RESEARCH PAPER

Temperature 1:3, 257–267; October/November/December 2014; Published with license by Taylor & Francis Group, LLC

Repeated nitrous oxide exposure in rats causes a thermoregulatory sign-reversal with concurrent activation of opposing thermoregulatory effectors

Douglas S Ramsay[1,2,3,*], Stephen C Woods[4], and Karl J Kaiyala[1]

[1]Departments of Oral Health Sciences; University of Washington; Seattle, WA USA; [2]Orthodontics; University of Washington; Seattle, WA USA; [3]Pediatric Dentistry at the University of Washington; Seattle, WA USA; [4]Department of Psychiatry and Behavioral Neuroscience; University of Cincinnati; Cincinnati, OH USA

Keywords: allostasis, drug addiction, drug tolerance, homeostasis

Abbreviations: DHL, dry heat loss; EHL, evaporative heat loss; HL, heat loss; HP, heat production; N_2, nitrogen; N_2O, nitrous oxide; O_2, oxygen; Tc, core temperature; Tsel, selected ambient temperature

Initial administration of 60% nitrous oxide (N_2O) to rats at an ambient temperature of 21°C decreases core temperature (Tc), primarily via increased heat loss (HL). Over repeated N_2O administrations, rats first develop tolerance to this hypothermia and subsequently exhibit hyperthermia (a sign-reversal) due primarily to progressive increases in heat production (HP). When rats initially receive 60% N_2O in a thermal gradient, they become hypothermic while selecting cooler ambient temperatures that facilitate HL. This study investigated whether rats repeatedly administered 60% N_2O in a thermal gradient would use the gradient to behaviorally facilitate, or oppose, the development of chronic tolerance and a hyperthermic sign-reversal. Male Long-Evans rats (N = 16) received twelve 3-h administrations of 60% N_2O in a gas-tight, live-in thermal gradient. Hypothermia (Sessions 1–3), complete chronic tolerance (Sessions 4–6), and a subsequent transient hyperthermic sign-reversal (Sessions 7–12) sequentially developed. Despite the progressive recovery and eventual hyperthermic sign-reversal of Tc, rats consistently selected cooler ambient temperatures during all N_2O administrations. A final 60% N_2O administration in a total calorimeter indicated that the hyperthermic sign-reversal resulted primarily from increased HP. Thus, rats did not facilitate chronic tolerance development by moving to warmer locations in the gradient, and instead selected cooler ambient temperatures while simultaneously increasing autonomic HP. The inefficient concurrent activation of opposing effectors and the development of a sign-reversal are incompatible with homeostatic models of drug-adaptation and may be better interpreted using a model of drug-induced allostasis.

Introduction

Drug tolerance, dependence and withdrawal are hypothesized to be manifestations of a common underlying 'adaptive' response that develops with repeated drug use.[1-7] Most adaptation models of drug tolerance and addiction trace their origin to the concept of homeostasis.[8] In brief, when a drug effect initially perturbs a homeostatically regulated variable, this triggers adaptive responses that oppose and eventually fully counter the drug-elicited perturbation while the drug is present (i.e., tolerance develops). With repeated drug use, the individual transitions to a dependent state wherein drug-induced perturbations are effectively countered by acquired compensatory responses. In the dependent state, if the drug effect dissipates more rapidly than the compensatory responses, symptoms of drug withdrawal occur.

As proposed by Walter Cannon, the core concept of homeostasis is captured by the title of his book, "The Wisdom of the Body"[9]; i.e., the body's homeostatic wisdom enables a coordinated array of physiological and behavioral responses to be elicited that stabilize and defend critical regulated physiological variables as they become perturbed by drugs or other stimuli.[10] Several aspects of homeostasis are especially relevant to adaptation models of drug addiction. Dworkin[11] reiterated a widely held belief about drug tolerance by stating "... even with very many administrations, drug effects sometimes diminish to zero but do not invert to the opposite" (p. xiv).

*Correspondence to: Douglas S Ramsay; Email: ramsay@uw.edu
Submitted: 03/31/2014; Revised: 06/19/2014; Accepted: 06/26/2014
http://dx.doi.org/10.4161/23328940.2014.944809

Thus, homeostatic adaptations "wisely" do not *over*respond, so as to *over*compensate for the disturbance. Another principle of homeostasis is the 'wise', efficient central coordination of corrective responses such that they work harmoniously together and are not in concurrent competition with one another.[12,13] In addition, it is commonly suggested that homeostasis preferentially recruits the least costly effector response available to correct a perturbation.

The objective of the present research was to rigorously evaluate these homeostatic concepts as they pertain to adaptation models of chronic drug use. The experimental model of tolerance development to nitrous oxide (N_2O)-induced hypothermia is well suited for this purpose. Thermoregulation is an archetype of a homeostatically regulated system. Core temperature (Tc), the regulated variable,[14,15] lends itself to accurate, continuous, and non-invasive telemetric measurement. Tc has an extensive history as a dependent measure in studies of drug tolerance.[16-18] Of great advantage for research focused on physiological regulation, much is known about the physics and physiological effects that underlie thermoregulation. In particular, the underlying determinants of Tc can be quantified accurately at the level of metabolic heat production (HP) and body heat loss (HL).[19] Telemetric measurement of Tc coupled with simultaneous total calorimetry (combining indirect and direct calorimetry) allows HP and HL to be measured noninvasively and continuously in undisturbed rats.[19-22]

N_2O is a pharmacologically active gas and an abused inhalant.[23] It is administered via inhalation and among other effects, causes hypothermia upon initial administration in rats.[24-26] N_2O's low solubility in blood and tissues means that a steady-state concentration can be quickly achieved and easily maintained.[27] Once equilibration occurs, the N_2O concentration in an animal simply equals the N_2O concentration in the chamber. The ability to "clamp" the drug concentration is an important advantage when interpreting acute and chronic adaptations to N_2O.

Acute (intrasessional) and chronic (intersessional) tolerance develop to 60% N_2O's hypothermic effect in the rat.[24,26] Total calorimetric assessments revealed that the marked drop in Tc during an initial administration of 60% N_2O is due primarily to a rapid elevation in HL that results in a state of negative heat balance.[19,20] Increases in HP can occur during the course of an initial N_2O administration and result in the development of acute tolerance,[19] while progressive increases in HP over subsequent N_2O administrations result in the development of chronic tolerance.[21,22]

In a previous study, a subset of rats that were relatively insensitive to the hypothermic effect of an initial 60% N_2O administration developed a *hyper*thermic Tc during subsequent N_2O administrations rather than merely becoming tolerant.[28] Subsequent research revealed that initially insensitive rats exhibited a prompt increase in HP when initially administered N_2O that was of sufficient magnitude to counter the increase in HL elicited by the N_2O[20]; i.e., the rats considered 'initially insensitive' at the level of Tc were actually initially *hyperresponsive* at the level of HP with the consequence that there was little or no change of Tc when first exposed to N_2O. The magnitude of the HP response increases progressively over repeated N_2O administrations, which contributes to chronic tolerance development, and with additional administrations causes rats to eventually exhibit a hyperthermic overcompensation of Tc.[21,22,29]

Rats recruit both autonomic and behavioral thermoeffectors to maintain Tc, although behavioral effectors often provide a quicker and more energetically efficient way to influence Tc.[30] Behavioral thermoregulation can be assessed by allowing animals to select their preferred ambient temperature (Tsel) in a thermal gradient.[30-32] Rats given an initial exposure to 60% N_2O while in a thermal gradient develop the usual hypothermia, and at the same time they move to a region of the gradient where the ambient temperature is cooler.[32] The goal of the present study was to determine whether chronic tolerance, with or without a sign-reversal hyperthermia, develops over repeated exposures to 60% N_2O in a thermal gradient. A second goal was to determine how behavioral and autonomic effectors contribute to the restoration and/or overcompensation of Tc and whether effector interaction is compatible with a homeostatic interpretation.

Materials and Methods

Subjects

Male Long-Evans rats (Charles River, N = 16; 8 squads of 2 each) arrived in the lab at 25–28 d of age. Both rats in a squad were housed together in a polycarbonate tub with free access to water and pelleted chow (5053 PicoLab Rodent Diet 20, Animal Specialties and Provisions, Quakertown, PA). The housing room and live-in thermal gradients had a 12-h:12-h light/dark cycle (lights on at 0700 h). The housing room had an ambient temperature of 22 ± 1°C. Following surgery and recovery, experimental testing began 12 d after the rats' arrival in the lab (141.1± 21.2 g, Mean ± SD) and lasted 31 d when the rats weighed 362.9 ± 26.7 g. All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Thermal gradient

The thermal gradient system allows a rat to select its preferred ambient temperature as a function of its choice of location within an alleyway. In brief, a removable rectangular acrylic alleyway is suspended within an insulated copper shell that is cooled at one end and heated at the other end, thereby creating a temperature continuum along the length of the alleyway. Our lab's 2 thermal gradients are based on a previously published design[30,31] that we modified to make the gradients gas-tight. Pelleted chow and water were freely available in the center of the alleyway. During the present study, the alleyway had an ambient temperature range of ~30°C, with 7.6°C ± 0.46 and 37.9°C ± 0.22 (Mean ± SD) at the 2 ends. The relationship between the temperature at each location along the length of the alleyway was similar to that described by Gordon and colleagues.[31] [A photograph of our thermal gradient system and additional details about its design and operation are available in Part I of the online supplement.]

One of 2 gas mixtures was delivered to each thermal gradient, i.e., either control gas consisting of room air, or 60% N_2O. Specifically, the control gas was made from room air that was

purified, dehumidified and compressed and then delivered to the thermal gradient at a flow rate of 10 L/min. The N_2O gas had the same flow rate and was composed of 60% N_2O, 21% oxygen (O_2), and 19% nitrogen (N_2). [A 10 L/min blend of 79% N_2O, 21% O_2, and 0% control gas was delivered for the first 12 min of the 60% N_2O gas condition to achieve the targeted 60% N_2O gas concentration more quickly.] Concentrations of N_2O, O_2, and CO_2 were measured using an infrared gas analyzer placed in the incurrent and excurrent gas lines connected to the gradient's copper shell.

Total calorimetry, Tc, and N_2O administration chambers

Independent total calorimetry chambers that also measure Tc telemetrically served as gas-tight exposure chambers for N_2O. Total calorimetry simultaneously measures the rates of total HL and metabolic HP, the 2 underlying determinants of Tc. [See Part II of the online supplement for additional details.]

Telemetric measurement of Tc, data acquisition and instrument control

Telemetric measurement of Tc was accomplished using a commercial system from Data Sciences International (Saint Paul, MN) that consists of a Data-Exchange Matrix, Physio-Tel Receiver (Model RPC-1), Dataquest ART 4.2 software, and an implantable battery-powered temperature sensor (model TA-F40) implanted in the rat's peritoneal cavity. The antenna wires surrounding the alleyway suspended inside the thermal gradient are exteriorized through a sealed port and connected to the commercial receiver base. The antenna system within the direct calorimeter consists of 2 radio ferrite coils oriented perpendicularly to each other that are epoxied underneath a Plexiglas platform that holds them ~2 mm above the floor of the calorimeter. Wires from these coils exit the calorimeter through a sealed port and are connected to the commercial receiver base. All other instrument control and data acquisition were performed using custom programs written in LabVIEW 6.8 (National Instruments, Austin, Texas).

Surgical placement of the telemetric temperature sensor

At least one week prior to the start of testing, a telemetric temperature sensor was implanted surgically into each rat's peritoneal cavity under isoflurane anesthesia while the rat was on a 39°C heating pad. Meloxicam (an NSAID) was provided in the drinking water (0.02 mg/ml H_2O) from 1 d before to 2 d after surgery.

Procedures

Each rat received 12, 3-h administrations of 60% N_2O in the thermal gradient over a 26-d period that commenced on a Monday, 13 d after arrival in the lab. The thermal gradients' 2 temperature-controlled recirculating water baths were set at 1°C and 42°C and circulated water around each end of the gradient's copper shell from Monday at 0900 h through Friday at 1600 h each week. Each rat was taken from the housing room and placed in the thermal gradient at 1600 h on Monday and returned to the housing room on Friday at 1600 h. Between 1200 and 1500 h on Tuesday, Thursday and Friday, 60% N_2O was administered in the thermal gradient. As a within-subject control condition, control gas instead of N_2O was administered on Wednesday between 1200 and 1500 h. Rats were briefly removed from the thermal gradient between 1600–1615 h on Wednesdays so that the waste trays could be cleaned, additional food provided as needed, and the alleyway inspected. Thermal gradient components were washed/sanitized each weekend. Rats were weighed on Monday, Wednesday and Friday.

After completing the thermal gradient phase of the experiment, rats were tested in the total calorimeters using a counterbalanced, cross-over design, so that each rat received both a control gas and a 60% N_2O exposure occurring on Tuesday and Thursday of the following week. Each rat was placed in the calorimeter at 1000 h. Control gas was delivered for a 2-h baseline period (1000–1200 h), followed by 3 h of the assigned gas condition (i.e., continued control gas or 60% N_2O). Control gas was delivered from 1500–1515 h and the rat was then returned to the housing room.

Data reduction

Thermal gradient data

The rat's position in the alleyway was recorded at 7-s intervals via infrared beam breaks from 24 locations, 7.62 cm apart. Position was computed as the average value of the location numbers of the interrupted infrared signals. Distance traveled (Dist.) was computed as the absolute value of the difference between successive time-stamped rat-position values multiplied by 7.62 cm. Distance was summed during each 6-min bin. Ambient temperature at the rat's position within the gradient (Tsel) was logged at the time the rat's position was recorded. Tsel was calculated as the mean temperature of the thermistor(s) corresponding with the interrupted infrared beam location(s). Tc data were recorded at 30-s intervals. Median Tc and mean Tsel values were computed within each 6-min bin. Gas concentration data were recorded from each gradient at 1-min intervals.

Total calorimetry and Tc

Dependent variables obtained from the calorimetry tests were Tc, HP, dry heat loss (DHL) and evaporative heat loss (EHL). Tc was recorded at 15-s intervals and mean Tc was calculated for each 6-min bin. HP and HL data were recorded at 10-s intervals. Average HP and HL were calculated for each 6-min bin. Gas concentration data were recorded from each calorimeter at 1-min intervals.

Statistical analyses

The correlated within-subjects longitudinal data were analyzed using the linear mixed-model program in SPSS Statistics 20 (IBM, Somers, NY). Session and condition were treated as fixed effects. Unless otherwise specified, unstructured covariance matrices were employed for statistical comparisons because

variances for thermal outcomes differed between N_2O and control-gas conditions. For comparisons between N_2O and control-gas conditions, means and 95% confidence intervals were adjusted for baseline values.

An experimental period was defined as the 3-h interval (0 to 180 min) in which 60% N_2O or control gas was administered. For thermal-gradient studies, statistical analyses involved 4 time periods: baseline (−60 to 0 min), early-experimental period (0 to 90 min), late-experimental period (90 to 180 min), and post-experimental period (180 to 240 min). For calorimetry data, early- and late-experimental periods were defined as above, and the baseline period was the 12 min immediately prior to the experimental period (the shorter baseline allowed for abatement of the initial hyperthermia associated with handling during placement into the calorimeter 120 min prior to the experimental period). The first 2 6-min bins of HP and HL data after the onset of N_2O were omitted from analysis due to their potential for artifact.[19] There was no post-experimental period for the calorimetry test sessions.

Normally-distributed data (Tc, Tsel, HP, HL and Δdistance) were summarized as means with 95% confidence intervals (CI) to convey the magnitude and uncertainty range of each outcome. Distance magnitudes were summarized in terms of medians ± 05th percentile (p05) and 95th percentile (p95). Baseline values were defined as the means over the 60 min prior to N_2O onset for normally distributed thermal gradient outcomes (median for distance) and the 12 min prior to N_2O onset for total calorimetry outcomes. The null hypothesis for baseline-adjusted comparisons was that N_2O = control. Accordingly, 95% confidence intervals for N_2O compared to control conditions that exclude zero are significant at $P < 0.05$, 2-tailed. We did not adjust for multiple comparisons due to the conundrums and misplaced emphasis that accompany this class of procedures when implemented in the context of basic preclinical research[33-35] [see Part III of the online supplement for additional details]. Readers are urged to judge our results on the basis of the 95% confidence intervals and their coherence across sessions.

Results

Qualitative patterns of Tc and Tsel during N_2O administrations

Figure 1 illustrates the temporal dynamics of Tc and Tsel over 3-h sessions with 60% N_2O in the thermal gradient. The evolution of patterns of Tc within and across N_2O inhalation sessions in the thermal gradient are similar to those we have observed in calorimetry experiments at typical lab temperatures (~21–22°C).[22] Specifically, in Session 1 the Tc of drug-naïve rats initially decreased rapidly from baseline, achieving a nadir by 30–45 min, and subsequently returned toward baseline, consistent with the development of acute tolerance. In subsequent sessions, rats developed chronic tolerance to the hypothermic effect of N_2O, and this stage of adaptation became fully expressed by sessions 4–6 (i.e., Tc remained commensurate with baseline during N_2O inhalation); subsequently, Tc exhibited a hyperthermic sign-reversal during N_2O inhalation (Sessions 7–12).

In Session 1, baseline Tsel was 28.1°C ± 1.61 (95% CI). N_2O promptly resulted in the rat's selecting a cooler ambient temperature, which, after an initial sharp decline, eventually settled to approximately the typical lab temperature during the early period of N_2O administration (see **Fig. 1**). Tsel gradually returned toward baseline during the latter half of N_2O exposure. During subsequent administrations, rats consistently selected a cool location throughout the first hour of N_2O inhalation and then gradually selected less cool temperatures during the remainder of the N_2O exposure.

Quantitative assessment of Tc, Tsel and distance traveled during N_2O inhalation

Figure 2 depicts mean ± 95% CI for Tc, Tsel and the median ± p05 and p95 distance outcomes for the early, late and post-N_2O periods averaged within each N_2O and control gas session. **Figure 3** provides the formal statistical analysis of the differences between control and N_2O sessions. Baseline Tc and Tsel did not differ between N_2O and control sessions. These baseline values gradually decreased over sessions as the rats gained body mass (as best visualized in **Figure 2**, baseline Tc and Tsel). On the initial N_2O inhalation, rats promptly moved to significantly cooler ambient temperatures that facilitated the development of hypothermia (**Fig. 3**, early period Tc and Tsel), as reported previously.[32] The rats continued to select a cooler ambient temperature during the early period across all sessions (**Fig. 3**, early period Tsel), even as Tc was exhibiting tolerance and eventually a *hyper*thermic sign-reversal during the early period of N_2O inhalation (**Fig. 3**, early period Tc). Indeed, in the early N_2O measurement interval, Tsel was consistently and substantially depressed by 5–7°C. Tsel did not differ from control levels during the late and post-exposure periods for the first several N_2O sessions. However, by the fifth or sixth N_2O session, Tsel started to become cooler during the late and post-exposure periods (**Fig. 3**, late and post-exposure periods Tsel). For example, Tsel was robustly depressed compared to control values during the post-exposure period for the final 3 N_2O sessions. During the late period, although chronic tolerance developed for Tc over the first several N_2O exposure sessions, Tc remained modestly decreased relative to control levels for 5 of the last 9 sessions (**Fig. 3**, late period Tc). During the post-exposure period, Tc recovered over the first 5 N_2O-exposure sessions and then remained modestly, yet significantly, decreased relative to control levels for 4 of the remaining 7 N_2O administrations (**Fig. 3**, post-exposure period Tc). It is notable that Tsel decreased over sessions during the post-exposure period while during that same interval, Tc recovered over sessions so as to be slightly cooler than control levels. In summary, over repeated N_2O-administration sessions tolerance developed and was followed by a hyperthermic overcompensation of Tc despite the persistent selection of a cooler Tsel. During the late and post-exposure periods of the later N_2O administrations, rats had a slightly reduced Tc (i.e., they were no longer hyperthermic) while exhibiting an increasing preference for cooler ambient temperatures.

There was a dramatic increase in locomotion in the early N_2O interval, a modest but significant increase during the late N_2O

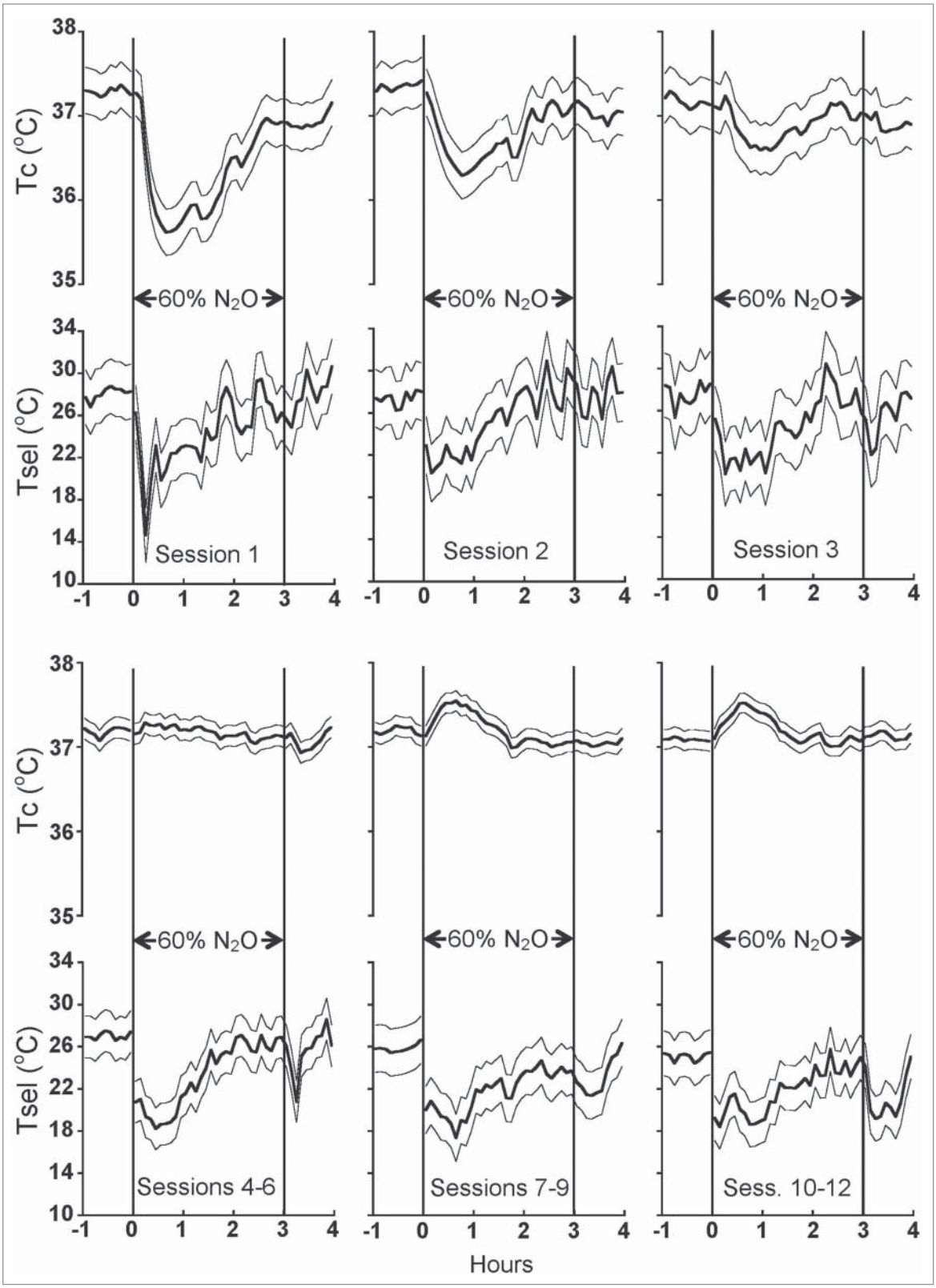

Figure 1. Temporal profiles of core temperature (Tc) and selected ambient temperature (Tsel) with 95% confidence bands during 60% N_2O administrations in the thermal gradient (n = 16). Rats were housed in the active thermal gradient for a minimum of 20 h prior to N_2O administration and had ad libitum access to food and water throughout. The data collected during the 6-min time bin prior to N_2O onset (plotted at −3 min) and during the 6-min time bin after N_2O onset (plotted at +3 min) are not connected by a line segment, which facilitates visualizing the rapid and large changes that can occur with drug delivery.

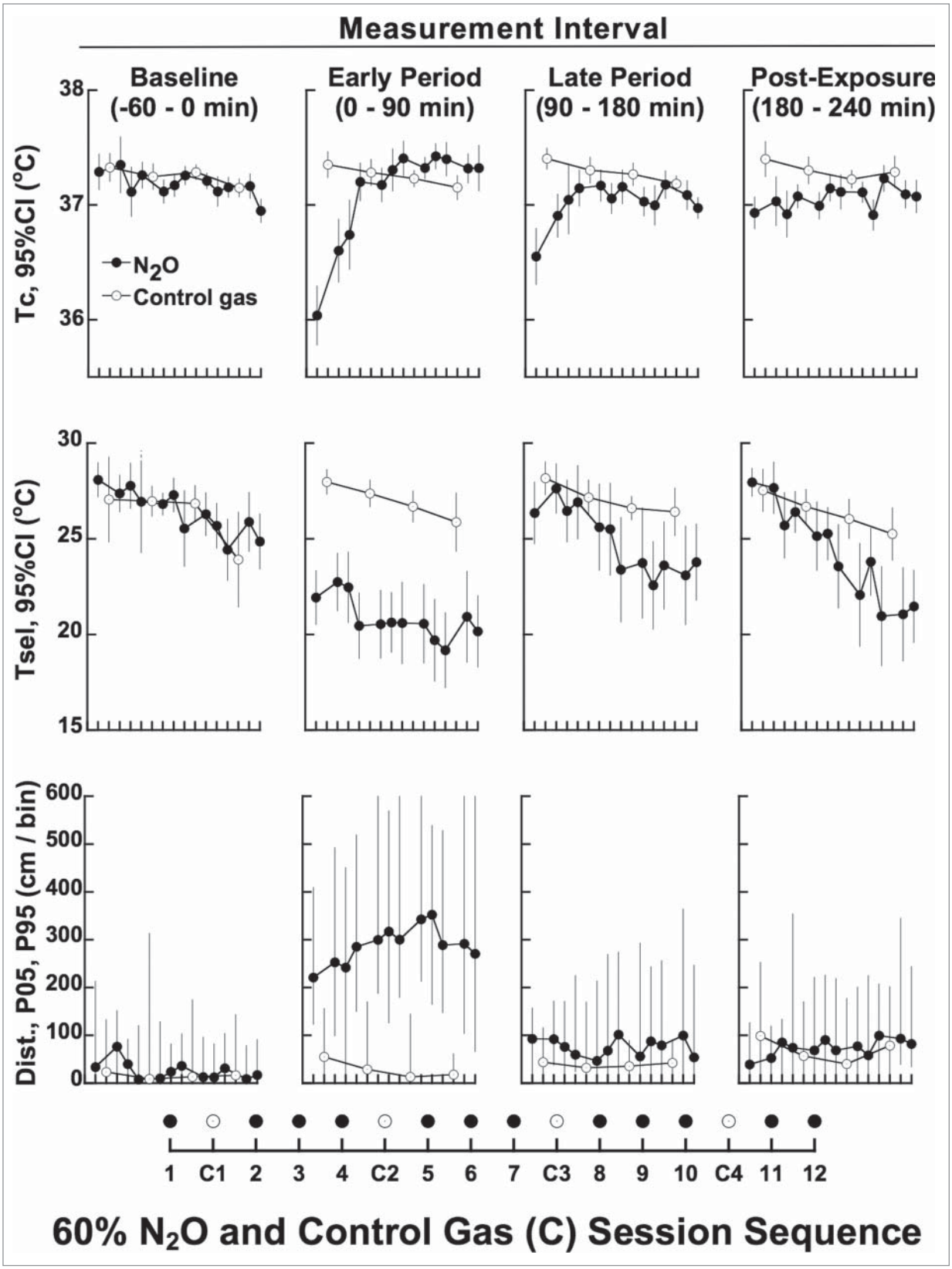

Figure 2. Core temperature (Tc), selected ambient temperature (Tsel) and distance (Dist.) traveled during N_2O and control gas sessions. Values for Tc and Tsel are unadjusted means with 95% confidence intervals based on repeated measures multiple linear regression analysis using unstructured covariance matrices within N_2O and control gas conditions computed separately for each measurement interval. Distance was non-normally distributed and is therefore presented as median with P05 and P95 limits (n = 16).

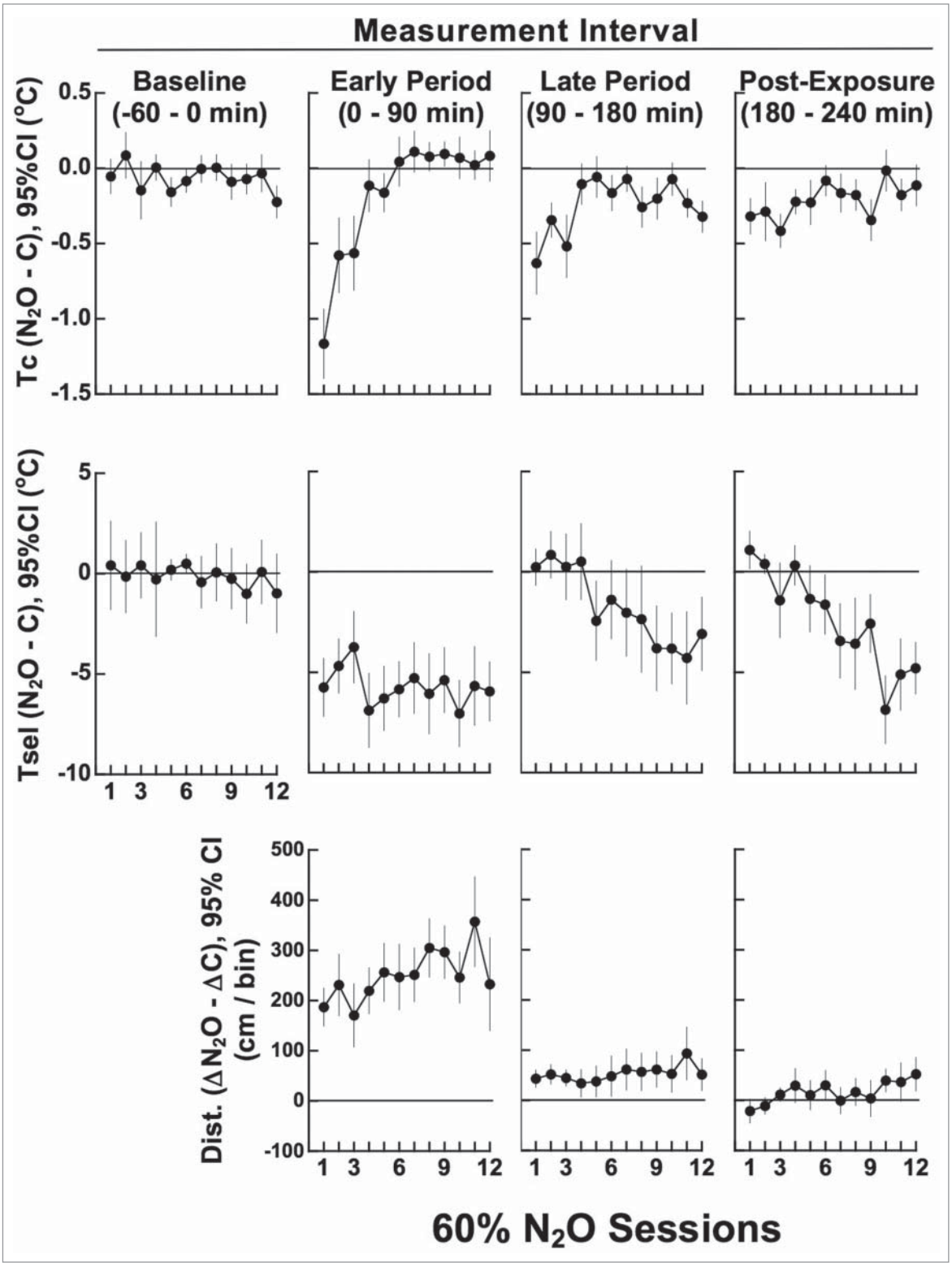

Figure 3. Statistical analyses involving linear mixed model repeated measures analyses of thermal and distance outcomes (n = 16). The top 2 rows depict means with 95% confidence intervals for N_2O minus control gas (C) differences for core temperature (Tc) and selected ambient temperature (Tsel) for each of the 12 N_2O sessions. For each outcome in each of the 12 N_2O sessions, the mean and confidence interval represents the contrast with the average effect of the 4 control gas sessions. Outcomes for the baseline pre-N_2O administration period were adjusted for N_2O and control gas session numbers, while outcomes for the early, late and post-N_2O intervals were adjusted for baseline values. The analysis for distance-traveled compared change (Δ) from baseline values between N_2O and control sessions adjusted for baseline (Δ distance scores were normally distributed). 95% confidence intervals that do not contain zero signify N_2O sessions that were significantly different than control gas sessions at $P < 0.05$.

interval, and little difference from control levels in the post-exposure interval (**Fig. 3**, Dist.). Increased metabolic rate yoked to locomotion would presumably generate heat, but is unlikely to explain tolerance or the hyperthermic sign-reversal of Tc. This is because: (1) a significant and substantial increase of activity occurred in early sessions in which hypothermia was maximal (**Fig. 3**); and (2) although the increase in activity during early N_2O administration was marked, the estimated metabolic cost of transport in rats of 2–3 m of locomotion per 6-min bin (~0.3–0.5 m/min) is estimated to be a modest ~0.1 W.[36]

Calorimetry testing following the thermal gradient sessions (**Fig. 4**) revealed a hyperthermic overcorrection of Tc during N_2O exposure similar to the hyperthermia that eventually developed during the early period of N_2O inhalation in the thermal gradient. Consistent with our previous work,[22] the early-period hyperthermic Tc in drug-adapted rats was primarily underlain by an increase of metabolic HP that exceeded the effect of the drug to augment HL. However, the late-period HL was not elevated compared to control values (**Fig. 4**) implying the existence of a gradual within-session adaptation that reduces heat conductance, as suggested previously.[22] This adaptation appears to work in concert with a gradual waning of HP during N_2O administration so as to favor an eventual rebalancing of Tc at or near its control value.

Discussion

The present work provides novel evidence that serial administrations of an initially hypothermic drug engender an adapted biobehavioral state in which autonomic and behavioral thermoeffector responses are pitted against each other during subsequent drug administration. This scenario is inconsistent with regulatory models according to which effector responses develop and act in coordination so as to efficiently defend homeostasis in the face of a disruptive agent.

A homeostatic model that includes an adjustable regulated level or 'set-point'[37] would describe N_2O as causing a "regulated hypothermia".[32,38] In this view, the initial N_2O administration causes the set-point for Tc to be reduced, eliciting a coordinated increase of HL and lowered Tsel to efficiently facilitate decreased Tc. During the continuous steady-state N_2O exposure, acute drug tolerance develops, gradually returning the set-point toward pre-drug values. The resulting discrepancy between the recovering set-point and the hypothermic Tc activates homeostatic corrective responses that raise Tc. Specifically, autonomic effectors are recruited that increase HP, and the rats move to less cool ambient temperatures (i.e., Tsel recovers from its nadir). Both of these adaptations contribute to the intrasessional recovery of Tc (**Fig. 1**, Session 1).

In contrast to this homeostatic interpretation of an initial N_2O exposure, the current findings implicate a different interpretation of the regulatory changes that occur over repeated N_2O exposures. When viewed over 12 individual N_2O administrations, it becomes evident that Tc can change independently of Tsel (**Fig. 1**). The pattern of Tsel during a 3-h N_2O administration is remarkably similar across all sessions, decreasing promptly with the onset of N_2O, reaching a nadir within the first hour and then gradually returning toward baseline value over the subsequent 2 h. In contrast, whereas Tc attains hypothermia during the initial N_2O session, chronic tolerance with no hypothermia is seen in Sessions 4–6, and this is followed by an early hyperthermia in Sessions 7–12. However, the cost-effective behavioral strategy of moving to a warmer environment is never utilized to facilitate the recovery or elevation of Tc across sessions. Rather, Tsel opposes the recovery and ultimate sign-reversal of Tc. The final N_2O session using the total calorimeter revealed that the rats were generating increased HP that mediated the hyperthermic Tc (**Fig. 4**). This finding is consistent with previous calorimetric research[21,22,29] suggesting that HP is an acquired compensation that grows over repeated administrations and contributes to the development of chronic tolerance as well as to the eventual hyperthermic overcompensation of Tc. Thus, in this situation, cool-seeking behavior is dis-coordinated with HP effector activity in the regulation of Tc.

Another example of dis-coordinated effector activity relates to the progressive decease in Tsel during the post-exposure period relative to the gradual increase of Tc toward control levels that occurs during that same period over the 12 sessions (**Fig. 3**). In contrast to the consistent effect of early-period Tsel across sessions, post-exposure Tsel changed over sessions and eventually became a cool preference of comparable magnitude to that observed during the early period of N_2O exposure. Thus, when N_2O delivery ceases, motivated behavior for cooler ambient temperatures increases over sessions while other concurrently active regulatory influences continue to support the recovery of Tc toward control levels.

These findings are not easily reconciled with traditional homeostatic interpretations as recently reviewed.[10] If Tc can be more efficiently regulated by adjusting Tsel than via changes in autonomic HP effector activity, why is HP rather than Tsel the primary mechanism accounting for chronic tolerance? Why should HP effectors and Tsel be in concurrent competition with each other when well-coordinated effector responses are a hallmark of homeostatic regulation? In Sessions 4–6, elevations in HP and possible heat-conserving adaptations are sufficient to offset the cool Tsel, thereby establishing a thermal balance that is able to maintain Tc at baseline/control levels during N_2O administration. Without a perturbation of Tc during Sessions 4–6, what drives the further adaptations that eventually lead to the thermoeffector imbalance that causes a transient hyperthermic sign-reversal of Tc during Sessions 7–12? While a transient hyperthermic overshoot could be interpreted as hysteresis resulting from time lags in homeostatic regulatory effector activity, this cannot explain why adaptations that effectively establish homeostasis during sessions 4–6 do not exhibit hysteresis.

We suggest that the explanation for these inconsistencies is that the principles of homeostatic regulation do not apply to all situations, especially to non-naturalistic experimental challenges such as those involving the delivery of pharmacological agents.[6,10,13,32] In fact, the current results are more consistent with the view that the thermoeffector loops regulating Tc are relatively independent of one another[14,15,39,40,41] and that Tc

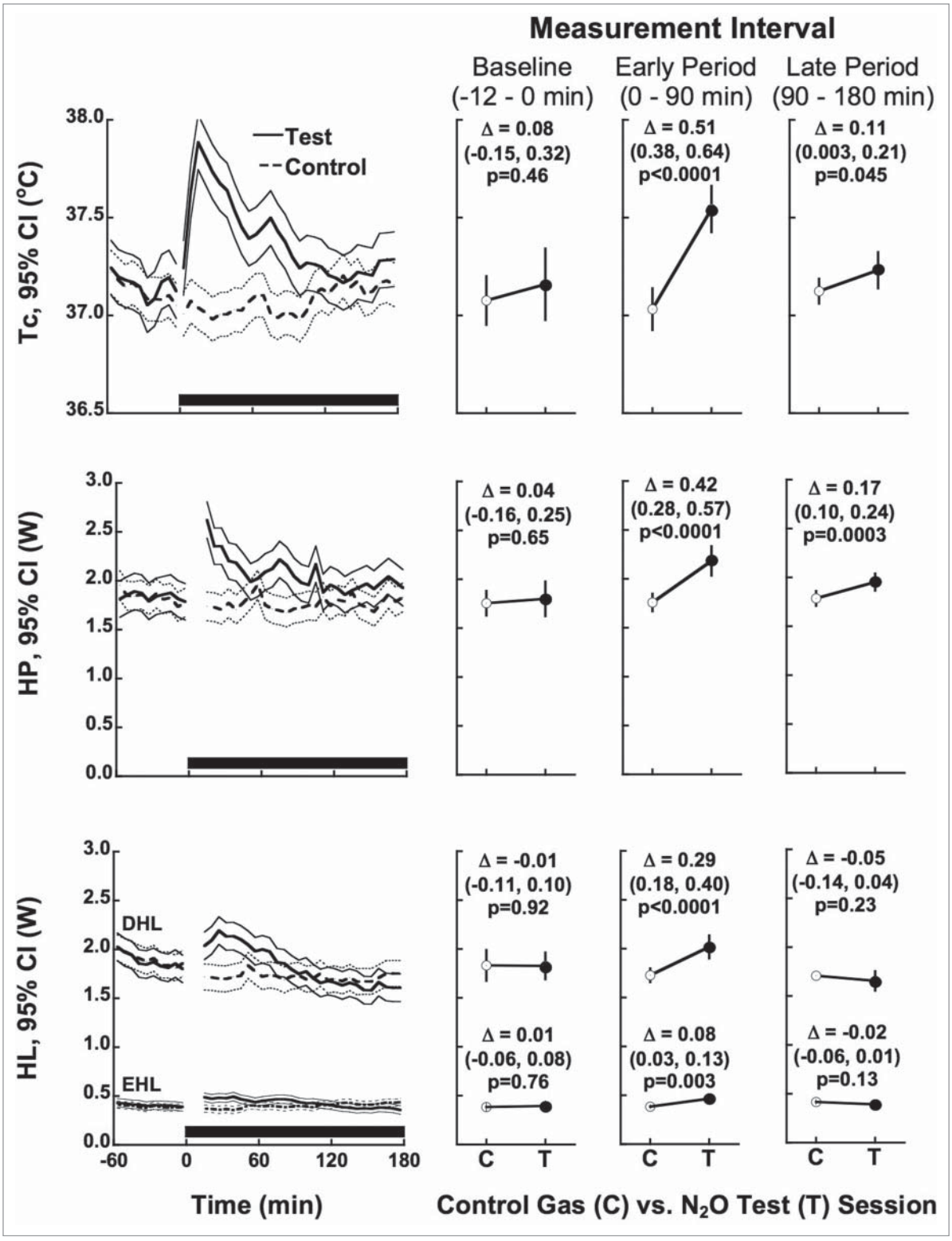

Figure 4. Core temperature (Tc), heat production (HP) and heat loss [HL, with evaporative HL (EHL) and dry HL (DHL) depicted separately] during total calorimetry and temperature testing at the conclusion of the 16 thermal gradient sessions. The black bar indicates the interval of 60% N_2O administration. Temporal profiles and line graphs are unadjusted means with 95% confidence intervals from repeated measures linear regression analysis with inhalation condition, and for temporal profiles, time, as repeated factors. HP and HL are not depicted for the first 12 min of the N_2O administration owing to a potential for artifactual changes therein (see Methods). Text in each line graph specifies effect size in terms of the difference (Δ) and 95% confidence interval (in parentheses) between the control gas and N_2O test sessions adjusted for baseline values based on linear mixed model repeated measures analysis (n = 14).

represents a balance point rather than a set-point defended by coordinated effector activity. The point is that there are current models of regulation that incorporate the relative independence of regulatory effectors and are thus able to accommodate occurrences of dis-coordinated effector responses that work in opposition to one another.[10] In this schema, aberrant challenges to evolutionarily-derived regulated systems can trigger dis-coordinated effector responses, and this has been suggested to be a characteristic of a non-homeostatic form of regulation called allostasis.[10] Goldstein[42] has described the inefficient cost of allostasis by analogy to regulating a home's temperature with competing effectors (e.g., the furnace and the air conditioner) being concurrently active. This is a fitting metaphor for the findings of the current study where concurrent motivated cool-seeking behavior opposes increased autonomic HP responses during the development of both chronic tolerance and the eventual transient hyperthermic sign-reversal. Importantly, sign-reversals of regulated variables have been suggested to reflect the existence of allostasis.[10]

An allostatic model of drug addiction can explain how motivational consequences arise that encourage drug-taking behavior.[10] Overactive compensatory responses that lead to sign-reversals have been suggested to motivate drug-taking behavior; i.e., increased drug-taking yields a greater pharmacological effect that can oppose the sign-reversal state. In other words, the behavioral effector of drug taking can oppose the overactive effectors that caused the sign-reversal state (i.e., there is concurrent opposing-effector activity). Taking additional drug may temporarily ameliorate the sign-reversal, but it also triggers increased effector activity that eventually restores the sign-reversal. Thus, an allostatic model can include a vicious cycle hypothesis for the escalation of drug taking seen in addiction.[43-49] The findings of the current study indicate that a cool Tsel opposes the hyperthermic Tc sign-reversal and may reduce the magnitude of hyperthermia. Presumably, the hyperthermic Tc could have been reduced further if the rat had selected an even cooler Tsel (i.e., there was not a floor effect at the cool end of Tsel). Since this did not occur, the magnitude and duration of the hyperthermic Tc may reflect an allostatic balance point that develops over repeated exposure with both autonomic effectors and the thermal gradient behavioral effector being concurrently available. A subsequent study[50] assesses this hypothesis in a different way by determining whether a sign-reversal state established during N_2O administration at typical laboratory temperatures (~21°C) is altered once a powerful behavioral effector provided by a thermal gradient becomes available during N_2O administration.

In conclusion, rats did not facilitate chronic tolerance development to N_2O-induced hypothermia by moving to warmer locations in the gradient, and instead selected cooler ambient temperatures while simultaneously increasing autonomic HP. The inefficient concurrent activation of opposing effectors and the development of a sign-reversal are incompatible with homeostatic models of drug-adaptation and may be better interpreted using a model of drug-induced allostasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We gratefully acknowledge Christopher W Prall and Hoang Yen Ho for their technical contributions to this study. The Helen Riaboff Whiteley Center, located at the University of Washington's Friday Harbor Laboratories, provided an ideal environment for completing this manuscript.

Funding

This investigation was supported by the National Institutes of Health (NIDA grant DA023484).

References

1. Haefely W. Biological basis of drug-induced tolerance, rebound and dependence. Contribution of recent research on benzodiazepines. Pharmacopsychiatry 1986; 19:353-61; PMID:2877468; http://dx.doi.org/10.1055/s-2007-1025061
2. Himmelsbach CK. Clinical studies of addiction. Arch Intern Med 1942; 69:766-72; http://dx.doi.org/10.1001/archinte.1942.00200170048004
3. Kalant H, LeBlanc AE, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 1971; 23:135-91; PMID:4398655
4. Peper A. Intermittent adaptation. A theory of drug tolerance, dependence and addiction. Pharmacopsychiatry 2009; 42(Suppl 1):S129-143; http://dx.doi.org/10.1055/s-0029-1202848
5. Poulos CX, Cappell H. Homeostatic theory of drug tolerance: a general model of physiological adaptation. Psychol Rev 1991; 98:390-408; PMID: 1891524; http://dx.doi.org/10.1037/0033-295X.98.3.390
6. Ramsay DS, Woods SC. Biological consequences of drug administration: implications for acute and chronic tolerance. Psychol Rev 1997; 104:170-93; PMID:9009884; http://dx.doi.org/10.1037/0033-295X.104.1.170
7. Siegel S, Ramos BM. Applying laboratory research: drug anticipation and the treatment of drug addiction. Exp Clin Psychopharmacol 2002; 10:162-83; PMID:12233979; http://dx.doi.org/10.1037/1064-1297.10.3.162
8. Siegel S. Learning and the wisdom of the body. Learn Behav 2008; 36:242-52; PMID:18683468; http://dx.doi.org/10.3758/LB.36.3.242
9. Cannon WB. The wisdom of the body. New York: W.W. Norton & Company; 1932.
10. Ramsay DS, Woods SC. Clarifying the roles of homeostasis and allostasis in physiological regulation. Psychol Rev 2014; 121:225-47; PMID:24730599; http://dx.doi.org/10.1037/a0035942
11. Dworkin BR. Learning and physiological regulation. Chicago: University of Chicago Press; 1993.
12. Bligh J. Mammalian homeothermy: an integrative thesis. J Therm Biol 1998; 23:143-258; http://dx.doi.org/10.1016/S0306-4565(98)00014-X
13. Cabanac M. Adjustable set point: to honor Harold T. Hammel. J Appl Physiol 2006; 100:1338-46; PMID:16540712
14. Kanosue K, Crawshaw LI, Nagashima K, Yoda T. Concepts to utilize in describing thermoregulation and neurophysiological evidence for how the system works. Eur J Appl Physiol 2010; 109:5-11; PMID:19882166; http://dx.doi.org/10.1007/s00421-009-1256-6
15. Werner J. System properties, feedback control and effector coordination of human temperature regulation. Eur J Appl Physiol 2010; 109:13-25; PMID:19787369; http://dx.doi.org/10.1007/s00421-009-1216-1
16. Lê AD, Poulos CX, Cappell H. Conditioned tolerance to the hypothermic effect of ethyl alcohol. Science 1979; 206:1109-10; http://dx.doi.org/10.1126/science.493999
17. Lovinger DM, Crabbe JC. Laboratory models of alcoholism: treatment target identification and insight into mechanisms. Nat Neurosci 2005; 8:1471-80; PMID:16251990; http://dx.doi.org/10.1038/nn1581
18. Mansfield JG, Cunningham CL. Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats. J Comp Physiol Psychol 1980; 94:962-69; PMID:7430477; http://dx.doi.org/10.1037/h0077824
19. Kaiyala KJ, Ramsay DS. Assessment of heat production, heat loss, and core temperature during nitrous oxide exposure: a new paradigm for studying drug effects and opponent responses. Am J Physiol Regul Integr Comp Physiol 2005; 288:R692-701; PMID:15563578; http://dx.doi.org/10.1152/ajpregu.00412.2004

20. Kaiyala KJ, Butt S, Ramsay DS. Direct evidence for systems-level modulation of initial drug (in)sensitivity in rats. Psychopharmacology (Berl) 2007a; 191:243-51; http://dx.doi.org/10.1007/s00213-006-0657-z
21. Kaiyala KJ, Butt S, Ramsay DS. Systems-level adaptations explain chronic tolerance development to nitrous oxide hypothermia in young and mature rats. Psychopharmacology (Berl) 2007b; 191:233-42; http://dx.doi.org/10.1007/s00213-006-0655-1
22. Kaiyala KJ, Chan B, Ramsay DS. Robust thermoregulatory overcompensation, rather than tolerance, develops with serial administrations of 70% nitrous oxide to rats. J Therm Biol 2012; 37:30-40; PMID:22247586; http://dx.doi.org/10.1016/j.jtherbio.2011.10.004
23. Balster RL. Neural basis of inhalant abuse. Drug Alcohol Depend 1998; 51:207-14; PMID: 9716942; http://dx.doi.org/10.1016/S0376-8716(98)00078-7
24. Kaiyala KJ, Leroux BG, Watson CH, Prall CW, Coldwell SE, Woods SC, Ramsay DS. Reliability of individual differences in initial sensitivity and acute tolerance to nitrous oxide hypothermia. Pharmacol Biochem Behav 2001; 68:691-99; PMID:11526966; http://dx.doi.org/10.1016/S0091-3057(01)00488-9
25. Quock RM, Panek RW, Kouchich FJ, Rosenthal MA. Nitrous oxide-induced hypothermia in the rat. Life Sci 1987; 41:683-90; PMID:3613837; http://dx.doi.org/10.1016/0024-3205(87)90447-4
26. Ramsay DS, Omachi K, Leroux BG, Seeley RJ, Prall CW, Woods SC. Nitrous oxide-induced hypothermia in the rat: acute and chronic tolerance. Pharmacol Biochem Behav 1999; 62:189-96; PMID:9972863; http://dx.doi.org/10.1016/S0091-3057(98)00156-7
27. Stenqvist O. Nitrous oxide kinetics. Acta Anaesthesiol Scand 1994; 38:757-60; PMID:7887090; http://dx.doi.org/10.1111/j.1399-6576.1994.tb03997.x
28. Ramsay DS, Kaiyala KJ, Leroux BG, Woods SC. Individual differences in initial sensitivity and acute tolerance predict patterns of chronic drug tolerance to nitrous-oxide-induced hypothermia in rats. Psychopharmacology (Berl) 2005; 181:48-59; PMID:15778887; http://dx.doi.org/10.1007/s00213-005-2219-1
29. Kaiyala KJ, Woods SC, Ramsay DS. Persistence of a hyperthermic sign-reversal during nitrous oxide inhalation despite cue-exposure treatment with and without a drug-onset cue.Temperature; 2014; 1:3
30. Gordon CJ. Temperature and toxicology: an integrative, comparative and environmental approach. Boca Raton: CRC Press; 2005.
31. Gordon CJ, Lee KL, Chen TL, Killough P, Ali JS. Dynamics of behavioral thermoregulation in the rat. Am J Physiol 1991; 261:R705-711; PMID:1887959
32. Ramsay DS, Seaman J, Kaiyala KJ. Nitrous oxide causes a regulated hypothermia: rats select a cooler ambient temperature while becoming hypothermic. Physiol Behav 2011; 103:79-85; PMID:21184766; http://dx.doi.org/10.1016/j.physbeh.2010.12.018
33. Feise RJ. Do multiple outcome measures require p-value adjustment? BMC Med Res Methodol 2002; 2:8; PMID:12069695; http://dx.doi.org/10.1186/1471-2288-2-8
34. Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998; 316:1236-38; PMID:9553006; http://dx.doi.org/10.1136/bmj.316.7139.1236
35. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990; 1:43-46; PMID:2081237; http://dx.doi.org/10.1097/00001648-199001000-00010
36. Tucker VA. Energetic cost of locomotion in animals. Comp Biochem Physiol 1970; 34:841-46; PMID:4952698; http://dx.doi.org/10.1016/0010-406X(70)91006-6
37. Hammel HT, Jackson DC, Stolwijk JA, Hardy JD, Strømme SB. Temperature regulation by hypothalamic proportional control with an adjustable set point. J Appl Physiol 1963; 18:1146-54; PMID:14080734
38. Gordon CJ. Autonomic nervous system: central thermoregulatory control. In: Encyclopedia of Neuroscience. Squire LR. (ed). Oxford, UK: Academic Press; 2009:891-98.
39. McAllen RM, Tanaka M, Ootsuka Y, McKinley MJ. Multiple thermoregulatory effectors with independent central controls. Eur J Appl Physiol 2010; 109:27-33; PMID:19949811; http://dx.doi.org/10.1007/s00421-009-1295-z
40. Romanovsky AA. Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. Am J Physiol Regul Integr Comp Physiol 2007; 292:R37-46; PMID:17008453; http://dx.doi.org/10.1152/ajpregu.00668.2006
41. Satinoff E. Neural organization and evolution of thermal regulation in mammals. Science 1978; 201:16-22; PMID:351802; http://dx.doi.org/10.1126/science.351802
42. Goldstein DS. Merging of the homeostat theory with the concept of allostatic load. In: Allostasis, Homeostasis, and the Costs of Physiological Adaptation. Schulkin J (ed). New York: Cambridge University Press; 2004:99-112.
43. Colpaert FC, Deseure K, Stinus L, Adriaensen H. High-efficacy 5-hydroxytryptamine 1A receptor activation counteracts opioid hyperallodynia and affective conditioning. J Pharmacol Exp Ther 2006; 316:892-99; PMID:16254131; http://dx.doi.org/10.1124/jpet.105.095109
44. Compton MA. Cold-pressor pain tolerance in opiate and cocaine abusers: correlates of drug type and use status. J Pain Symptom Manage 1994; 9:462-73; PMID:7822886; http://dx.doi.org/10.1016/0885-3924(94)90203-8
45. Edwards S, Koob GF. Neurobiology of dysregulated motivational systems in drug addiction. Future Neurol 2010; 5:393-401; PMID:20563312; http://dx.doi.org/10.2217/fnl.10.14
46. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001; 24:97-129; PMID:11120394; http://dx.doi.org/10.1016/S0893-133X(00)00195-0
47. Koob GF, Le Moal M. Addiction and the brain antireward system. Annu Rev Psychol 2008; 59:29-53; PMID:18154498; http://dx.doi.org/10.1146/annurev.psych.59.103006.093548
48. Ossipov MH, Lai J, King T, Vanderah TW, Malan TP, Jr, Hruby VJ, Porreca F. Antinociceptive and nociceptive actions of opioids. J Neurobiol 2004; 61:126-48; PMID:15362157; http://dx.doi.org/10.1002/neu.20091
49. White JM. Pleasure into pain: the consequences of long-term opioid use. Addict Behav 2004; 29:1311-24.
50. Ramsay DS, Woods SC, Kaiyala KJ. Drug-induced regulatory overcompensation has motivational consequences: implications for homeostatic and allostatic models of drug addiction. Temperature; 2014; 1:3