Am J Physiol Regul Integr Comp Physiol 314: R43–R48, 2018.
First published September 6, 2017; doi:10.1152/ajpregu.00170.2017.

RESEARCH ARTICLE | *Neural Control*

Circadian variability of body temperature responses to methamphetamine

Abolhassan Behrouzvaziri,[1] Maria V. Zaretskaia,[2] Daniel E. Rusyniak,[2,3] Dmitry V. Zaretsky,*[2,3] and Yaroslav I. Molkov*[,1,4]

[1]*Department of Mathematical Sciences, Indiana University Purdue University, Indianapolis, Indiana;* [2]*Department of Emergency Medicine, Indiana University School of Medicine, Indianapolis, Indiana;* [3]*Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana; and* [4]*Department of Mathematics and Statistics, Georgia State University, Atlanta, Georgia*

Submitted 4 May 2017; accepted in final form 1 September 2017

Behrouzvaziri A, Zaretskaia MV, Rusyniak DE, Zaretsky DV, Molkov YI. Circadian variability of body temperature responses to methamphetamine. *Am J Physiol Regul Integr Comp Physiol* 314: R43–R48, 2018. First published September 06, 2017; doi:10.1152/ajpregu.00170.2017.—Vital parameters of living organisms exhibit circadian rhythmicity. Although rats are nocturnal animals, most of the studies involving rats are performed during the day. The objective of this study was to examine the circadian variability of the body temperature responses to methamphetamine. Body temperature was recorded in male Sprague-Dawley rats that received intraperitoneal injections of methamphetamine (Meth, 1 or 5 mg/kg) or saline at 10 AM or at 10 PM. The baseline body temperature at night was 0.8°C higher than during the day. Both during the day and at night, 1 mg/kg of Meth induced monophasic hyperthermia. However, the maximal temperature increase at night was 50% smaller than during the daytime. Injection of 5 mg/kg of Meth during the daytime caused a delayed hyperthermic response. In contrast, the same dose at night produced responses with a tendency toward a decrease of body temperature. Using mathematical modeling, we previously showed that the complex dose dependence of the daytime temperature responses to Meth results from an interplay between inhibitory and excitatory drives. In this study, using our model, we explain the suppression of the hyperthermia in response to Meth at night. First, we found that the baseline activity of the excitatory drive is greater at night. It appears partially saturated and thus is additionally activated by Meth to a lesser extent. Therefore, the excitatory component causes less hyperthermia or becomes overpowered by the inhibitory drive in response to the higher dose. Second, at night the injection of Meth results in reduction of the equilibrium body temperature, leading to gradual cooling counteracting hyperthermia.

circadian; body temperature; methamphetamine; modeling

INTRODUCTION

Vital parameters of living organisms exhibit circadian rhythmicity. Daily variations of body temperature in rats may exceed 1°C, with body temperature at night being higher than during the daytime. Accordingly, temperature fluctuations in response to low doses of amphetamines appear comparable to daily variations of body temperature; intraperitoneal injection of 1 mg/kg of methamphetamine (Meth) increases body temperature by ~1°C (11, 15). Intermediate doses of Meth (5 mg/kg ip) evoke a response of similar magnitude but delayed in time (11, 12). Therefore, it is reasonable to hypothesize that the temperature responses to psychostimulants may differ depending on the time of day. However, despite that rats are nocturnal animals, most of the studies of drug abuse in rodents are performed during the day. To the best of our knowledge, this is the first study to describe circadian variability of responses to Meth.

There is a clear interaction between circadian rhythmicity and the effects of amphetamines. First, amphetamines induce arousal, which could be due to alterations in the neuronal activity controlling circadian rhythms. Administration of Meth results in the activation of orexin-containing neurons (1–3), which are critical for normal circadian rhythmicity and are activated during the active phase of the day cycle. Chronic use of Meth leads to disruption of normal circadian rhythmicity. If Meth is freely provided to rats in the drinking water, they establish an oscillatory pattern of activity (7). Although the period of these oscillations is close to circadian (24–36 h), they are independent of the circuits mediating normal circadian rhythmicity, as these oscillations also emerge in rats with a lesioned suprachiasmatic nucleus (SCN) (6).

To explain the circadian variability of temperature responses to Meth, in this study, we used a mathematical model that was previously developed to explain complex, multiphasic responses of body temperature to Meth during the daytime (11, 12). The model is based on the premise that body temperature variations after Meth administration have both an excitatory and inhibitory drive. The difference in strength between these two signals determines the dynamics of body temperature. Using this model, we assimilated the experimentally obtained nighttime temperature responses to Meth. We then used Bayesian inference analysis to find which parameters of the model are significantly different between phases of the circadian cycle and interpreted the differences we found.

METHODS

Biological Experiments

Animals. Male Sprague-Dawley rats (280–350 g) were individually housed with a 12-h:12-h light/dark cycle at room temperature of 23–25°C with free access to food and water. All animals for which data are reported remained in good health throughout the surgical procedures and experimental protocols as assessed by appearance, behavior, and maintenance of body weight. All procedures described

* Dmitry V. Zaretsky and Yaroslav I. Molkov contributed equally to this work.

Address for reprint requests and other correspondence: Y. Molkov, Dept. of Mathematics and Statistics, Georgia State Univ., 30 Pryor St., 750-COE, Atlanta, GA 30303 (e-mail: ymolkov@gsu.edu).

here were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

Surgical preparation. We anesthetized animals with 1.5–2.0% isoflurane in oxygen, adjusting the concentration of isoflurane as needed, and monitored heart rate and oxygen saturation during surgery using a Pulse Oximeter monitor (model LS1P-10R; Nonin, Plymouth, MN). The depth of anesthesia was checked using withdrawal and corneal reflexes. To measure core body temperature, we placed TA-F40 telemetric transmitters (DSI, St. Paul, MN) in the peritoneum of the animal. Briefly, animals were placed in a dorsal recumbent position with abdominal skin shaved along the midline. We made a 2-cm-long longitudinal medial skin incision, followed by a longitudinal incision of the muscular wall along the white line. We inserted the body of the transmitter into the abdominal cavity and sutured the muscle closed followed by the skin. Carprofen (5 mg/kg sc) was administered immediately after surgery. Animals were returned to their cage for at least 1 wk for recovery. By the end of the recovery period, all animals displayed weight gain, normal levels of activity and mobility, and normal grooming patterns.

Drugs. Methamphetamine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO). It was dissolved in sterile saline at the time of injections and injected at a volume of 1 ml/kg.

Experimental protocol. After 7 days of recovery, rats were brought to the experimental room in their home cages. The cages were placed on telemetry receivers (model RPC-1; Data Sciences International, St. Paul, MN), and the animals were given 1 h to adapt to the new environment. Physiological parameters were sampled for 10 s every minute, and all cages with the animals were left at the receivers of the telemetric system for at least 24 h. The animal in the cage was not disturbed for at least 4 h before injection.

To determine temperature responses to Meth, three groups of animals were studied in home cages at room temperature in rooms with lights on at 8 AM and off at 8 PM. In the first group (Meth-1), we injected Meth at 1 mg/kg ip, in the second (Meth-5) 5 mg/kg ip, and the third normal saline. For each group, half of the animals were assigned to the experiment during the light phase (intraperitoneal injection between 10 and 11 AM), and others were injected in the dark (between 10 and 11 PM). Each animal received only one injection of Meth. The number of animals in each group was chosen so that the amplitude of Meth-induced temperature deviation from the baseline was statistically significantly different between the day and night groups. In our pilot experiments, we found this difference to be ~0.5°C. In our existing daytime data, the standard deviation of the temperature near the maximum response was 0.3°C for both doses of Meth. Therefore, we estimated the number of animals to not exceed P value of 0.05 as $n \geq 5$. In our experiments, saline and Meth-5 groups had $n = 6$; Meth-1 groups had $n = 7$.

Statistical Analysis

For the analysis, we selected the interval starting 1 h before injection and 5 h after injection (total of 360 min). Data were transferred to Microsoft Excel, and 10-min averages were calculated using a template in Excel. Values are shown as means ± SE. Data were compared using a one-way ANOVA with repeated measures followed by Fisher's least-significant-difference post hoc test, where appropriate. $P < 0.05$ was considered to indicate a significant difference in all comparisons.

Mathematical Modeling

Model design. Experimental data on dose dependence of temperature responses to Meth during the daytime were described with acceptable precision by a mathematical model we previously published (11). In short, the model consisted of three components, pharmacokinetics describing concentration of Meth in the blood after injection, a neural network that had Meth-dependent inputs, and a temperature control system driven by a signal from the neural network (Fig. 1).

We assumed that after the injection the drug was absorbed in the blood from the peritoneum and was also simultaneously being eliminated from the blood. Accordingly, drug concentrations were described by the following equations:

$$\frac{d[M_p]}{dt} = -\frac{[M_p]}{\tau_u} \quad (1a)$$

$$\frac{d[M]}{dt} = \frac{[M_p]}{\tau_u} - \frac{[M]}{\tau_d} \quad (1b)$$

where t is time in minutes, M_p is the intraperitoneal drug concentration, and M is the blood drug concentration (both in mg/kg). $[M_p]/\tau_u$ represents drug absorption with time constant τ_u, and $[M]/\tau_d$ represents drug elimination with time constant τ_d. Initial conditions are $[M_p](0) = d$, $[M](0) = 0$ for injections made at *time 0*, where d is the dose of the drug administered. System 1 can be solved explicitly as

$$[M](t) = d\left(\frac{\tau_u}{\tau_d} - 1\right)^{-1}\left(e^{-t/\tau_u} - e^{-t/\tau_d}\right) \quad (2)$$

for $t > 0$ and $[M](t) = 0$ if $t < 0$.

In our study, we use exactly the same formalism as previously (11) to model the neural circuitry as a feed-forward artificial neural network. The outputs of Meth-sensitive neural populations are calculated as follows:

$$P_{Exc}(t) = \sigma\left(w_{Exc} \cdot [M](t) + \gamma_{Exc}\right) \quad (3a)$$

$$P_{Inh}(t) = \sigma\left(w_{Inh} \cdot [M](t) + \gamma_{Inh}\right) \quad (3b)$$

$$P_{HD}(t) = \sigma\left(w_{HD} \cdot [M](t) + \gamma_{HD}\right) \quad (3c)$$

where $\sigma(x) = (1 + tanhx)/2$ is a sigmoid activation function, $w_i(t)$ is the sensitivity of $P_i(t)$ to Meth, and γ_i is the parameter defining baseline activity of the population P_i, $i \in \{Exc, Inh, HD\}$. The activity of medullary population Med is described as follows:

$$P_{Med}(t) = w_{Exc\to Med} \cdot P_{Exc}(t) - w_{Inh\to Med} \cdot P_{Inh}(t) \quad (4)$$

where $w_{Exc\to Med}$ and $w_{Inh\to Med}$ are the weights of the excitatory and inhibitory projections from Exc to Med and Inh to Med, respectively. Inframedullary population sympathetic preganglionic neuron (SPN) duplicates the activity of Med but is additionally excited by the Meth-sensitive high-dose (HD) population as follows:

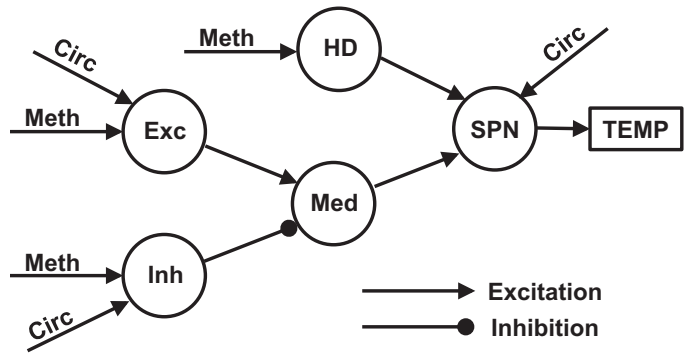

Fig. 1. Schematic of the network mediating the temperature responses to methamphetamine (Meth) suggested by Molkov et al. (11) incorporating circadian phase-dependent inputs. Each circle represents a neural population. Meth-sensitive populations (see "Meth"-labeled arrows) are modeled as an artificial neuron with sigmoidal activation function applied to its input (see text for a detailed description). "Circ"-labeled arrows represent circadian phase-dependent inputs. Exc, excitatory; Inh, inhibitory; HD, high dose; Med, medulla; SPN, sympathetic preganglionic neuron; TEMP, temperature.

$$P_{SPN}(t) = P_{Med}(t) + w_{HD\to SPN} \cdot P_{HD}(t) + \gamma_{SPN} \quad (5)$$

where $w_{HD\to SPN}$ is the weight of the excitatory projection from HD to SPN, and γ_{SPN} is the activity of SPN in absence of inputs from Med and HD.

The temperature dynamics is modeled by a first-order linear ordinary differential equation driven by the SPN signal as follows:

$$\tau_T \frac{dT}{dt} = P_{SPN}(t) - (T - T_0) \quad (6)$$

where T is the body temperature in degrees Celsius, τ_T is the time constant of the temperature response, and T_0 is the baseline body temperature. See previous work (11) for all parameter values.

The equilibrium temperature was defined as the temperature at which the right-hand side of (*Eq. 6*) is equal to zero after Meth is completely washed out. Accordingly, it is a function of baseline activities of the nodes, which can be calculated as follows:

$$T_{eq} = w_{Exc\to Med}\sigma(\gamma_{Exc}) - w_{Inh\to Med}\sigma(\gamma_{Inh}) + \gamma_{SPN} + T_0 \quad (7)$$

Circadian variability. The major assumption of the study was that animals have different status of the Meth-response system described above during active and inactive phases of the day. Specifically, we assumed that the responses are mediated by the same network, but the baseline activity of its nodes may be different during the day and at night. Accordingly, we used the experimental data sets we obtained for the day and nighttime to estimate the parameters of the model defining baseline activities of the nodes, i.e., γ_{Exc}, γ_{Inh}, and γ_{SPN}. All other parameters were assumed independent of the circadian phase, so we used their values estimated in our original Meth-response study (11). In our model, the baseline activity levels of the nodes are supposed to be defined by synaptic and neuromodulatory inputs independent of Meth, so the day versus night differences in the values of parameters γ_{Exc}, γ_{Inh}, and γ_{SPN} can be interpreted as circadian-related (changes of) external inputs to the network.

Statistical Analysis. We used the Bayesian approach for inferring the model parameters. We then employed statistical analysis to calculate statistical significance of changes in γ_{Exc}, γ_{Inh}, and γ_{SPN} between day and night. To do this, we constructed a corresponding posterior probability density function (PDF) of the parameter vector $\gamma = (\gamma_{Exc}, \gamma_{Inh}, \gamma_{SPN})$. According to Bayes's rule (4), this probability distribution is proportional to the probability that an observed temperature time series $\{T_i\}$ is produced by our model with the given γ, usually referred to as a likelihood. Assuming that the residuals are normally distributed and noninformative priors for the parameters, the posterior PDF, $p(\gamma \mid \{T_{i,d}\})$, is given by the following formula:

$$p(\gamma \mid \{T_{i,d}\}) \sim \exp\left\{ -\sum_{d=1,5} \sum_{i=30}^{300} \frac{[T_{i,d} - T(\gamma, i, d)]^2}{2\sigma_{i,d}^2} \right\} \quad (8)$$

where $T(\gamma,i,d)$ is the temperature calculated by our model at time i for dose d with the given $\gamma \cdot T_{i,d}$ and $\sigma_{i,d}$ are respectively a mean and a standard deviation of the body temperature over a corresponding group of animals for dose d at time i by increments of 10 min. We used the data starting from 30 min after the injection when the effect of the stress becomes negligible.

We used the Markov Chain Monte Carlo (MCMC) approach, specifically the Metropolis-Hastings algorithm adapted for reconstruction of dynamical systems (5, 8–10, 13, 14), to create a statistical ensemble of points distributed according to the PDF (8). Once we had the ensembles, we calculated mean values and standard errors for the three parameter estimates for each combination of time of day and dose of Meth. We then used a two-sample *t*-test to evaluate the statistical significance of differences between the groups. P value <0.05 was accepted as a statistically significant effect of the daytime on the corresponding parameter of the model.

RESULTS

At Night Rats Have Higher Body Temperature and Stronger Stress Response

Baseline (initial) body temperature of animals before the injections was 0.8°C higher on average at night for all groups (saline, Meth-1, and Meth-5, Fig. 2). The intraperitoneal injection of saline evoked a transient increase of the body temperature (Fig. 2*A*). This increase developed faster and was almost twice as great during the nighttime. The body temperature returned to initial values approximately 1 h after the injection and did not change significantly for the remaining 5 h after that.

At Night Rats Exhibit a Weaker Hyperthermic Response to 1 mg/kg of Meth

A lower dose of Meth (1 mg/kg) induced monophasic hyperthermia both during the day and at night (Fig. 2*B*). However, the maximal increase of the temperature was approximately half at night compared with the day. The maximal hyperthermia was observed 30 min after the injection at night, whereas it occurred after 90 min during the day.

At Night, There Is No Hyperthermic Response to the Intermediate Dose of Meth

A higher dose of Meth (5 mg/kg) when administered during the daytime caused a delayed hyperthermic response peaking at ~200 min after the injection (Fig. 2*C*). In contrast, at night, the same dose did not produce hyperthermia. Moreover, the temperature had a clear tendency to decrease, which was not observed during the day.

At Night, the Excitatory Node Has Substantially Higher Baseline Activity

We used *Eqs. 1–6* to fit the above experimental data (see Fig. 3 and its legend for the most probable model responses and

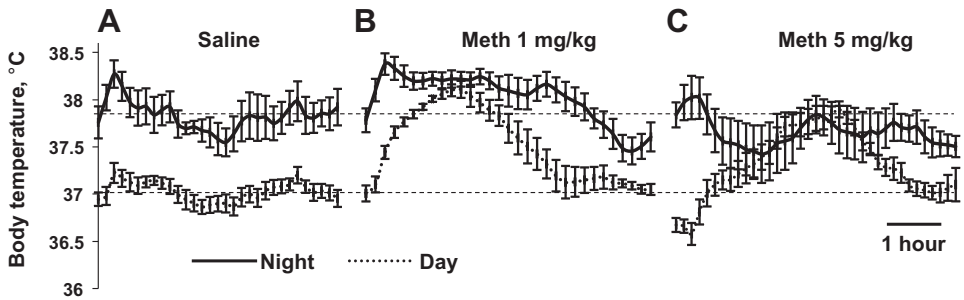

Fig. 2. Experimental data on body temperature responses to intraperitoneal injections of saline (*A*) and 2 different doses of Meth (*B* and *C*) at night (solid lines) and during the day (dotted lines). Data are shown as means ± SE ($n = 6$). Dashed lines show approximate initial temperature.

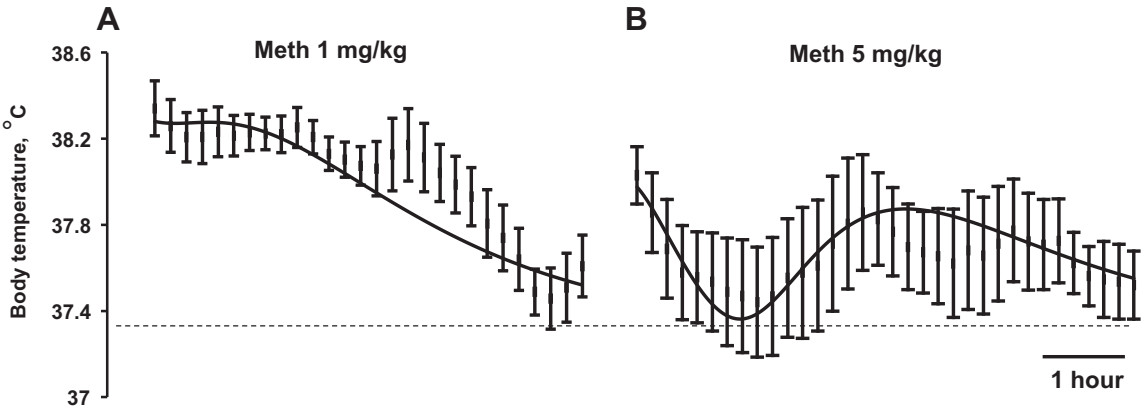

Fig. 3. Temperature dynamics as reproduced by the model (solid curves) with parameters corresponding to the mode of probability density function (*Eq. 8*) superimposed on experimental data obtained at night (error bars) in the 30 min after the injection of 1 mg/kg of Meth (*A*) and 5 mg/kg of Meth (*B*). χ^2 test (16), sum squared standardized residual, $\chi^2 = 33.4$; number of degrees of freedom, $\nu = 51$; *P* value = 97%. Coefficients of determination: $R^2 = 0.74$ for 1 mg/kg; $R^2 = 0.51$ for 5 mg/kg. Dashed line shows the equilibrium temperature estimate.

conventional goodness-of-fit measures). Then we sampled the PDF (*Eq. 8*) to estimate parameters γ_{Exc}, γ_{Inh}, and γ_{SPN} and their standard errors during the day and at night (Fig. 4) as described in METHODS. We found that, at night, the parameters defining baseline activities of Exc and Inh nodes, γ_{Exc} and γ_{Inh}, respectively, have statistically significantly higher values (Fig. 4, *A* and *B*). Specifically, from day to night, the drive to excitatory node, γ_{Exc}, changes from about -0.35 to ~0.05 (Fig. 4*A*), which corresponds to an increase in baseline activity of the Exc from ~30% to above 50% of maximum. The day-to-night change in the drive to the inhibitory node is relatively small from ~ -1.3 to -1.0 (Fig. 4*B*) corresponding to the increase in its baseline activity from ~7 to 12%. Therefore, the baseline activity of the excitatory node is increased to a substantially greater extent than of the inhibitory node, which has a net positive effect on the baseline activity of the medullary node (see Fig. 1). There was no statistically significant difference in γ_{SPN} values between day and night (Fig. 4*C*).

Meth Reduces the Equilibrium Temperature in a Dose-Independent Manner

As mentioned, during the day, the average baseline temperature was ~0.8°C lower than at night. Specifically, the initial temperatures during the day and at night were 37.02 ± 0.04°C vs. 37.81 ± 0.09°C, respectively. In rats injected with saline, the equilibrium temperature (see *Eq. 7*) was equal to the baseline temperature. However, after Meth injections, the equilibrium temperature appeared to be significantly lower. Specifically, during the day, the equilibrium temperature was 36.66 ± 0.09°C, and at night it was 37.34 ± 0.06°C (Figs. 3 and 5). Hence, on average, Meth injection caused a statistically significant decrease in the equilibrium temperature by 0.36°C during the day and by 0.47°C at night.

To check whether this effect is dose dependent or not, we estimated the parameters γ_{Exc}, γ_{Inh}, and γ_{SPN} separately for the groups of rats injected with 1 mg/kg and 5 mg/kg of Meth at night (Fig. 6). We did not find a statistically significant difference between the two doses in individual parameter values (Fig. 6, *A*–C), as well as in the equilibrium temperatures (Fig. 6*D*).

DISCUSSION

Mechanisms of Circadian Variations of Meth Responses

As we previously described (11, 12), the temperature response to the administration of Meth has three distinct components putatively mediated by three distinct neuronal populations/nodes (Fig. 1) that have different activation thresholds for Meth and, hence, get activated at different Meth concentration levels.

The node most sensitive to Meth is labeled Exc in Fig. 1. It is an excitatory node that can be activated by a low dose of Meth. This node mediates virtually immediate increase in the body temperature after injection of 1 mg/kg Meth during the day (Fig. 2, *middle*, dotted curve). By estimating the parameters of the model using the data obtained at night (Fig. 2, *middle and right*), we found that, at night, the excitatory node has much higher baseline activity (~30% of max during the day vs. ~50% of max at night, see also Fig. 3). We speculate that, at night, the corresponding population of neurons receives

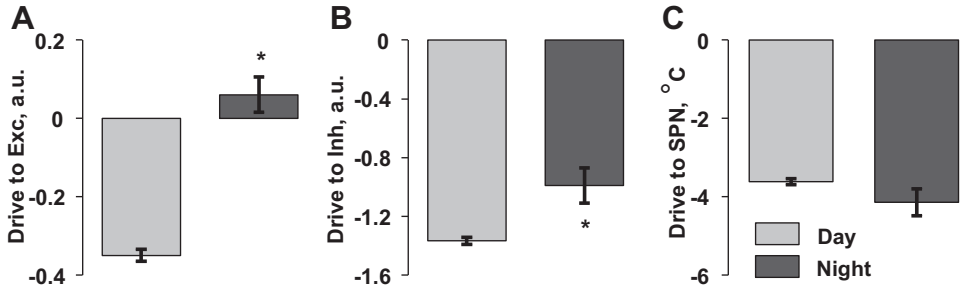

Fig. 4. Inferences of circadian phase-dependent parameters during the day (light gray) and at night (dark gray) shown as means ± SD of the posterior probability density function (see METHODS). *Statistically significant difference of daytime and nighttime values. Exc, excitatory; Inh, inhibitory; SPN, sympathetic preganglionic neuron.

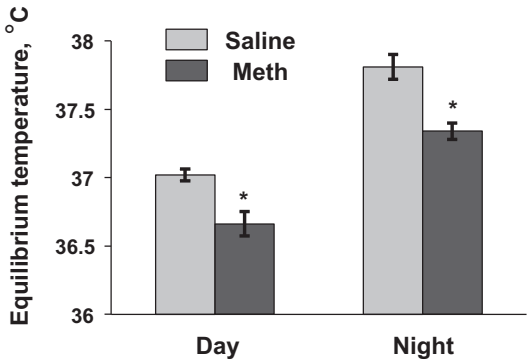

Fig. 5. Estimates of equilibrium temperature for each combination of saline vs. Meth and day vs. night. Groups injected with either dose of Meth are pooled together.

additional excitatory drive, which may be treated as circadian rhythm-related input.

Because of saturation, higher baseline activity of the excitatory node leaves less room for this population to further increase its activity after Meth injection. Specifically, during the day, 70% of the maximal activity is still available, whereas only 50% remains available at night. Accordingly, the low dose of Meth (1 mg/kg) additionally activates this population to a lesser extent at night, which contributes to the attenuation of hyperthermia following Meth injection (Fig. 2, *middle*).

The intermediate dose of Meth used in our study (5 mg/kg) is sufficient to activate, not only the excitatory, but also the inhibitory node (Fig. 1). During the day, the excitatory and inhibitory components are almost level until the concentration of Meth falls below the threshold of the inhibitory component activation (11). However, at night, because of saturation of the excitatory node activity as described above, the inhibitory node starts dominating during the initial phase of the response, thus causing a transient reduction in temperature (Fig. 4, *right*).

Another mechanism underlying a smaller increase of the temperature after the low dose of Meth and a slightly hypothermic response to the intermediate dose at night is a reduction in the equilibrium temperature after the Meth injection. Because the initial temperature at night appears almost a half degree higher than the equilibrium temperature (37.81°C vs. 37.34°C, respectively), the already weakened effect of the excitatory node activation occurs on the top of the declining trend toward the lowered equilibrium temperature.

Mechanisms of Higher Baseline Temperature at Night

In this study, we found that the excitatory node (Exc) of the neuronal network responsible for temperature fluctuations after Meth injections (Fig. 1) has higher baseline activity. Specifically, Exc increases its baseline activity from ~30% of max during the day to ~50% at night. Such an increase, according to *Eq. 7*, would result in an ~2°C increase in temperature. At night, the baseline temperature was elevated by 0.8°C only, so some compensatory mechanisms should be involved. In Fig. 3*B*, one can see that parameter γ_{Inh}, which defines the baseline activity of the inhibitory node (see Inh in Fig. 1), is also increased at night. This has a negative effect on the equilibrium temperature of ~0.5°C. The remaining compensation (~0.7°C) must have been provided by other, e.g., thermoregulatory, mechanisms.

Mechanisms of Lower Equilibrium Temperature After Injection of Meth

We found that, after either dose of Meth, the equilibrium temperature reduces relative to the baseline temperature by 0.36°C and 0.47°C during the day and at night, respectively (Fig. 5). The equilibrium temperature (as described by *Eq. 7*) depends on baseline activities of three nodes in the network, $\sigma(\gamma_{Exc})$, $\sigma(\gamma_{Inh})$, and γ_{SPN}. Accordingly, the reduction in the equilibrium temperature can result from either lower $\sigma(\gamma_{Exc})$ and/or γ_{SPN} or higher $\sigma(\gamma_{Inh})$. Unfortunately, for obvious reasons, it is impossible to infer these quantities in our experimental paradigm without injecting Meth. However, we can speculate that, by design of the model, the dependence of Exc and Inh nodes on Meth concentration is already explicitly described by *Eq. 3*, and the reduction in equilibrium temperature occurs due to long-lasting alterations in the thermoregulatory system manifesting themselves by a reduction in γ_{SPN}.

Putative Neuronal Structures and Neurotransmission

Our original model was based on existing but rather limited experimental identification of structures responsible for temperature variations after Meth injections (11). We speculated that the excitatory component (Exc node in Fig. 1) of the response is mediated by dorsomedial hypothalamus, which projects to the presympathetic neurons in the ventromedial medullary region (raphe pallidus, Med node) relaying the signal further down to sympathetic preganglionic neurons in the spinal cord (SPN node). The inhibitory drive (Inh) puta-

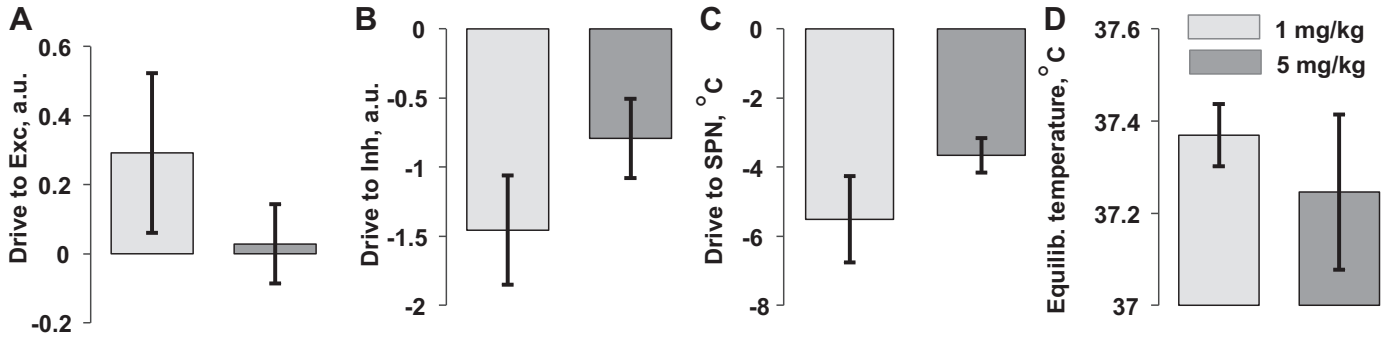

Fig. 6. Comparison of circadian phase-dependent parameter estimates at night for experimental groups injected with 1 mg/kg of Meth (light gray) and 5 mg/kg of Meth (dark gray). No statistically significant differences observed. Exc, excitatory; Inh, inhibitory; SPN, sympathetic preganglionic neuron.

tively originates from supramedullary (preoptic nucleus or ventrolateral periaqueductal gray matter) and/or intramedullary (rostroventral lateral medulla) structures.

Previously, we demonstrated that temperature responses to Meth are mediated by orexinergic neurotransmission (1). Moreover, using our mathematical model (11), we inferred that both the excitatory and inhibitory components are likely to be activated by orexinergic signals induced by Meth administration. In the present study, we show that, at night, the excitatory and inhibitory nodes receive additional drive. Importantly, the night is an active time for rats when orexinergic neurons are firing. Therefore, it is reasonable to suggest that additional drive to the excitatory and inhibitory nodes emerging at night is orexinergic. This suggestion leads to an interesting prediction. We previously reported that blockade of orexinergic neurotransmission during the day did not evoke any thermal responses by itself (1). However, at night, when the status of the thermoregulatory network is modulated by orexinergic inputs, the same manipulation should lead to significant body temperature variations.

Perspectives and Significance

In this study, we were the first to measure and compare the body temperature responses in rats to injections of Meth during daytime and at night. We found that, at night, which is their active time of the day, rats exhibit much weaker temperature fluctuations after Meth administration compared with their time of rest.

Using mathematical modeling, we inferred that, at night, the neuronal circuitry mediating thermal responses to Meth receives additional excitatory inputs primarily converging at the excitatory node, which has the lowest activation threshold to Meth. These inputs substantially increase the baseline activity of this node. Therefore, its additional activation after Meth administration at night turns out to be much weaker compared with the daytime because of saturation.

Furthermore, we observed that, in the long run, the temperature tends to stabilize at lower levels after Meth washes out. This reduction in the equilibrium temperature was similar for both doses of Meth used in this study and was substantially stronger at night, which further reduced hyperthermia and exaggerated hypothermic components of the response.

GRANTS

This work was supported by National Institute on Drug Abuse Grant R01-DA-026867, IUPUI iM2CS-GEIRE, IUPUI Research Support Fund Grants (RSFG) to Y. Molkov and D. Zaretsky, and a Purdue Research Foundation Award to Y. Molkov. Furthermore, this work was conducted in a facility constructed under support from National Center for Research Resources Grant C06-RR-015481-010.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.B., D.V.Z., and Y.I.M. analyzed data; A.B., D.V.Z., and Y.I.M. interpreted results of experiments; A.B., D.V.Z., and Y.I.M. prepared figures; A.B. and Y.I.M. drafted manuscript; A.B., M.V.Z., D.E.R., D.V.Z., and Y.I.M. approved final version of manuscript; M.V.Z. and D.V.Z. performed experiments; D.E.R., D.V.Z., and Y.I.M. conceived and designed research; D.V.Z. and Y.I.M. edited and revised manuscript.

REFERENCES

1. **Behrouzvaziri A, Fu D, Tan P, Yoo Y, Zaretskaia MV, Rusyniak DE, Molkov YI, Zaretsky DV.** Orexinergic neurotransmission in temperature responses to methamphetamine and stress: mathematical modeling as a data assimilation approach. *PLoS One* 10: e0126719, 2015. doi:10.1371/journal.pone.0126719.
2. **Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper CB, Scammell TE.** Fos expression in orexin neurons varies with behavioral state. *J Neurosci* 21: 1656–1662, 2001.
3. **Fadel J, Bubser M, Deutch AY.** Differential activation of orexin neurons by antipsychotic drugs associated with weight gain. *J Neurosci* 22: 6742–6746, 2002. doi:20026632.
4. **Jeffreys H.** *Scientific Inference*. Cambridge, UK: Cambridge University, 1973.
5. **Loskutov EM, Molkov YI, Mukhin DN, Feigin AM.** Markov chain Monte Carlo method in Bayesian reconstruction of dynamical systems from noisy chaotic time series. *Phys Rev E Stat Nonlin Soft Matter Phys* 77: 066214, 2008. doi:10.1103/PhysRevE.77.066214.
6. **Mohawk JA, Baer ML, Menaker M.** The methamphetamine-sensitive circadian oscillator does not employ canonical clock genes. *Proc Natl Acad Sci USA* 106: 3519–3524, 2009. doi:10.1073/pnas.0813366106.
7. **Mohawk JA, Menaker M.** A new (and different) circadian pacemaker. *Cell Cycle* 8: 2861–2862, 2009. doi:10.4161/cc.8.18.9402.
8. **Molkov YI, Loskutov EM, Mukhin DN, Feigin AM.** Random dynamical models from time series. *Phys Rev E Stat Nonlin Soft Matter Phys* 85: 036216, 2012. doi:10.1103/PhysRevE.85.036216.
9. **Molkov YI, Mukhin DN, Loskutov EM, Feigin AM, Fidelin GA.** Using the minimum description length principle for global reconstruction of dynamic systems from noisy time series. *Phys Rev E Stat Nonlin Soft Matter Phys* 80: 046207, 2009. doi:10.1103/PhysRevE.80.046207.
10. **Molkov YI, Mukhin DN, Loskutov EM, Timushev RI, Feigin AM.** Prognosis of qualitative system behavior by noisy, nonstationary, chaotic time series. *Phys Rev E Stat Nonlin Soft Matter Phys* 84: 036215, 2011. doi:10.1103/PhysRevE.84.036215.
11. **Molkov YI, Zaretskaia MV, Zaretsky DV.** Meth math: modeling temperature responses to methamphetamine. *Am J Physiol Regul Integr Comp Physiol* 306: R552–R566, 2014. doi:10.1152/ajpregu.00365.2013.
12. **Molkov YI, Zaretsky DV.** Balanced excitation and inhibition in temperature responses to meth. *Temperature (Austin)* 1: 154–156, 2014. doi:10.4161/2167549X.2014.968483.
13. **Morozova E, Yoo Y, Behrouzvaziri A, Zaretskaia M, Rusyniak D, Zaretsky D, Molkov Y.** Amphetamine enhances endurance by increasing heat dissipation. *Physiol Rep* 4: 4, 2016. doi:10.14814/phy2.12955.
14. **Mukhin DN, Feigin AM, Loskutov EM, Molkov YI.** Modified Bayesian approach for the reconstruction of dynamical systems from time series. *Phys Rev E Stat Nonlin Soft Matter Phys* 73: 036211, 2006. doi:10.1103/PhysRevE.73.036211.
15. **Myles BJ, Jarrett LA, Broom SL, Speaker HA, Sabol KE.** The effects of methamphetamine on core body temperature in the rat--part 1: chronic treatment and ambient temperature. *Psychopharmacology (Berl)* 198: 301–311, 2008. doi:10.1007/s00213-007-1061-z.
16. **Press WH.** *Numerical Recipes: The Art of Scientific Computing*. Cambridge, New York: Cambridge University, 2007.