ɛs in Operant Behaviors of Rats With Acute Exposure to High Ambient Temperature: A Potential Role of euptake Transporters

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e functions are known to be influenced by thermal stress from the change in ambient temperature (Ta). ⁄n about how increased Ta (i.e., when the weather becomes warm or hot) may affect operant and the neural substrates involved. The present study thus investigated the effects of high Ta on operant on a fixed-ratio 1 (FR1) and a differential reinforcement for low-rate responding 10 s (DRL 10-s) nent. The rats were randomly assigned to three groups receiving acute exposure to Ta of 23°C, 28°C, y, for evaluating the effects of high Ta exposure on four behavioral tests. Behavioral responses in an locomotor activity were not affected by Ta treatment. Regarding operant tests, while the total responses decreased only under 35°C when compared with the control group of 23°C, those of DRL 10-s behavior ɹced in both groups of 28°C and 35°C. Distinct patterns of inter-response time (IRT) distribution of DRL nong the three groups; between-group differences of behavioral changes produced by high Ta exposure antitative analyses of IRT data. Western blot assays of dopamine (DA) D1 and D2 receptor, DA brain-derived neurotrophic factor (BDNF) were conducted for the sample tissues collected in six brain jects after acute high Ta exposure. Significant Ta-related effects were only revealed in the dorsal In which, the DAT levels were increased in a Ta-dependent fashion that was associated with operant ɗer high Ta exposure. And, there as an increased level of D1 receptors in the 28°C group. In summary, at the performance of operant behavior affected by the present high Ta exposure is task-dependent, and rant behaviors cannot be attributed to gross motor function or anxiety being affected. The regulation of olved in this operant behavioral change under high Ta exposure.

receptors (D1Rs; A), D2Rs (B), DA reuptake transporters (DATs; C), and brain-derived neurotrophic
ıe dorsal hippocampus (dHIP) following the exposure of high ambient temperatures (n = 6 per group).…

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Task-Dependent Differences i Operant Behaviors of Rats Wit Acute Exposure to High Ambie Temperature: A Potential Role Hippocampal Dopamine Reup Transporters

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Behavioral or cognitive functions are known to be influenced by therm change in ambient temperature (Ta). However, little is known about h (i.e., when the weather becomes warm or hot) may affect operant cor and the neural substrates involved. The present study thus investiga high Ta on operant behaviors maintained on a fixed-ratio 1 (FR1) reinforcement for low-rate responding 10 s (DRL 10-s) schedule The rats were randomly assigned to three groups receiving acute e 23°C, 28°C, and 35°C, respectively, for evaluating the effects of h on four behavioral tests. Behavioral responses in an elevated T-maz activity were not affected by Ta treatment. Regarding operant test responses of FR1 behavior were decreased only under 35°C when c control group of 23°C, those of DRL 10-s behavior were significantly groups of 28°C and 35°C. Distinct patterns of inter-response time of DRL behavior appeared among the three groups; between-gro behavioral changes produced by high Ta exposure were confirme analyses of IRT data. Western blot assays of dopamine (DA) D1 and transporter (DAT) and brain-derived neurotrophic factor (BDNF) were sample tissues collected in six brain areas from all the subjects af exposure. Significant Ta-related effects were only revealed in the dor (dHIP). In which, the DAT levels were increased in a Ta-dependent associated with operant behavior changes under high Ta exposure. increased level of D1 receptors in the 28°C group. In summary, th

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this operant behavioral change under high Ta exposure.

Keywords: warm ambient temperature, schedule-controlled behavior, FR-typed, DRL-type

UCTION

to physiological responses monitored by the noregulation system (Nakamura, 2011), behavioral influenced by thermal stress resulting from in ambient temperature (Ta; Cheshire, 2016). navioral performance affected by Ta can be more unpredictable than the thermoregulation processes rolled by certain levels of brain mechanisms (e.g., ic or medullary). While previous studies showing eness of varied Ta's at behavioral level have uited the test models based on reflexive system l., 1995; Gallup, 2010; Suwanapaporn et al., 2017), the effects of Ta on the associative conditioning aradigms are scarce. It is, thus, important to ether the conditioned behavior can be influenced on-thermoneutral environment. Individuals and mic animals living in the tropical regions frequently a when exposed to excessive natural heat and heat late, how different degrees of high Ta may affect conditioned or schedule-controlled behavior and its neural mechanisms is still largely unknown (but see 69; Thomas et al., 1991).

deration of the functional relationship potentially veen the operant behavior and high Ta, the present lesigned to assess the effects of high Ta exposure ormances of operant behaviors maintained in a (FR1) and a differential reinforcement for low-rate 10 s (DRL 10-s) schedule of reinforcement in erant behaviors trained on these two different f reinforcement are distinctively characterized by task difficulty but also the behavioral component gical construct (Ferster and Skinner, 1957). That is vioral inhibition and timing process are especially the subject to perform on the DRL-typed behavior d Rilling, 1970; Sanger and Blackman, 1975; Neill, lure and McMillan, 1997; Bayley et al., 1998; and Ainslie, 1999; Paule et al., 1999; Cheng). Thus, unlike a relatively high response rate rally measurable in FR-typed schedule, a low-rate on operant manipulandum is typically elicited by chedule. A previous study has shown that dissociable ar in operant behaviors maintained on DRL and rat under stress of tail-pinch and psychoactive ents (Chang et al., 2000). Based on the premise ature stress causes a physiological response with a n body temperature in rats exposed to warm or nment (Long et al., 1990), the effects of high Ta these two operant behaviors are expected to be distinctively different. With regard to the Ta being examined, the relatively high de exposure, e.g., 36–40° Celsius (°C), have be affect behavioral manifestation (Carlisle and Bouali et al., 1995). Furthermore, behavio thermoregulations have been shown to be following exposures to mild and severe heat 2010). Accordingly, 28 and 35°C were ch as two different degrees of high Ta to be control Ta of 23°C. The acute Ta exposur to determine the effects of different levels o and FR-typed operant behaviors. In additi response in an elevated T-maze and the locc the acute exposure to high Ta were assessed. has been used to examine anxiety-like beha is validated by pharmacological tests show attenuated by anxiolytic drugs (Graeff et al., Graeff, 2014). Whether acute exposure to h anxiety-like response on the elevated T-maz understood.

Substantial evidence has been accur that a functional relationship exists be brain dopamine (DA) systems (Roth et 2000). Considering that exposure to high stress, physiological and neurochemical fu correspondingly respond as a part of therm subject during environmental temperature several studies have shown that experime of DA receptors and the release and re produce thermoregulatory responses in m Lee, 1977, 1980; Cox et al., 1978; Brown et 1982, 1992, 1995; Lin and Tsay, 1985; Cha Neurotrophins, including brain-derived n (BDNF), are altered in stressful conditi psychosocial and physical factors (Alleva a Alleva and Francia, 2009). Assuming that may act as a stressor and affect the DA- an brain regions, we collected tissues from s were subjected to Western blot assay to de expression of DA D1 and D2 receptors respectively), DA transporter (DAT), and areas included the medial prefrontal cort striatum (dSTR), nucleus accumbens (AMG), dorsal hippocampus (dHIP) a (HYPO).

The objective of this study was to examin Ta on a FR and a DRL operant tasks alon response in an elevated T-maze and the loc addition, Western blot assays of D1R, D2R

:ted for the sample tissues collected in six brain areas subjects following acute high Ta exposure after the ·ioral tests.

ALS AND METHODS

s

ale Wister rats (BioLASCO Taiwan Co., Ltd.),)proximately 250 g of body weight and 6 weeks old , were housed individually. The rats were handled owed 10 days of acclimation to the colony. Food and provided *ad libitum*, except for the experiments of avior. The rats were maintained on a water restriction h that there was 5 min access to tap water in the occurring no sooner than 30 min after the end of experimental session of operant behavior. During the body weight was monitored and allowed to throughout the course of operant experiment on a vth curve. Food pellets were continuously available e cage. Training and/or test sessions were conducted same time (10:00–15:00) during the light portion um's 12 h/12 h light/dark cycle (lights on at 7:30). ature of the colony and the behavioral test room ned at 23 ± 1°C throughout the experiment. All were conducted in accordance with the NIH Guide and Use of Laboratory Animals and approved by an review committee of animal use and care at National niversity.

us

aviors were measured using a custom-made operant four chambers located in a room separate from colony. The interior dimensions of each chamber 1 × 25 cm × 30 cm (MED Associates Inc., St. USA). Aluminum panels formed the front and back ear Plexiglas comprised the remaining sides and the s steel rods (with a diameter of 5 mm) were set t to provide flooring. Each chamber was equipped positioned 7.3 cm above the floor and 4 cm from rner of the front panel. A liquid dispenser was of the front panel of the chamber. The reinforcer chanism provided 0.04 ml of tap water at each . The water was delivered into a receiving dish meter) located at the center of the front panel and the floor. The chamber was illuminated by a small cated 10 cm above the floor and positioned 5 cm ft corner of the front panel. Each chamber was a plywood box with a fan to provide the necessary nd to mask any outside noise. The four operant ere serviced and controlled by a microcomputer house designed program to control the operant t as well as to allow data collection (Cheng and Liao,

locomotor activity test, an acrylic box point of the box floor. The imaging data to measure the traveling distance of each calculated using a commercial software Trace System, version 1.17, Taipei, Taiwan).

The elevated T-maze was set 50 cm abo made of wood and had three arms with ar (50 cm × 10 cm each). The stem of the T- with 40 cm high walls denoted as the clos perpendicular to the two open arms. This up in a behavioral test room separate from chambers and locomotor activity arena.

Procedures

Following the adaptation to colony and the between colony and behavioral test roo randomly assigned to three groups (n = acute Ta exposure of 23°C, 28°C, and 35 experimental manipulation before the behavi this between-subject design, each rat rece exposure throughout behavioral testing. T were conducted in the following order: th locomotor activity, FR1 behavior, and DRL Ta conditions in each test room were establ commencement of behavioral test. In the test apparatus was located, each Ta was mainta reverse-cycle air conditioner. The maintena run by using an oil-filled radiator heater. Te taken from two thermometers inside behavi always within ± 1°C of Ta.

The natural escape and conditioned avoid in open-arm area were measured in the ele the subject was placed on the far end of escape latency (in seconds) from lingerin area to entering the closed area was meas rat was placed in the most inside part of begin the test trail. The latency of inhibi measured as the time that the rat left the maximum inhibitory latency was set at 300 Four trials were conducted to measure the co avoidance in the elevated T-maze. A week l activity test was carried out for 30 min by the test arena where the distance (in cent measured.

The experiments of operant behavior b the locomotor activity test. During this adapted to the water restriction regimen. I the operant behavioral experiment, the rats of FR1 training where each lever press reinforcer (a water drip in 0.04 ml). Th operant behavioral training and test was DRL 10-s behavioral task. The criterion to performance of FR1 behavior was 120 res that was consecutively observed over 3 days criterion, the subjects underwent a 3-day t

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› > 0.05; **Figure 2A**). Subsequently, the subjects the colony for 2 days before entering the second operant behavioral experiment. With a retraining onse, the rats were then trained to respond on the chedule of reinforcement, wherein a reinforcer was ntingent upon a lever press if at least 10 s had e the previous press. Premature responses led to a cement contingency and a resetting of the interval dexed by the non-reinforced response. Each lever ner reinforced or not, reset the delay timer. The d the DRL 10-s behavior and reached a baseline nately 20 daily sessions of training. The criterion baseline of DRL 10-s behavior was less than 10% the response rate for three consecutive sessions. a 3-day test of DRL 10-s behavior, including a day a day after the Ta treatment, was conducted. No up difference was observed on the total responses s on the day before the Ta treatment ($p > 0.05$;

e temperature was recorded by using a digital r (HP-100, DRI, Taiwan) with a rectal thermistor inserted to a depth of 30 mm when the rat was lightly The rectal temperature of each rat was measured before and after the 2 h exposure of Ta, after or DRL 10-s behavioral test began. One week after ion of DRL 10-s behavior test, the subjects of each went Ta treatments for 2 h before being sacrificed by . Tissues were then collected from the specified brain uding mPFC, dSTR, NAcc, AMG, dHIP, and HYPO 2014). The collected brain tissues were treated with gen and stored in a −80°C freezer until preparation blot analysis.

Blot Analysis

d brain tissues were sonicated by lysis buffer (50 mM 50 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, h protease inhibitor Cocktail Set I (Calbiochem) atase inhibitor cocktail tablets (PhosSTOP). The e then centrifuged at 14,000× *g* for 10 min at sates were diluted with lysis buffer and the protein ns were determined by Bradford assay with a protein eagent (Bio-Rad). Equal amount of each sample s heated for 10 min at 90°C before loading for t by 10% sodium dodecyl sulfate polyacrylamide gel sis (SDS-PAGE). The proteins were then transferred vinylidene fluoride (PVDF) membrane (Millipore, by wet electroblotting systems. The membrane ining the target proteins (according to molecular β-actin (1:100,000; Millipore). The membr 0.1% Tween 20-TBST for three times (5 and then incubated with either anti-mouse conjugated-horseradish-peroxidase (HRP) (1:5,000) for 1 h under room temperatu membrane was washed again for three times i detection by chemiluminescent reaction w Western chemiluminescent HRP substrate intensity of protein band was quantified 1.47, National Institutes of Health, Bethes normalized with the endogenous actin pro control.

Statistical Analysis

The number of total responses was recorded f of FR1 behavior. With regard to the DRL l press was classified in terms of its associ time (IRT; the time in millisecond elaps response). The resulting dataset on IRT plotted into a distribution consisting of re for 21 consecutive 1 s time bins (**Figure 3A** analyses, six dependent variables were responses; (2) reinforced responses, lever pr (3) non-reinforced responses, lever press (4) burst responses, lever response with I rate; and (6) peak time. The burst response responses with IRTs that were <2 s (as sh 2 of the IRT distribution curves in **Figure** and peak rate were calculated from the de-bu in which a moving average based on four c with a 1-s step size was applied to smooth After identifying the maximum frequencie the peak time was the average value (in IRTs that fell within the four bins (i.e., th The peak time measure indicated at which pressed the lever with the highest IRT fr expected time for obtaining the reinforcer. calculated from the summed responses in th by four, indicating how strongly the rats press the lever at the expected criterion tir procedure has been previously used (e.g., Ch 2017).

The effects of high Ta exposure or variable from behavioral measures and bioc separately subjected to one-way analysis of with the Ta as the between-subject factor. In mixed ANOVA was used to evaluate the inte and the test trials or days for certain cases. An

ibody overnight at 4°C. The primary antibodies ti-DAT (1:2,000; Abcam, Cambridge, MA, USA), :1,000; Santa Cruz Biotechnology, Santa Cruz, CA, D2R (1:1,000; Santa Cruz Biotechnology, Santa USA), anti-BDNF (1:1,000; Millipore), and anti-

operant behavior and each of the protein le biochemical assay. Statistical significance w All analyses were conducted using a com program (SPSS version 18.0, SPSS Inc., Chi values are expressed as means ± SEM.

S

of High Ta Exposure on Elevated and Locomotor Activity

l to the data collected from the elevated T-maze, **,B** presents the escape latency and inhibitory avoidance, respectively. No significant Ta ef the escape latency ($p > 0.05$). A two-way AI analyzing the data of inhibitory avoidance, not yield any significant main effect or intera **Figure 1C** shows the effects of high Ta on and there was no significant Ta effect on ($p > 0.05$).

Effects of High Ta Exposure o Responses of FR1 and DRL 1

Figure 2 presents the total responses of schedules as measured on the day with hig the days before and after this treatment. F in **Figures 2A**, a two-way ANOVA revealed effect of test day ($F_{(2,30)} = 22.291$, $p <$ and a significant day-by-group interactio $p < 0.001$; $\eta_p^2 = 0.671$). The follow-up s showed that the group with Ta of 35°C sig total responses when compared with its the pre-test day ($p < 0.001$). For DRL 1 **Figure 2B**, via a two-way ANOVA, the m test day and group were significant: $F_{(2,30)}$ = ($\eta_p^2 = 0.840$) and $F_{(2,15)} = 10.541$, $p = 0.$ respectively. Additionally, the day-by-gro

ehavioral responses to exposure of high ambient temperatures up) on the escape latency **(A)** and inhibitory avoidance **(B)** an elevated T-maze and the locomotor activity **(C)** measured in a All data are displayed as mean ± SEM.

FIGURE 2 | Mean (±SEM) total responses of fixed-rati 10-s **(B)** schedule on the days before, during, and afte ambient temperatures. ***p <0.001 (Bonferroni Test) a pre-test day for each group (n = 6).

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$F_{(4,30)} = 45.129$, $p < 0.001$; $\eta_p^2 = 0.857$. The sample revealed that total responses in both groups of 28°C re significantly decreased when compared with their day baselines (both $p < 0.001$). As noted, in either 10-s, the total responses measured in the post-day ter the Ta treatment) were not significantly different f the pre-test day ($p > 0.05$).

tive Analyses of the DRL 10-s Data est Day of High Ta Exposure

of high Ta exposure on DRL 10-s task are shown . **Figure 3A** shows the IRT distribution curves of DRL behavior from the 18 subjects assign under different degrees of Ta exposure. A t distribution was shown in the control grou group of 28°C. One peak yielded in the bir burst responses) and another one appeared 10 s. By contrast, the IRT distribution curv the group of 35°C exposure. Quantitative a of DRL behavior are shown in **Figures 3B-** the group of 35°C emitted less than 5 respo the outliers, their data were excluded from c of IRT data for DRL 10-s behavior. Via the effects of Ta manipulation were sign

ıter-response time (IRT) distributions of DRL 10-s during the test day of high ambient temperatures **(A)**, and quantitative measur
, non-reinforced **(D)**, and burst **(E)** responses as well as peak rate **(F)** and peak time **(G)**. Details of these dependent variables a
alysis" section. The number inside the bar (in **B–G**) depicts the sample size of the corresponding group. All data are expressed a
ɔ < 0.01, and ***$p < 0.001$ as compared with the control group of 23°C (Bonferroni test).

ures: total responses, $F_{(2,13)} = 23.470$, $p < 0.001$
); reinforced responses, $F_{(2,13)} = 7.543$, $p = 0.0067$
7); non-reinforced responses, $F_{(2,13)} = 11.055$,
($\eta_p^2 = 0.630$); burst responses, $F_{(2,13)} = 4.608$,
($\eta_p^2 = 0.415$); and peak rate, $F_{(2,13)} = 22.752$,
($\eta_p^2 = 0.778$). Following *post hoc* tests, the rats
5°C exhibited total responses ($p < 0.001$), reinforced
ɔ < 0.05), non-reinforced responses ($p < 0.01$),
ıses ($p < 0.05$), and peak rate ($p < 0.001$) less
in the 23°C group. The rats exposed to 28°C
ınly a lower peak rate compared with those exposed
< 0.05), indicating DRL behavior being slightly
ntly affected by 28°C and profoundly disrupted by

ɔtal responses of DRL decreased for the 28°C and
s, the other three types of responses (reinforced,
ɛed, and burst) would be also decreased. Further
normalize each of these three types of responses
he total responses was conducted and then tested
ıy ANOVA. The ratio of reinforced responses was
ıd that of non-reinforced responses was decreased
high, but these two trends were not significantly
both $F_{(2,13)} = 3.785$, $p = 0.0506$). The normalized
st responses was not significantly changed by Ta,
6, $p > 0.05$.

emperature Changes Following Exposure During Operant ral Tests

n **Table 1**, a significant Ta-dependent increment

There was a significant correlation betwe
and total responses of FR1 ($r_{(16)} = -0.5$
so was for that of DRL 10-s ($r_{(16)} = -$
indicating lower operant responding is ass
level of DAT expressed in the dHIP after
The relationship between the total respons
task and the dHIP D1 receptor level wa
confirmed (both $p > 0.05$). Furthermore, t
correlation was significantly appeared for the
D1 receptor, level in associated with quantita
data for DRL 10-s behavior including the r
($r_{(14)} = -0.674$, $p = 0.004$), the non-re
($r_{(14)} = -0.733$, $p = 0.001$), the burst respor
$p = 0.032$), and the peak rate ($r_{(14)} = -0.80$
relationship corresponding to the peak time
($r_{(14)} = -0.493$, $p = 0.052$).

In contrast to the results of the dHIP, no
was confirmed for each protein level expres
brain regions (all $p > 0.05$; data not presente

DISCUSSION

Behavioral changes following high Ta exposu
revealed by the use of tasks related to th
conditioned, type of behavioral response. I
acute exposure to high Ta (28°C and 35
the control of 23°C) produced different
two types of operant behavior respectively
FR1 and DRL 10-s schedule of reinforcem
responses of FR1 behavior were decreased on

was higher than that of the control, ANOVA revealed
ıt Ta effect ($p > 0.05$).

of High Ta Exposure on the Protein f D1R, D2R, DAT, and BDNF

corresponding to the dHIP are presented in **Figure 4**.
ray ANOVA, significant Ta effects were detected on
levels of D1R (**Figure 4A**) and DAT (**Figure 4C**)
P, $F_{(2,15)} = 6.292$, $p = 0.0104$ ($\eta_p^2 = 0.456$) and
23, $p < 0.001$ ($\eta_p^2 = 0.638$), respectively. Compared
ntrol of 23°C from *post hoc* tests, the 28°C and
nents increased in D1R ($p < 0.05$) and DAT
, respectively. No Ta effect was observed on D2R
or BDNF (**Figure 4D**) in the dHIP ($p > 0.05$).

n rectal temperature increase (± SEM; °C) from 2 h exposure to
d 35°C ($n = 6$ each group) as measured before the fixed-ratio 1
10-s behavioral tests.

t 23°C	28°C	35°C
7 0.80 ± 0.28	2.13 ± 0.34*	
± 0.47 1.22 ± 0.16	1.88 ± 0.26	

npared with the control group of 23°C (Bonferroni post hoc test).

of behavioral changes produced by high
confirmed by quantitative analyses of IRT d
Western blot assays, the Ta-related increm
the protein levels of D1 receptors and DA
dHIP.

Differential Effects of Two Lev Ta on FR1 and DRL 10-s Beha

In this study, operant responses to FR1 and
were significantly affected by the acute ex
being manipulated in 28°C and 35°C. The
profound in operant responses to DRL
in the FR one. The operant responses of
significantly decreased in both the 28°C
when compared with their corresponding
the pre-test day (**Figure 2**). For the FR1
decrease in total responses was detected
group. In other words, acute exposure to 28
response to DRL 10-s schedule, whereas t
FR1 schedule was preserved. These result
performance operant behavior can be disti
acute exposure of different degrees of hig
task-dependent fashion. The results presente

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rotein levels of D1 receptors (D1Rs; **A**), D2Rs **(B)**, DA reuptake transporters (DATs; **C**), and brain-derived neurotrophic factor (BI (dHIP) following the exposure of high ambient temperatures (n = 6 per group). Representative protein pattern profiles depicting n high ambient temperatures are shown on the *top* panel. All data are displayed as mean ± SEM. $^{*}p < 0.05$ and $^{***}p < 0.001$ a of 23°C (Bonferroni test).

r understanding about how the performance of avior can be affected by acute exposure to high Ta. erature on this topic is scarce, exposure to 38°C has ed to decrease the response rate measured in both RL 18-s components of a multiple DRL-FR schedule- ehavior (Thomas et al., 1991). In the other study (1969), during the exposure to 35°C, response and nt rates of DRL 15-s behavior profoundly decreased um level within 40–50 min of the 90 min test pite that a significant adverse effect on both FR sks was consistently observed in high Ta exposure 38°C, some methodological discrepancies (e.g., l design) warrant consideration for comparing the een studies. First, descriptive, but not inferential, s presented in previous studies (Barofsky, 1969; al., 1991). The use of rather small sample size vitably forced the experiments run in a within- gn for testing both FR and DRL behaviors over nditions of thermal stress, even from cold to warm s et al., 1991). Second, only one degree of high ed in comparison with the control Ta (e.g., 38°C vs. 24°C or 35°C vs. 25°C) in previous s data in the current study were collected subject design for the exposure to 23°C, 28° four behavioral tests including the separa DRL operant behavior. The current data informative for clarifying the previously re were influenced by certain confounding fa in the present study, the performance of significantly affected by acute exposure to Ta given in 28°C, whereas that of FR beha These behavioral changes may be associat differences between DRL and FR schedule-c as mentioned in the Introduction. That is, the FR task, the DRL task known with task difficulty that requires for behavioral in process. More sessions are normally neede response in DRL schedule than that in the reaching an acceptable baseline. It is th cognitively demanding DRL behavior is mo affected by high Ta exposure than a simpl behavior.

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also be noted that the aforementioned effects of ure to high Ta on operant behaviors were short- e the behavioral performance that was measured in t day returned to the level of the pre-test day for DRL task. The present findings that the adverse L 10-s behavior induced by 35°C treatment reversed ne level is consistent with the report by Barofsky estingly, differential effects of two levels of high Ta ., 30°C and 35°C) have been recently observed in a test for 1 h but not daily (Suwanapaporn et al., 2017).

e Effects of High Ta on Locomotor and Elevated T-Maze Test

nentioned changes of operant behaviors cannot be he alteration of gross motor function or anxiety-like owing acute exposure to high Ta. Statistical analyses t the locomotor activity measured in the group 35°C was not significantly different from that rol group of 23°C, despite the presence of an mechanism have been used in the field of ar certain debated issues being concerned (En et al., 2017). Thus, caution should be taken of the results regarding the negative effects on the anxiety test using the elevated T-maz required before any conclusion can be ma issue.

Neural Basis of Operant Beha by High Ta Exposure

The phenomenon where body temperature organism is confronted with a stressor is induced hyperthermia. This hyperthermia been observed even as mildly induced by a et al., 2003) or psychological threat (Naka rodent. Given high Ta exposure as a stres rectal temperature after the high Ta exposu the subjects of the 35°C group of the prese this effect was not the case for the subjects

noregulatory and hyper-locomotion responses to drugs (Wright et al., 2012; Miller et al., 2013). a decrease in locomotor activity at 30°C (vs. been reported in vehicle-treated rats, which were with 3,4-methylenedioxymethamphetamine treated argreaves et al., 2007). No severe depression of ctivity (e.g., akinesia) was observed in the rats of °C groups in the present study. Thus, the profound otal numbers of responses measured in either FR1 or ere not attributed to locomotor activity disrupted or by high Ta exposure.

sure to high Ta is a kind of stressor, and the stress a precipitating factor for anxiety-related behavior rs. Therefore, the appearance of anxiety or panic increase as the temperature also increases. However, this notion, the present high Ta exposure did not cape latency and inhibitory avoidance as measured ty-like responses in the elevated T-maze. By using e decrement of escape latency and the increment avoidance have been shown to model panic and iety disorders, respectively, in the rat (Zangrossi 2014). During the present test of escape latency , the subjects in the experimental groups with high went into the closed area with a latency a bit he control subjects, but there was no between-group lso, a null result of high Ta exposure was obtained of inhibitory avoidance. Thus, these data reflected e anxiety-like response in this task for the rats of °C groups compared with the controls in this study. ance of the control subjects on elevated T-maze in study is akin to that of the normal subjects being this laboratory and the others (Silveira et al., 2001; iao, 2005). Nevertheless, it is noted that various types odels with distinct behavioral constructs and brain

dissociable on behavioral and physiological 2010; Suwanapaporn et al., 2017). The reason Ta-related increase of rectal temperature ap not DRL 10-s, test is unclear. The adaptation given in multiple trials (i.e., the third time fourth time in DRL 10-s test) may be related research is needed to confirm this hypothesis

Conversely to the adaptation view, a pos the observation of two operant behaviors c but no significant Ta effect in the tests of e locomotor activity, may be due to the test multiple sessions of high Ta exposure (as a could have carryover effects into the subseq to stressors (e.g., disrupted sleep, predator c sensitize the animal's responses (e.g., elevate increased anxiety, deficits in cognition, etc.) We acknowledge several limitations in this this issue. But just with the rectal temper two operant behavioral tests, if this issue is rise of rectal temperature would be expecte higher in DRL 10-s test than that in FR ANOVA (3-group-by-2-test) revealed a sign $F_{(2,15)} = 6.113$, $p = 0.011$, neither the test interaction was significant ($p > 0.05$). The h of body temperature along with the repeated may be depedentent on experimental conditi Poddar (2001) reported that rectal temperatu increased by a daily 2 h exposure of 40°C for in their normal control rats. In the present to high Ta were manipulated for five times separated in 1 week or longer. Thus, given might be potentially existed, the effectiveness

In addition to the central circuitries fo regulation (Nakamura, 2011), the midbrai

ted to be critically important for autonomic and hermoregulation (Brown et al., 1982; Lee et al., theless, how Ta affects or modifies the DA-involved atory system is largely unknown to date. The t of DA release has been shown in the rodent under ls of stressors (Roth et al., 1988; Feenstra, 2000). In eurochemical evidence regarding the synaptic levels tly examined in the rat under high Ta exposure, body of research suggests that increased central including the neurotransmitter release is essential activity (e.g., exercise) linked with hyperthermia Hasegawa, 2016). Therefore, exposure to high Ta enhance the release of DA in terminal areas of

With regard to BDNF, no detectable T revealed in any of the six areas tested in this of BDNF change could be due to the degree in this study is lower than that typically app of heat shock or heat-related illness (e.g., > Johanson, 2007). It is also possible that BDN chronic, but not acute, exposure to high Ta. have also been shown to be altered by the to high Ta in adult rats (Matsuzaki et al., during their postnatal stage (Katz and Mei acute exposure, rather than chronic/long-te high Ta applied in this study may be attribut outcome.

AT were shown in distinct patterns following high . In terms of the Ta-dependent effect, we found that levels of D1 receptors and DAT were significantly ı the dHIP in the 28°C and the 35°C groups, Despite the appearance of Ta-related increment in s of the dHIP, it was not significantly confirmed l test. In contrast to the results of dHIP, those of eceptors and DAT expressed in the other five regions ;nificantly detected in high Ta treatment. That the ıre to high/warm Ta had a significant adverse effect DRL 10-s behaviors may be involved with stress- erthermia by increasing the protein levels of DAT ı the dHIP following the synaptic release of DA temperature stress. This notion is supported by the relations between the DAT level and all behavioral cept the peak time of DRL test. In this region, the of Ta-related increment of DAT implicates that ptic reuptake mechanism is critical for the neural ollowing the impact of high level of warm Ta (e.g., ersely, the postsynaptic D1 receptors may be more alterations during low levels of warm Ta (e.g., ırding the role of the hippocampus in the DRL evious studies showed impaired performance of a sk in hippocampectomized rats (Rawlins et al., 1983) in acquiring a DRL 18-s behavior after cytotoxic l lesion (Bannerman et al., 1999). Moreover, with logical recording, the vast majority of hippocampal ed event-related profiles in association to DRL or (Young and McNaughton, 2000). Together, the essions of D1 receptor and DAT in the dHIP may d with the adverse effect on DRL 10-s behavior high Ta exposure. Further investigation is needed to cause-effect mechanisms underlying this proposed One more issue worth noting is the present cal data were obtained in separate to behavioral (i.e., after at the end of behavioral testing). It tially reflect a summed effect of all five-time high . A factorial design of high Ta exposure on both ıviors with brain samples collected immediately after esting may be applied to tackle this issue.

be distinctly affected by exposure to differen in a task-dependent fashion. The operant b on DRL task is more sensitive to be alter FR task by high Ta exposure. These beha not the result of motor suppression or ar induced by high Ta exposure. The change c is associated to these changes of operant be results complement those from studies in physiological and behavioral thermoregulati provide a better understanding of the effects on operant conditioned behavior.

DATA AVAILABILITY

All datasets generated for this study ar manuscript.

AUTHOR CONTRIBUTIONS

S-FC, C-YChu and R-ML conceived experiments. S-FC, C-YChuang, Y-HY, Y-CS and Y-HH performed the behavioral C-YChuang and C-CC ran biochemical and R-ML analyzed the data and contribu manuscript.

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