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Procedure of rectal temperature measurement affects brain, muscle, skin and body temperatures and modulates the effects of intravenous cocaine

David D. Bae, P. Leon Brown, and Eugene A. Kiyatkin*

Behavioral Neuroscience Branch, National Institute on Drug Abuse - Intramural Research Program, National Institutes of Health, DHHS, 5500 Nathan Shock Drive, Baltimore, Maryland 21224

Abstract

Rectal probe thermometry is commonly used to measure body core temperature in rodents because of its ease of use. Although previous studies suggest that rectal measurement is stressful and results in long-lasting elevations in body temperatures, we evaluated how this procedure affects brain, muscle, skin and core temperatures measured with chronically implanted thermocouple electrodes in rats. Our data suggest that the procedure of rectal measurement results in powerful locomotor activation, rapid and strong increases in brain, muscle, and deep body temperatures, as well as a biphasic, down-up fluctuation in skin temperature, matching the response pattern observed during tail-pinch, a representative stressful procedure. This response, moreover, did not habituate after repeated day-to-day testing. Repeated rectal probe insertions also modified temperature responses induced by intravenous cocaine. Under quiet resting conditions, cocaine moderately increased brain, muscle and deep body temperatures. However, during repeated rectal measurements, which increased temperatures, cocaine induced both hyperthermic and hypothermic responses. Direct comparisons revealed that body temperatures measured by a rectal probe are typically lower ($\sim 0.6^{\circ}\text{C}$) and more variable than body temperatures recorded by chronically implanted electrodes; the difference is smaller at low and greater at high basal temperatures. Because of this difference and temperature increases induced by the rectal probe *per se*, cocaine had no significant effect on rectal temperatures compared to control animals exposed to repeated rectal probes. Therefore, although rectal temperature measurements provide a decent correlation with directly measured deep body temperatures, the arousing influence of this procedure may drastically modulate the effects of other arousing stimuli and drugs.

Keywords

body core temperature; thermorecording; stress; metabolic brain activation; skin hypothermia; rats

1. Introduction

Body temperature is a basic physiological parameter, which is often measured in awake rodents by rectal probe thermometry (Lomax, 1966). While it is known that rectal probe measurements are less accurate and less reliable than other methods that involve surgical implantation of

*Correspondence should be addressed to Eugene A. Kiyatkin at the above address. Fax: (410) 550-5553; tel.: (410) 550-5551; e-mail: ekiyatki@intra.nida.nih.gov

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temperature recording devices (Dallman et al., 2006), the procedure also results in long-lasting elevations in body core temperature (Clark et al., 2003; Poole and Stephenson, 1977; Van der Heyden, 1977), suggesting stress-induced hyperthermia. This procedural influence may be especially crucial in pharmacological studies because the effects of many drugs depend upon basal temperature and the activity state of animals (see Kiyatkin, 2005 for review). Cocaine, for example, induces hyperthermia at low basal temperatures but no change at higher basal temperatures (Brown and Kiyatkin, 2005). This observation as well as the dependence of cocaine effects upon dose and ambient temperature (Crandall et al., 2002; Long et al., 1994; Lomax and Daniel, 1990; Gonzalez, 1993) may explain contradictory findings regarding temperature responses induced by cocaine.

This study was designed to evaluate how the traditional procedure of rectal measurement affects temperature and drug-induced temperature fluctuations in the brain (nucleus accumbens or NAcc), non-locomotor muscle, skin, and body core as continuously evaluated by chronically implanted thermocouple probes. First, we examined temperature effects of a single rectal measurement and compared them with those induced by a tail-pinch, a traditional stressogenic procedure. Second, we compared how temperature effects of rectal probe insertion and tail-pinch changed following repeated procedures. Third, we examined temperature changes during repeated rectal probe measurements that were performed at the rate typically used in pharmacological experiments. Fourth, we compared temperature effects induced by intravenous (iv) cocaine, a psychostimulant drug that induces behavioral activation and hyperthermia (Brown and Kiyatkin, 2005), in two conditions: with and without rectal temperature measurement. Finally, we evaluated the extent to which body temperatures measured by a rectal probe differ from body temperatures continuously recorded from the same area by a chronically implanted electrode.

2. Results

2.1. Temperature fluctuations induced by rectal probe insertion and tail-pinch in naive and habituated, experienced rats

As shown in Fig.1, both rectal probe insertion and tail-pinch induced a similar pattern of temperature changes and locomotor activation in naive rats. Temperatures in the NAcc, body core and temporal muscle rapidly increased (~ 0.4 - 0.6°C), peaked at ~ 5 - 10 min, and slowly returned to baseline within ~ 20 - 30 min after both procedures (A and B). In contrast, subcutaneous temperature transiently decreased for 4-5 min after both procedures and then returned to baseline or slightly higher values (A). There were significant differences between temperatures recorded from different locations with maximal values in the NAcc ($36.96 \pm 0.09^{\circ}\text{C}$), slightly lower in body core ($36.82 \pm 0.10^{\circ}\text{C}$), significantly lower in the muscle ($36.11 \pm 0.18^{\circ}\text{C}$, $p < 0.01$ vs. NAcc and body) and minimal values in the skin ($34.73 \pm 0.16^{\circ}\text{C}$; $p < 0.01$ vs. all other locations). Among all parameters, locomotion and skin temperature changed most rapidly, peaking at one min after both procedures. Brain, deep body and muscle temperatures also increased at the highest rate during and immediately after the procedures but peaked at later times. Although the pattern of temperature elevation was generally similar for each parameter, NAcc showed shorter latencies than muscle and body. While the changes in all parameters were slightly higher for tail-pinch, they were comparable for both procedures.

Habituated, experienced rats tested on Day 5 (after 15 rectal measurements performed during previous days) had lower basal temperatures in each recording location, with significant differences in the NAcc (36.56 ± 0.09 vs. $36.98 \pm 0.12^{\circ}\text{C}$; $p < 0.05$) and body (36.39 ± 0.16 vs. $36.82 \pm 0.10^{\circ}\text{C}$, $p < 0.05$). Although basal muscle and skin temperatures were also lower on Day 5 than Day 1, differences were not significant for either of these parameters (muscle: 35.70 ± 0.18 vs. $36.11 \pm 0.18^{\circ}\text{C}$; $p = 0.10$; skin: 34.59 ± 0.13 vs. $34.73 \pm 0.16^{\circ}\text{C}$). Despite a decrease in basal temperatures, temperature responses induced by both the rectal probe and tail-pinch were

similar in pattern to those seen on Day 1 but stronger in amplitude and duration. Both rectal probe insertion and tail-pinch induced phasic locomotor activation, which was quite similar to that induced by the initial procedures. Similar to Day 1, NAcc showed a more rapid and strong temperature increase than body and muscle, a similar biphasic, down-up, skin temperature fluctuation, and a stronger and more prolonged increase for tail-pinch than for the rectal probe. In the case of rectal probe, the relative amplitude of hyperthermic response on Day 5 was significantly higher than on Day 1 (peak for NAcc: 0.62 vs. 0.44°C, $p<0.05$) and its duration was relatively similar (23 vs. 24 min for Day 5 and 1, respectively). In contrast, brain hyperthermic response induced by tail-pinch on Day 5 was significantly stronger and more prolonged than on Day 1 (peak and duration of significant increase in NAcc: 0.88°C for 49 min vs. 0.70°C for 37 min, respectively).

As shown in Fig. 2, brain temperature responses to both rectal probe and tail-pinch on Day 1 had high individual variability (from 0.1 to 2.4°C) and were negatively correlated with basal brain temperatures ($r=-0.64$ and -0.68 , respectively). Although responses were also variable on Day 5, their relative change was independent of basal brain temperatures for both stimuli.

2.2. Temperature changes induced by repeated rectal probe insertion

Figure 3 shows changes in NAcc, muscle, body core and skin temperatures as well as locomotion during repeated rectal temperature measurements over a period of four hours. Each rat was exposed to 13 identical procedures with 20-min intervals.

As can be seen, repeated rectal probe insertion had a strong effect on temperature and locomotion. While NAcc, body and muscle temperatures tonically increased about 0.6-0.7°C (A and B), bouts of locomotor activation were seen after each procedure (C). Phasic increases in NAcc, body and muscle temperature also occurred after each repeated rectal probe insertion, and these fluctuations were superimposed on large temperature elevations that developed after the first probe. Skin temperature also showed biphasic, down-up fluctuations with each probe and its values gradually increased during the session. While NAcc and muscle temperatures returned to baseline 20-30 min after the last rectal probe, body and skin temperatures remained higher at the final point of observation (+50 min after the last rectal probe).

2.3. Changes in temperature and locomotion induced by iv cocaine with and without rectal temperature measurements

Fig. 4 shows changes in NAcc, skin, muscle and body temperatures (A and B) as well as locomotion (C) following an iv injection of cocaine (0.75 mg/kg) both without (left panel) and with (right panel) repeated rectal probe insertions. When the drug was injected in control conditions (without rectal tests), it induced powerful but relatively short locomotion (~20 min) and more prolonged brain and muscle hyperthermia (~0.6°C for ~45 min). Skin temperature after cocaine injection decreased rapidly, being the lowest at 1-3 min and correlating with the peak of locomotor activation (1-3 min). Temperature in NAcc increased slower than in the skin, but quicker than in the muscle, resulting in a rise of NAcc-muscle temperature gradient for ~10 min after cocaine injection.

In contrast, cocaine induced minimal brain and muscle hyperthermia (~0.2°C) when it was administered 10 min after the rectal probe, when brain and muscle temperatures were significantly increased (NAcc: 37.49 ± 0.15 vs. 37.06 ± 0.18 °C; muscle: 36.49 ± 0.18 vs. 36.09 ± 0.22 °C, $p<0.01$ in both cases). With respect to a new baseline established after the first rectal probe, NAcc and muscle temperature showed up-down phasic fluctuations after each subsequent probe with a slight progressive decrease in tonic baseline. Skin temperature also showed phasic down-up fluctuations, which were similar for both rectal probes and cocaine.

Both rectal probes and cocaine induced phasic increases in locomotion (Fig. 4C), but the effect of cocaine was clearly stronger than the effects of each probe.

Consistent with our previous data (Brown and Kiyatkin, 2005), NAcc temperature increase induced by iv cocaine was dependent upon basal temperature. Figure 5 shows correlative relationships between basal NAcc temperatures (mean for last min before the injection) and peak temperature (mean for the 20th min) determined for both conditions of drug administration. While in both conditions cocaine-induced temperature increase was negatively correlated with basal temperatures, temperature preferentially increased when cocaine was injected in quiet resting conditions (range 0.16-1.30, mean $0.61 \pm 0.09^{\circ}\text{C}$), but temperatures both increased and decreased ($n=11$ and 5 , respectively; range -0.45 to 1.08 , mean $0.23 \pm 0.11^{\circ}\text{C}$; $p < 0.01$ vs. quiet rest) when drug was injected after rectal probe at higher brain temperatures. The correlation was stronger for cocaine effects evaluated during repeated rectal probes.

Profound differences in the effects of cocaine were also seen in body temperatures (Fig. 6A). If cocaine induced $\sim 0.6^{\circ}\text{C}$ increase in body temperature vs. quiet resting baseline, the increase was marginal during repeated rectal probes. Rectal temperatures significantly increased ($\sim 0.6^{\circ}\text{C}$) after the first probe in both cocaine (+10 min) and no treatment groups (Fig. 6B). This increase was virtually identical with no changes between cocaine and control, suggesting that iv cocaine has no effect on body temperature as evaluated by repeated rectal measurements.

2.4. Differences in rectal and body temperatures

To evaluate the extent to which rectal temperatures differ from body temperatures, we analyzed 96 pairs of values obtained from 8 cocaine-administered rats (12 values during 2 days in each rat). As shown in Figure 7, rectal temperatures are consistently lower than body temperatures and the difference depends significantly on baseline temperature values. At lower temperatures, the difference is weakest, but it became stronger at higher temperatures. Despite attempts to standardize the procedure of measurement and its duration, $\sim 13\%$ of tests showed rectal temperature more than 1°C lower than body temperature and the difference was more than 1.5°C in 3/96 tests. The majority of cases of under-valued temperatures were seen at higher basal temperatures.

3. Discussion

Our study revealed that rectal probe insertion induces strong locomotor activation and significantly affects central and peripheral body temperatures following single and repeated tests. The pattern of locomotor activation and temperature responses induced by a single rectal probe was very similar to those induced by tail-pinch, a traditional stress-inducing challenge. Both procedures induced rapid motor activation, rapid and transient skin hypothermia, and prolonged increases in NAcc, muscle and body temperatures. Although brain temperature elevation peaked well after the 1-min procedures, the increase was most rapid within one to two minutes (Fig. 1), preceding a slower change in muscle temperature. Although these changes are usually interpreted as stress-induced hyperthermia (Briese and Cabanac, 1991; Kluger et al., 1987; Van der Heyden et al., 1977), they occur following quite different environmental challenges that range from simple somato-sensory stimuli to various motivationally significant stimuli and events (see Kiyatkin, 2005 for review). Therefore, this response may reflect neurally triggered metabolic brain activation, which underlies behavioral activation and triggers, via the effector pathways, a peripheral metabolic response. The increase was clearly more delayed and weaker in the temporal muscle (Fig. 1 and 3), which, as a non-locomotor muscle, has no heat production of its own and is warmed primarily by the inflow of arterial blood. In contrast, body warming occurred more rapidly, but with some delay vs. NAcc temperature, suggesting that body is warmed by its own heat production and may affect brain temperature via warming of arterial blood. Although temperature in the body is only slightly

lower than in NAcc, arterial blood is cooler than body and brain (Delgado and Hanai, 1966; Kiyatkin et al., 2002; Nybo et al., 2002). Therefore, although warming of arterial blood may diminish heat dissipation from the brain, brain hyperthermia results from its own heat production. Importantly, increases in brain and muscle temperatures were associated with rapid and transient skin hypothermia, which reflects peripheral vasoconstriction (Baker et al., 1976), limiting heat dissipation to the external environment. Therefore, hyperthermia induced by both rectal probe and tail-pinch is a part of the adaptive organism's response under conditions that require behavioral activation. While the duration of rectal measurement and tail-pinch were 1 min, temperature changes induced by these procedures both in brain and body locations were long-lasting, varying from 30 to 60 min (see Fig. 1). Rebound-like skin hyperthermia that followed the initial rapid, transient hypothermia was evident for more than 1 hour.

We found that thermal responses to both rectal probe insertion and tail-pinch did not show habituation with repeated use. Although basal temperatures in each location (except skin) became lower on Day 5 vs. Day 1, locomotor activation induced by both stimuli was virtually identical and relative temperature increases became stronger and more prolonged. While this increase may be viewed as "sensitization", it appears that animal habituation to the testing environment and progressive decrease in basal temperatures are the cause of these "sensitized" responses. Our previous work revealed that the amplitude of brain and body temperature increases induced by various arousing stimuli correlate negatively with basal brain temperatures (Kiyatkin et al., 2002; Kiyatkin and Mitchum, 2003). Following day-to-day habituation to the same testing environment, basal brain and body temperature became progressively lower and, because of this, the response to the same salient stimulus (i.e., a relative change vs. a new, lower baseline) became relatively stronger. However, this process may co-exist with "true" habituation (or decreasing the arousing effect of repeatedly presented stimulus) since brain and body core temperature elevation induced by rectal probe on Day 5 (after preceding 15 tests), while increased in relative magnitude, was not higher in absolute magnitude and not longer in duration than in Day 1, but the response to tail-pinch on Day 5 (after 2 tests on Day 1) was stronger both in magnitude and duration. While we cannot exclude that mechanisms of conditioning may contribute to altered responses to both arousing stimuli, it appears that this process intimately involves day-to-day changes in basal activity state—an essential component in determining the response.

We found that repeated rectal probe insertion also increased rectal temperatures (see Fig. 6B for control group) and had an effect on all temperatures recorded with chronically implanted electrodes (Fig. 3). While NAcc, muscle, and deep body temperatures increased rapidly by about 0.6-0.8°C after the first probe, this increase was maintained at significant levels following repeated probe insertions. There were, however, fluctuations associated with each repeated test. These fluctuations were greatest for locomotor activity and skin temperature, but they were also evident for NAcc, muscle and deep body temperatures. Although locomotor activation triggered by each repeated probe insertion was relatively stable during the experiment (see Fig. 3C), NAcc and muscle temperatures robustly increased after the first insertion, and showed much weaker relative increases with each subsequent insertion, and a slight decrease in tonic baseline (Fig. 3B). Therefore, the responses to rectal probe insertion show a certain within-session habituation, but it manifests as a slow decrease in elevated temperature baseline.

To evaluate how the procedure of rectal temperature measurement affects drug-induced temperature responses, we used cocaine—a psychomotor stimulant drug that induces powerful locomotor activation and hyperthermia (Brown and Kiyatkin, 2005). Consistent with our previous work (Brown and Kiyatkin, 2005), the magnitude of the cocaine-induced hyperthermic response was variable and inversely correlated with baseline brain temperatures

when the drug was administered at quiet rest. Interestingly, the pattern of hyperlocomotion and temperature fluctuations induced by cocaine at a typical self-administering dose (0.75 mg/kg, iv) was very similar to that induced by rectal measurement and tail-pinch procedures (compare Fig. 1 and 4). The differences were evident only in skin temperature, which decreased by cocaine stronger and for a longer time than by rectal probe and tail-pinch. It is likely that transient skin hypothermia is related to peripheral vasoconstriction, a known centrally-mediated effect of cocaine (Knuepfer and Branch, 1992; Tella and Goldberg, 1998). When cocaine administration was preceded by the rectal probe and pre-drug brain, muscle and body temperatures increased, cocaine induced differential effects varying from hypo- to hyperthermia. Mean temperatures slightly increased but the elevation was about three-fold weaker than that occurring in quiet resting conditions. Although rectal temperature increased after cocaine injections, a similar increase occurred after the first rectal probe (see Fig. 6B). Therefore, the effect of cocaine on deep body temperature as a difference between drug and control group was absent as evaluated by rectal measurements.

Consistent with our previous observations (Brown and Kiyatkin, 2005; Kiyatkin and Brown, 2004), iv cocaine could either increase or decrease brain temperature depending upon basal brain temperatures (see Fig. 5). While the elevation in individual tests varied from 0°C to ~1.5 in control conditions, both increases and decreases (range - 0.7°C to 0.75°C) were seen after cocaine was injected during repeated rectal probing and mean Nacc temperature was about 0.5°C higher. Four of five cases of temperature decreases were seen at higher temperatures (37.8-39.1°C), which were not seen in control conditions. Similar inverse relationships of brain temperature response upon basal brain temperature were also evident for both rectal probe and tail-pinch on Day 1 and they were previously reported for other arousing stimuli (Kiyatkin and Mitchum, 2003). Although these observations may be viewed as examples of the so-called «law of initial values», which postulates that the magnitude and even direction of autonomic response to a stimulus is related to the pre-stimulus, basal values (Wilder, 1956), these relationships did not exist for other drugs (i.e., methamphetamine and MDMA; see Kiyatkin, 2005 or review) and were absent for the same rectal probe and tail-pinch on Day 5. While cocaine to some extent mimics natural arousing stimuli in their ability to induce brain temperature responses that are dependent upon basal levels, the mechanisms underlying this phenomenon are obviously different and currently poorly understood.

We found that the traditional procedure of rectal measurement (40 mm depth with 1-min test duration) produces values consistently lower than body temperature assessed by chronically implanted electrodes located in approximately the same area. While rectal temperature on average was about 0.6°C lower than body temperature, between-technique differences were smaller at low temperatures and larger at higher temperatures ($r=0.58$). This measurement difference is close to that recently reported by Dallmann et al (2006), who compared values produced by Thermochron iButtons chronically implanted into the abdominal cavity and a commercially prepared thermistor probe (CMA/150 Controller: CMA Microdialysis, Sweden) at a depth of 40-50 mm and a duration “up to 20 s”. In contrast to other studies comparing temperature values assessed by rectal probe and temperature sensors located in the abdominal cavity, a body thermocouple electrode in this study was chronically implanted in the retro-peritoneal space very close to the top of the rectum—the area presumably measured by a rectal probe. Although the two factors, depth of insertion and duration of measurement, are usually considered the most important to provide a good match between rectal and body core temperatures, our previous studies (Kiyatkin and Brown, 2004) revealed the importance of proper temperature insulation of the metal probe. While deeper probe insertion in the colon (>6.5 cm) provides a better match with body core temperature (Lomax 1966; Romanovsky et al., 1998), this procedure is not less, but presumably more, stressful and potentially more harmful for the animal than traditional rectal insertion. Therefore, despite providing a better

match with real body temperature, the procedure of colonic temperature measurement has the same inherent limitations as the traditional procedure of rectal temperature measurement.

Hence, despite the simplicity of rectal temperature measurement and the relatively close match with real body temperature, this procedure significantly affects an animal's activity state, inducing brain and body hyperthermia. This influence should be considered in the interpretation of data that rely exclusively on rectal measurements. This influence may be especially important for environmental stimuli and drugs (i.e., cocaine), the effects of which depend upon basal temperature. Other approaches, which rely on the use of chronically implanted electrodes and thermo-sensors, may provide a more accurate measure of this important physiological parameter.

4. Experimental Procedures

4.1. Subjects and surgery

Seventeen male Long-Evans rats (Taconic, Rockville, MD), 4-5 months in age and 470 ± 20 g in weight, housed individually (12-h light cycle beginning at 7:00) with free access to food and water, were used. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication 865-23) and were approved by the Animal Care and Use Committee, NIDA-IRP.

Under Equithesin anesthesia (3.3 ml/kg), each rat was implanted with four thermocouple probes. The probes were prepared from twin copper and constantan wires (diameter 125 μ m) obtained from Physitemp Instruments (Clifton, NJ, USA) and described in detail elsewhere (Brown et al., 2003). The first probe was stereotactically implanted in the nucleus accumbens, shell (NAcc; 1.2 mm anterior to bregma, 0.9 mm lateral to bregma, 7.4 mm deep; Paxinos and Watson, 1996). The second probe was implanted in the temporal muscle, a non-locomotor head muscle that receives blood from the *a. carotis*, as does the brain. The third probe was implanted subcutaneously (skin) along the skull's center line approximately 9-12 mm in front of bregma. This location minimizes thermocouple probe movement and recording artifacts typical to other locations (i.e., tail and external body surfaces). The fourth probe was implanted into the retroperitoneal space between the peritoneum and trunk muscular wall (body), approximately 4 cm from the base of the tail, via a ~ 12 mm skin incision between the rectum and tail base. In this probe, thermocouple wires, except the tip, were covered by a plastic thermo-isolated material. Connecting wires were fed subcutaneously from the tail base to the head assembly. This recording site prevented movement of the thermocouple probe, minimizing recording artifacts, and placed the probe in close proximity to the tip of the inserted rectal probe, allowing a between-technique comparison. All four probes were secured with dental cement to three stainless steel screws threaded into the skull. Eight animals were also implanted with a jugular intravenous (iv) catheter during the same surgery session. For jugular catheter implantation, a 10 mm incision was made in the neck to expose the jugular vein. A catheter was then inserted into, and secured to, the vein, and the catheter was run subcutaneously to the head mount and secured with dental cement.

4.2. Experimental protocol

All rats were given three days of recovery following surgery, plus one day of habituation (6 hrs) prior to testing. On each test day, rats were also given a two-hour habituation period before any procedures. This period is essential for stabilization of temperature values altered by the procedure of cage exchange (Kiyatkin et al., 2003). During experiments, a four-channel cable connected the probes on the head mount, via electric commutator, to a computer running temperature collection software (Thermes-16, Physitemp Instruments, Clifton, NJ). In pharmacological experiments, a catheter extension was also attached to the internal catheter,

thereby allowing remote, unsignalled iv injections. All recordings took place during the light phase of the rat's cycle (10:00-17:00) in a Plexiglas chamber (32×32×32 cm), equipped with four infrared motion detectors (Med-PC IV, Med Associates, Burlington, VT), and placed inside of a sound-attenuating box. Four pairs of individual sensors (four receivers and four transmitters) were placed at regular intervals on each outer wall of the test chambers at 5 cm above the cage floor level. The resulting grid consisted of four beams. A count was recorded whenever a beam was tripped, and measurements were stored as cumulative values for each minute.

The environmental temperature was maintained at $23\pm0.5^{\circ}\text{C}$, and was monitored by a thermal probe placed inside of the experimental chamber.

During the first testing session (Day 1), animals from the first group (n=9) were exposed to both the tail-pinch and rectal probing procedures. Both procedures were executed twice, 1.5 hrs apart, and alternated in presentation order. The tail-pinch procedure consisted of placing a wooden clothespin at the base of the tail for one minute. The rectal measurement procedure was executed by lifting the tail and inserting a rectal probe thermometer (RET-2, Physitemp Instruments, Clifton, NJ, USA), with minimal restraint achieved by holding the base of the tail. The rectal temperature measurement procedure was executed over one minute. Because our preliminary data suggested that both the depth of insertion and, more importantly, reliable thermo-isolation of the metal body of the probe affect temperature values, our commercially prepared probe was thermo-isolated and inserted ~40 mm from tail base. This positioning allowed us to obtain rectal temperature values from the same body location, where the tip of the chronically implanted body electrode was located. Approximately 20 s were required to position the rat for rectal probe insertion. After insertion, the probe remained in the rectum for 40 s, allowing for temperature stabilization. During the next testing session (Day 3), rats, after a similar 2-hour habituation period, were exposed to a series of repeated rectal measurements (n=13) executed at a rate of 20 min and spanning a period of 4 hours. The final testing session (Day 5) repeated the protocol of Day 1 and rats were exposed twice to tail-pinch and rectal probing.

In animals of the second group (n=8), Day 1 was used for additional habituation. On Day 2, after a 2-hour habituation, rats received two iv cocaine injections (0.75 mg/kg, in 0.15 ml saline; inter-injection interval at least three hours) without and with additional rectal measurements. In the second case, the first rectal probe was made 10 min before cocaine injection and rectal measurements were repeated five more times with 20-min intervals. The same procedures were repeated again on Day 4 after one free day, which was used for habituation. The order of comparisons was alternated in both days to eliminate the influence of treatment day (Day 4 vs. 2) and injection number within the session (1 vs. 2). At 0.75 mg/kg dose, cocaine induces profound locomotor activation and is self-administered by rats (Ettenberg et al., 1982).

4.3. Histology

The day following the last test, rats were euthanized by pentobarbital overdose and brains were removed for subsequent histological verification of the location of NAcc thermal probes. The locations of the recording sites were determined from cryostat cut, 50 μm slices mounted on glass slides. In each of the tested animals, histological verification confirmed that the tips of brain probes were located within the medial NAcc, shell.

4.4. Data analysis

Temperature changes were presented as absolute and relative changes with respect to the moment of stimulus presentation or drug injection. One-way ANOVA with repeated measures followed by Fisher test was used for statistical evaluation of temperature changes and

locomotor activity. Student's t-test was used for comparing between-group and between-structure differences. Both 1- and 2-min time-course averages were used for data analysis. For evaluating the relationships between temperatures produced by different techniques (rectal vs. chronically implanted electrode) and cocaine-induced temperature responses depending basal temperatures, we used standard correlative and regression analyses.

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Fig 1. Changes in NAcc, skin, muscle and body temperatures (A, absolute and B, relative) as well as locomotion (C) induced by rectal probe insertion and tail-pinch in naive (Day 1) and experienced (Day 5) rats. Filled symbols show values significantly different from the last pre-stimulus value. Each group represents an average of 18 tests performed in 9 rats.

Fig 2.

Relationships between basal NAcc temperatures and their relative changes induced by rectal probe and tail-pinch on Day 1 and Day 5. Each graph shows two regression lines and coefficients of correlation (r ; **, $p < 0.01$) for rectal probe and tail-pinch.

Fig 3.

Changes in NAcc, muscle, skin and body temperatures (A, absolute, B, relative with respect to baseline preceding the first probe) as well as locomotion (C) during repeated rectal probe insertion. Each line represents an average of data obtained in 6 rats. Filled symbols show values significantly different from either pre-first probe baseline (A) or values preceding each rectal probe (C).

Fig 4.

Changes in NAcc, skin, muscle and body temperatures (A, absolute values; B, relative change) as well as locomotion (C) induced by iv cocaine (0.75 mg/kg) with (right panel) and without (left panel) additional rectal temperature measurement. Filled symbols indicate values significantly different from baseline (left panel and first rectal probe in right panel) or pre-cocaine values (right panel). Vertical solid line shows the moment of cocaine injection and vertical hatched lines on the right graphs show the moments of rectal probes (n=6).

Fig 5.

Changes in NAcc temperature induced by iv cocaine depending pre-injection NAcc temperature. “Control” represents the effects of cocaine in quiet resting conditions and “rectal” represents cocaine-induced changes during repeated rectal probes. In both groups, the change was dependent upon baseline ($r=-0.49$ and -0.73). Solid lines are regression lines. Two vertical lines show mean basal temperatures in each group.

Fig 6.

A. Changes in body temperature induced by iv cocaine in quiet resting conditions (control) and during repeated rectal probes (rectal). Filled symbols mark values significantly different vs. baseline and pre-injection ($p < 0.05$). B. Changes in rectal temperatures evaluated following repeated tests and shown as a relative change vs. the first value. In “cocaine” group, cocaine was injected at 10th min after the first rectal probe. In both cases, the effect was significant (cocaine: ANOVA $F_{15,95}=6.05$, $p < 0.01$; control: ANOVA $F_{15,95}=3.02$, $p < 0.05$), but between-group difference was absent.

Fig 7.

Relationships between body and rectal temperatures. A shows absolute temperatures and B shows body-rectal difference. In both cases, there was significant correlation between body and rectal temperatures (see regression lines and coefficients of correlation) and the difference grew at higher temperatures. Interrupted hatched lines show the line of no effect and horizontal interrupted lines determine the degree of difference (0.5, 1.0 and 1.5°C).