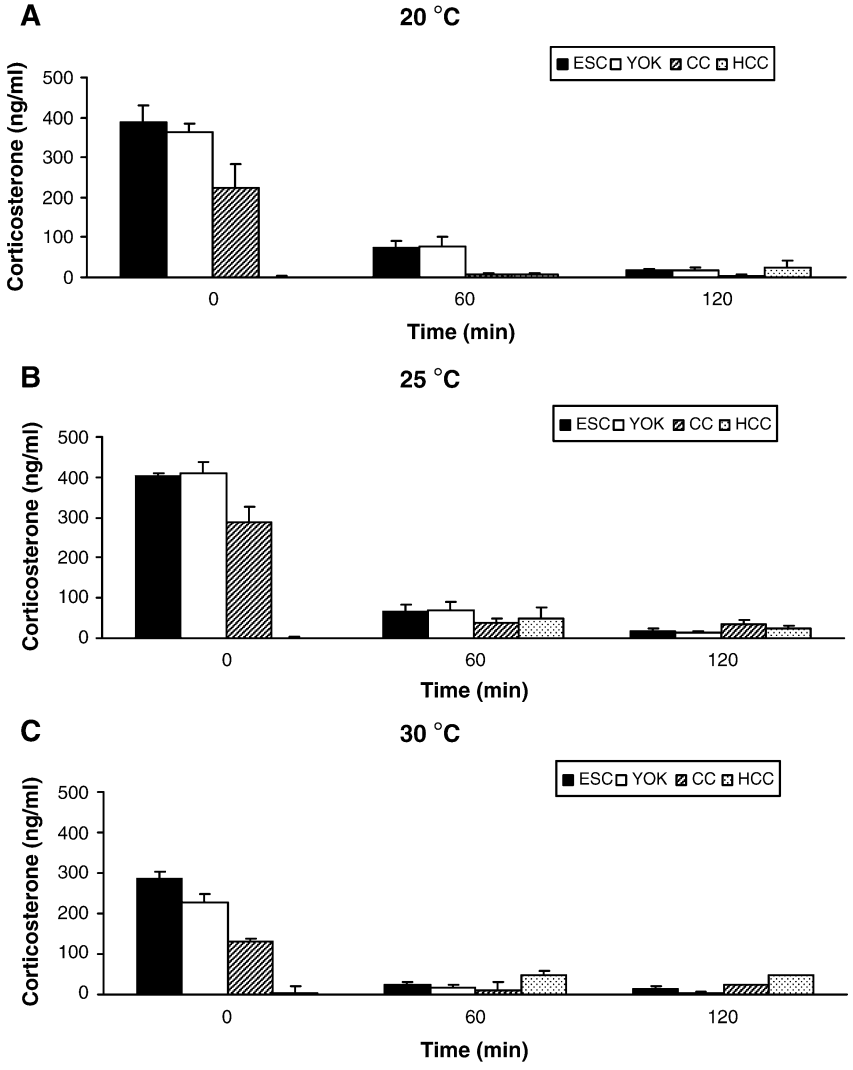

Fig. 4. Mean serum corticosterone levels (±SEM) for all four groups (escape, yoke, confined and home cage) at 0, 60 and 120 min following the 80 min ISS session in either 20 (Panel A), 25 (Panel B) or 30 °C (Panel C) water.

3.3. Swim stress-induced corticosterone release

The physical stress of hypothermia coupled with the psychological stress of ISS was reflected by the serum levels of the stress hormone, CORT (Fig. 4). At all three T_ws both the ESC and YOK groups show a significant elevation in serum CORT compared to both CC and HCC groups. As can be seen in Fig. 4, at all three T_ws, the ESC and YOK groups exhibit a marked elevation immediately post-stress that returns to basal levels by 2 h post-stress. Conversely, the CC group has an intermediate elevation between the two stressed groups and the HCC. This CORT increase in the CC group dissipates more quickly to basal levels at 60 min post-stress.

A 2-way repeated measures ANOVA at 20 °C revealed main effects of treatment [$F(3,18)=18.37$, $p<.001$], time [$F(1.49,26.87)=126.48$, $p<.001$], and their interaction [$F(4.48,26.87)=17.27$, $p<.001$]. Post hoc comparisons with Tukey's HSD test indicated that ESC and YOK groups were significantly different from both the CC and HCC ($p<.001$ to .012). Also, the CC rats were significantly different from the HCC ($p<.035$). The ESC and YOK groups were not different from one another. Similar effects were observed at a T_w of 25 °C with ANOVA demonstrating main effects of treatment [$F(3,19)=18.58$, $p<.001$], time [$F(2,38)=379.96$, $p<.001$], and their interaction [$F(6,38)=55.91$, $p<.001$]. Tukey's HSD determined that the HCC rats were different from the 3 other treatments ($p<.001$), but in contrast to 20 °C, the CC rats were not different from either the ESC or YOK groups. At 30 °C ANOVA still found significant main effects for treatment [$F(3,20)=23.51$, $p<.001$], time [$F(1.81,36.10)=151.19$, $p<.001$], and their interaction [$F(5.42,36.10)=37.33$, $p<.001$], however, the CC rats had CORT levels significantly lower than the stressed rats ($p=.001$ and .042) and not different from the HCC.

3.4. Forced swim behaviors

Swim stressor controllability had a significant impact on behaviors in the FST. There was a significant main effect of treatment on time spent immobile during the FST [$F(2,33)=4.81$, $p=.015$]. Post-hoc comparisons revealed a significant increase in immobility in the YOK group compared with both the ESC and CC groups ($p<0.05$), which did not differ from one another (Fig. 5).

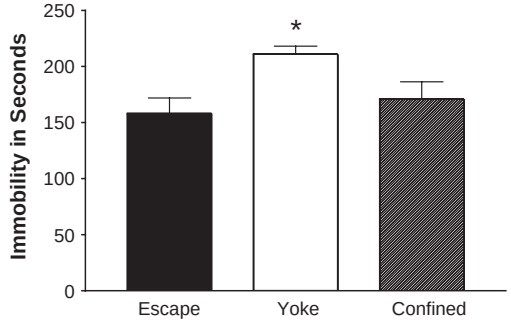

Fig. 5. Mean time spent immobile (+SEM) for rats previously exposed to intermittent escapable swim stress, yoked-inescapable swim stress, or confinement ($n=12$/group). * indicates significantly different from both escape and confined groups ($p<0.05$).

4. Discussion

The current study tested the impact of water temperature and stressor controllability on CORT, T_b, and forced swim responses to stress. Variation in the T_w from 20 to 30 °C did not significantly influence the escape acquisition across the entire swim stress session. However, reducing the water temperature from 30 to either 25 or 20 °C in the ISS paradigm resulted in significant hypothermia. Other studies have reported hypothermia-induced impairment in escape performance in tasks, such as the Morris water maze (Iivonen et al., 2002; Rauch et al., 1989). However, several factors unique to the current paradigm most likely afforded protection against these deficits: 1) reduced physical demand and restricted swim space (small diameter of cylinder, 21 cm); 2) the 15 s maximum exposure to the cold water on each trial; and 3) the heater blowing warm air on the rats during the ITI. Nonetheless, it is still impressive that there was no deterioration in responding across the session given that the escape rats were required to perform up to an FR-4 response while experiencing a mean reduction in core T_b of 5–7 °C by the end of the stress session for the 25 and 20 °C temperatures.

The physical stress of hypothermia coupled with the psychological stress of ISS is reflected in the serum CORT measures obtained immediately following stress. The highest levels of serum CORT were observed in both swim groups immediately following exposure to 20 and 25 °C water. Serum CORT also increased immediately after the 30 °C swim stress session. Although the increase was not as large as at that at the two cooler temperatures, the elevation in the two swim groups was still significantly larger than both the CC and HCC. This elevation in CORT could be due to the combination of swimming in 30 °C water and/or the loss of body heat due to convection. Although there is a clear impact of T_w on the endocrine stress measure, no systematic effect of stressor controllability was observed. Rats exposed to escapable or inescapable swim stress showed equivalent changes in hypothermia and serum CORT levels post-stress. Both of these groups show significant stress-induced changes in comparison to confined and home cage controls. These data confirm and extend the work of others showing no difference of stress controllability on CORT release (Maier et al., 1986).

The confined group exhibited a significant serum CORT response in the 25 and 20 °C conditions. We feel that this represents a combination of confinement and a heat stress reaction resulting from the residual warm air exposure from the adjacent fans directed at the ESC and YOK rats. The increase in ambient air temperature in the confined chamber during the session was confirmed using a probe thermometer indicating a temperature in excess of 35 °C during the session. This effect is reduced significantly in the 30 °C condition, where ambient

rather than warm air is blown on all 3 groups of rats. However, it is also possible that the increase in CORT in the CC group is caused by ultrasonic vocalizations (USVs) emitted by the swim stressed rats communicating the presence of danger (Knutson et al., 2002). This alternative explanation seems rather untenable given recent observations in our lab indicating that less than 25% of rats exposed to cold water ISS emit USVs as detected via high frequency microphone at the bottom of the chamber (Soucy and Drugan, unpublished observations). Given the broader context of stress communication between animals that includes USVs, sounds, and smells (Van den Berg et al., 1998), the CC rats might need to be run separately from the stress group to avoid these potential influences on subsequent physiology and behavior. Alternatively, confinement or novelty alone are sufficient to induce stress hyperthermia as reported by Oka et al. (2001).

The fact that 30 °C water 1) provides the escapable swim stressed rat with adequate motivation to efficiently perform the swim escape task, 2) minimizes the confound of hypothermia, and 3) results in a significant stress-induced elevation in serum CORT in comparison to controls following the stress exposure points to this T_w as the optimal temperature for evaluating the impact of coping on behavioral measures following this ISS paradigm. Although some investigators have pointed to brain and core body hypothermia as a factor underlying immobility (Arai et al., 2000; Taltavull et al., 2003), others have shown a clear dissociation of hypothermic and behavioral effects of forced swimming (Porsolt et al., 1979). We sought to verify this dissociation using the 30 °C ISS paradigm.

In the final experiment, swim stressor controllability markedly influenced subsequent immobility when tested 24 h post-stress. More specifically, YOK rats show significantly more immobility than both ESC and CC rats. Importantly, the ESC and CC groups did not differ indicating that swim stress controllability and not merely swim stress per se has a marked impact on this well-accepted measure of behavioral depression. These observations are quite different from the results reported by Brown et al. (2001) indicating increased immobility in both ESC and YOK groups compared to confined and naïve controls. Two procedural differences between the two studies may account for the divergent results: the current study employed 80 trials of ISS at a T_w of 30 °C whereas Brown et al. (2001) used 100 trials and a T_w of 23 °C. Therefore, the extended number of trials at 23 °C and subsequent hypothermia may have masked the swim stress controllability effect observed in the current study. These results also demonstrate that prior stress-induced hypothermia is not necessary for the expression of behavioral despair in the ISS paradigm. The current findings add to a body of literature indicating the importance of controllability in altering subsequent behavioral reactivity to stressors.

Acknowledgements

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