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Differential effects of dopamine and opioid receptor blockade on motivated Coca-Cola drinking behavior and associated changes in brain, skin and muscle temperatures

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Abstract

Although pharmacological blockade of both dopamine (DA) and opiate receptors has an inhibiting effect on appetitive motivated behaviors, it is still unclear which physiological mechanisms affected by these treatments underlie the behavioral deficit. To clarify this issue, we examined how pharmacological blockade of either DA (SCH23390 + eticlopride at 0.2 mg/kg each) or opioid receptors (naloxone 1 mg/kg) affects motor activity and temperature fluctuations in the nucleus accumbens (NAcc), temporal muscle, and facial skin associated with motivated Coca-Cola drinking behavior in rats. In drug-free conditions, presentation of a cup containing 5 ml of Coca-Cola induced locomotor activation and rapid NAcc temperature increases, which both transiently decreased during drinking, and phasically increased again after the cup was emptied. Muscle temperatures followed this pattern, but increases were weaker and more delayed than those in the NAcc. Skin temperature rapidly dropped after cup presentation, remained at low levels during consumption, and slowly restored during post-consumption behavioral activation.

By itself, DA receptor blockade induced robust decrease in spontaneous locomotion, moderate increases in brain and muscle temperatures, and a relative increase in skin temperatures, suggesting metabolic activation coupled with adynamia. Following this treatment (~180 min), motor activation to cup presentation and Coca-Cola consumption were absent, but rats showed NAcc and muscle temperature increases following cup presentation comparable to control. Therefore, DA receptor blockade does not affect significantly central and peripheral autonomic responses to appetitive stimuli, but eliminates their behavior-activating effects, thus disrupting appetitive behavior and blocking consumption.

Naloxone alone slightly decreased brain and muscle temperatures and increased skin temperatures, pointing at the enhanced heat loss and possible minor inhibition of basal metabolic activity. This treatment (~60 min) had minimal effects on the latencies of drinking, but increased its total duration, with licking interrupted by pauses and retreats. This behavioral attenuation was coupled with weaker than in control locomotor activation and diminished temperature fluctuations in each recording location. Therefore, attenuation of normal behavioral and physiological responses to appetitive stimuli appears to underlie modest inhibiting effects of opiate receptor blockade on motivated behavior and consumption.

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Keywords

appetitive behavior; brain metabolism; dopamine antagonists; naloxone; neural activation; arousal; motivation; consumption; reward

It is known that pharmacological blockade of dopamine (DA) receptors has a powerful inhibiting effect on different types of motivated behaviors established by positive and negative reinforcers (Le Moal and Simon, 1989; Wise and Bozarth, 1987); the effect is especially strong with respect to drinking and feeding behaviors (Dourish, 1983). Appetitive motivated behaviors are also affected by the blockade of opiate receptors; these effects are less robust, more delicate, but also are especially evident with respect to consumption of palatable foods and drinks (Bolles and Fanselow, 1982; Billing et al., 1985; Cleary et al., 1996; Holloway et al., 2004; Holtzman, 1974). While these data suggest that both DA and endogenous opioid peptides are involved in organization and performance of motivated behavior, a highly energetic debate is continued for the last 30 years to define this role in psychological terms (Barbano and Cador, 2006, 2007; Benninger, 1983; Berridge, 1996; Berridge and Robinson, 1998; Di Chiara, 2002; Hayward et al., 2002; Kelley et al., 2005; Robbins and Everitt, 1996; Salamone et al., 1991; Salamone and Correa, 2002; Schultz et al., 1997; Schultz, 2002; Wise et al., 1978; Wise, 1982). This debate is based on enormous volume of experimental data that analyze animal behavioral output in different models (varying in nature and quality of the reinforcer, the means of its obtainment, animal homeostatic state, concomitant environmental conditions) and various types of genetic and pharmacological interventions. While this discussion has sparked from a famous “anhedonia hypothesis” (Wise et al., 1978; Wise, 1982) that explained the behavior-inhibiting effects of DA antagonists by their ability to reduce the affective or pleasurable actions of food and other rewards (i.e., to make them less reinforcing), further experimental evidences were more consistent with drug-induced deficits of motivational or preparatory aspects of appetitive behavior (Berridge and Robinson, 1998; Di Chiara, 2002; Robbins and Everitt, 1996; Salamone et al., 1991, 2009; Salamone and Correa, 2002). In contrast, endogenous opioids were more implicated in the evaluation of the hedonic, pleasant effects of positive reinforcers that make them desired and wanted (Berridge, 1996; Hayward et al., 2002; Kelley et al., 2005; Barbano and Cador, 2007).

In the present study we explored another approach to examine the role of DA and endogenous opioid mechanisms in appetitive motivated behavior by defining physiological mechanisms that are affected by DA and opioid receptor blockade and could underlie drug-induced behavioral deficit. As a behavioral model, we choose a simple paradigm of learned drinking of sugar-containing caffeine-free Coca-Cola®. Rats quickly establish Coca-Cola drinking after a few days of passive training and maintain this behavior for many repeated sessions without food and water deprivation. In addition to quantifying the latencies to initiate drinking and drinking durations, we examined the pattern of locomotor activity and temperature fluctuations in the brain (NAcc), facial skin and temporal muscle. These tests were conducted in both drug-free conditions and after systemic blockade of either opioid (naloxone 1 mg/kg) or DA (a mixture of D1- [SCH23390 0.2 mg/kg] and D2- selective [eticlopride 0.2 mg/kg]) antagonists. Therefore, we were able to evaluate how either treatment affects (a) basal motor activity and temperatures, (b) drinking behavior, and (c) temperature and motor effects associated with key events of drinking behavior. Temperatures were recorded with a high temporal resolution (5-s), allowing for the analysis of both tonic and phasic, event-related, temperature fluctuations.

Brain temperature is closely related to neural metabolism, reflecting a dynamic balance between metabolism-related heat production and heat loss by cerebral circulation to the rest of the body and then to external environment. In contrast to widespread beliefs on stability and tight regulation, brain temperature shows relatively large (~3°C) and rapid (5-10 s) fluctuations

associated with various environmental stimuli and occurring during different behaviors (see Kiyatkin, 2005, 2010 for review). While phasic increases in brain temperature primarily reflect metabolic neural activation, fluctuations in skin temperature provide a valuable measure of peripheral vasoconstriction—another sensitive, centrally mediated response triggered by various arousing and reinforcing stimuli (Baker et al., 1976). The temporal muscle is a non-locomotor head muscle that receives the same blood supply as the brain (via the carotid artery); this recording site is important for evaluating the contribution of arterial blood supply to alterations in brain and skin temperatures. Thus, simultaneous recordings of locomotion and temperature fluctuations in different central and peripheral locations in drug-free and treatment conditions could add new information on the effects of DA or opiate receptors blockade on basal physiological state and alterations in basic physiological mechanisms that underlie drug-induced behavioral deficit.

Materials and Methods

Subjects

Six Long-Evans male rats (Taconic, Germantown, NY), weighing 400-460 g and housed under a 12 h light cycle (lights on at 0700), with *ad libitum* food and water, were used. Prior to surgery, rats were put through a pilot trial of the Coca-Cola drinking procedure for both training and selective purposes (see below). Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publications 865-23) and were approved by the Animal Care and Use Committee, NIDA-IRP.

Surgery

All animals were implanted with three thermocouple electrodes as previously described (Kiyatkin and Brown, 2005). Animals were anesthetized intraperitoneally (ip) with 3.3ml/kg of Equithesin (sodium pentobarbital, 32.5 mg/kg and chloral hydrate, 145 mg/kg) and mounted in a stereotaxic apparatus. Four holes were drilled through the skull: three for securing screws and one for thermocouple insertion in the NAcc shell (1.2 mm anterior to bregma, 0.9 mm lateral to bregma) using the coordinates of Paxinos and Watson (1998). The *dura mater* was retracted and the thermocouple probe was slowly lowered to the desired target depth (7.4 mm, measured from the skull surface). A second thermocouple probe was implanted subcutaneously along the nasal ridge with the tip approximately 15 mm anterior to bregma. Although the tail is the primary organ of heat dissipation in rats, facial skin has dense vascularization and this location provides mechanical stability of the electrode, an essential condition for long-term, artifact-free temperature recordings. A third thermocouple probe was implanted in the deep temporal muscle (*musculus temporalis*), which has a similar arterial blood supply as the brain and not directly involved in motor activity. Because temperature fluctuations in skin and muscle depend upon two variables: the state of vessels (vasoconstriction/vasodilatation) and the temperature of arterial blood inflow, these two locations were important for evaluating the source of temperature fluctuations and their underlying mechanisms. By tracking temperature responses in different brain and body sites with a short collection interval, one is able to follow the dynamics of heat generation and flows within the organism. The probes were secured with dental cement to the three stainless steel screws threaded into the skull. Rats were allowed three days recovery and two more days of habituation (6 h sessions) to the testing environment before the start of testing.

Experimental Protocol

During three training sessions that preceded surgeries, rats were placed in experimental chamber and, after 2-3 hour habituation period, were given a container (a small plastic bottle cap, ~3.0×1.6 cm, fixed on a metal plate for stability) holding 5 ml of caffeine-free Coca-Cola Classic (Coke®). This drink contains 11% of sugars, with 1.97 calories or 0.55 g of sugars in

a 5-ml portion. Coca-Cola samples were de-gassed by intense shaking and had temperature equal to room temperature (22-23°C). The cup with Coca-Cola was presented for one hour and the procedure was repeated twice during the ~6-hour session. Rats were not food- or water-deprived before the sessions, but had no food or water except two Coca-Cola drinks each presented for 1-hour intervals. If the rats learned to consume the beverage entirely during two consecutive presentations, they were selected for surgery. Three rats, which showed inconsistent Coca-Cola drinking were excluded from further work.

All tests occurred inside a Plexiglas chamber (32×32×32 cm) placed inside a sound- and light-attenuated plastic box (60 × 56 × 70 cm) under continuous weak white light insulation (15 W) and in view of a small video camera mounted above the cage. These chambers were equipped with four infrared motion detectors (Med Associates, Burlington, VT, USA), allowing for monitoring locomotor activity. Rats were brought to the testing chamber at ~09:30 AM and attached via a flexible cord and electrical commutator to thermal recording hardware (Thermes 16, Physitemp, Clifton, NJ, USA). Temperatures were recorded with a 5-s time resolution and movement was recorded as the number of infrared beam breaks per 1 min. Room temperature was maintained at 22-23°C and controlled by another thermocouple located inside of the recording chamber.

During the first 2-3 recording sessions, each rat was exposed to a similar basic testing protocol, which included two presentations of 5 ml Coca-Cola samples. After 2-3-hour habituation period and stabilization of basal temperatures, a drink-filled container was first presented to rats, resulting, with some onset latency, in drinking behavior. Empty cup was always removed from the cage ~60 min after its presentation. The same procedure was repeated in the afternoon, about 2-3 hours after the first Coca-Cola presentation. The moments of cup introduction, cup removal and behavioral events (start of drinking, end of drinking) were recorded for further analyses. Independently of animal location in the chamber, the cup was placed in the same location right below the view of video camera, allowing visual observation of animal behavior from another room without any influences from the experimenter. Cup was introduced and removed from the chamber by an experimenter.

In addition to 2-3 identical control sessions in drug-free conditions, each rat experienced at least once treatment sessions in which the same testing program was used following administrations of either naloxone (1 mg/kg) or a mixture of D1- (SCH23390 0.2 mg/kg) and D2-selective (eticlopride 0.2 mg/kg) DA antagonists. Drugs were freshly dissolved in saline and injected subcutaneously (sc) in 0.3 ml bolus. The order of drug treatments was counterbalanced and each session with drug injection was always followed by drug-free regular session, when the rat received two Coca-Cola samples. SCH-23390 [R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, or SCH] and eticlopride (ETI) have strong antagonistic activity at D1-like or D2-like DA receptors, respectively (relative D1:D2 affinity, SCH=2,500:1 and ETI 1:51,400; Neve and Neve, 1997). Naloxone has an extremely high affinity for μ -opioid receptors, producing their transient competitive blockade. Naloxone also has an antagonist action, though with a lower affinity, at κ - and δ -opioid receptors. The blocking action of naloxone on opiate receptors is relatively short, being evident not more than two hours after sc administration (1 mg/kg) and followed by transient, rebound-like receptor hypersensitivity (Kiyatkin, 1989). Both these drugs were obtained from Sigma (St. Louis, MO). The first Coca-Cola test after pharmacological treatments (either DA antagonists or naloxone) was always conducted 40 min post-injection, with cup removal one hour later (100 min post-drug). The second test was conducted ~3-hrs after injections, following by cup removal 60 min later. Since the injection procedure results in transient behavioral activation and significant temperature responses (Brown et al., 2003), rats during control, drug-free sessions received at least one sc injection of saline (0.3 ml); in these tests the first Coca-Cola was presented 40 min following saline injections.

Histology and Data Analysis

When recording experiments were completed, all rats were anesthetized, decapitated, and had their brains removed for sectioning and confirmation of probe placement. Brains were cut on a cryostat into 50 μ m slices and placed on glass slides. All probes were located within the medial portion of the ventral striatum (NAcc shell), as described in Paxinos and Watson (1998). Temperature data were analyzed with 5-s and 60-s time bins and were presented as both absolute and relative changes with respect to the moment of cup presentation, cup removal as well as the start and end of drinking. For most conditions, we also calculated NAcc-muscle and skin-muscle temperature differentials, relative between-site temperature differences with respect to the event of interest. While an increase in NAcc-muscle differential, which reflects more rapid and strong increase in the brain vs. muscle site, consistently occurs following various arousing stimuli (serving a measure of brain metabolic activation), the skin-muscle differential is more accurate measure of vascular response (vasoconstriction/vasodilatation), corrected for temperature changes in arterial blood inflow (see Kiyatkin, 2010). Since rapid skin temperature decrease due to vasoconstriction is opposed by a slow temperature increase due to inflow of warmer blood, skin-muscle temperature differentials were instrumental for better representation of peripheral vasoconstriction and organism's heat retention and loss. Locomotor activity was analyzed with 60-s bins. One-way ANOVA with repeated measures, followed by post-hoc Fisher tests, was used for statistical evaluation of temperature and locomotion changes preceding and following events of interest. Student's t-test was used for comparisons of between-site and between-condition differences in temperature and locomotion. Between-state differences were evaluated based on statistical comparisons of basal temperatures and locomotion averaged for 6 min preceding the container's presentation. The use of the words "increase" or "decrease" as well as "significant" refers to the presence of a statistically significant change in the parameter or differences between the compared groups or conditions (with at least $p < 0.05$) revealed by either ANOVA or Student's t-test. Most quantitative results of statistical evaluations are shown in figure captions.

Since rats showed different latencies to initiation of drinking and different drinking durations and, in some cases, refused to drink or their drinking was transient or intermittent, different types of statistical analyses were used to accurately represent temperature dynamics associated with the events of interest. First, we analyzed basal temperatures and basal locomotor activity preceding the events of interest. For this analysis, temperature and locomotor data were averaged for different specified durations preceding these events. Second, we analyzed temperatures and locomotor activity following presentations of a Coca-Cola-containing cup and its removal from the cage one hour later. This slow time-course analysis was performed, using consecutive 1-min values of recorded parameters. This analysis provided a general representation of tonic changes in temperature and locomotion induced by different stimuli, allowing between-stimulus comparison. To reveal fine time-course dynamics of temperature fluctuations in different conditions, this analysis for critical time intervals of interest was also performed with 5-s temporal resolution. Finally, high temporal resolution (5-s bins) was used to evaluate changes associated with drinking behavior. Because of high variability of both latencies to drinking and its durations of drinking in each rat and session, fine temperature dynamics was analyzed with respect to the initiation of drinking and the end of drinking. These two events were set at zero and temperature values preceding and following these events were averaged for all rats within specified timing intervals and statistically evaluated with respect to these events. A similar strategy was used for statistical evaluating of highly variable timing intervals between cup presentation and initiation of drinking behavior.

Results

1. Changes in temperature and locomotion during motivated drinking behavior

Although the rats were trained to drink Coca-Cola, they always showed certain latency to initiate drinking behavior after cup presentation; this latency to the first lick in control, drug-free conditions varied from 20 to 310 s (mean 53.3 ± 8.4 s). Drinking behavior consisted of a continuous chain of licking movements until all Coca-Cola volume was consumed; duration of drinking varied from 150 to 390 s, with a mean 229.1 ± 11.6 s.

As shown in Fig. 1 (left panel), motivated Coca-Cola drinking was associated with robust locomotor activation and significant temperature fluctuations in each recording location. Averaged with 1-min quantification bin for all drinking tests in drug-free conditions ($n=34$ tests), NAcc and muscle temperatures significantly increased and skin temperature significantly decreased during this procedure (see quantitative results of statistical comparisons in figure captions). At the initial part of the ascending curve, NAcc showed more rapid and stronger increases than the muscle (B1), resulting in significant increase in NAcc-muscle temperature differential (C1). In contrast, skin temperature decreased rapidly and strongly immediately after cup presentation; this effect was most rapid, transient, and followed by a rebound-like skin warming. Skin-muscle differential showed a rapid and prolonged decrease maintained for ~30 min following cup presentation. Locomotion response (D1) had two peaks: the first that preceded drinking and the second that followed it; a between-peak decrease in locomotion (2-4 min) was associated with Coca-Cola consumption.

Removal of an empty cup (one hour after its presentation and ~50 min after drink was consumed) also resulted in locomotor activation and temperature fluctuations that mimicked a general pattern seen with motivated Coca-Cola drinking (Fig. 1, right panel). However, all these changes were weaker, with a significant difference for NAcc (mean increase for 25 min: 0.22 ± 0.04 vs. $0.41 \pm 0.08^\circ\text{C}$ for full cup presentation; see Table 2 below). There were also some minor changes in temperature dynamics. Following the removal of an empty cup, locomotor activation was monophasic (D2), increases in NAcc and muscle temperatures occurred with shorter latencies (B2), and a NAcc-muscle differential increased within the first few minutes after the event (C2). This change as well as the change in skin-muscle differentials were weaker and more transient than those seen with drinking behavior. An averaged locomotor activation following empty cup removal was also about two-fold weaker ($p < 0.05$) than that for full cup presentation (see Table 2 below).

Figure 2 (left panel) shows temperature dynamics associated with motivated drinking behavior analyzed for the NAcc and temporal muscle with a high temporal resolution (5-s bins). In this case, data were averaged with respect to three events: cup presentation (first vertical hatched line), initiation of drinking (second vertical line) and its termination (third vertical line). Because of variable latencies to drinking and its duration, temperature data are not continuous in time, but they accurately represent the changes preceding and following these three events. As can be seen, NAcc temperature rapidly increased following cup presentation and before the initiation of drinking behavior, while muscle temperature did not grow during this period (A1). Because of these differences, NAcc-muscle temperature differential rapidly increased and peaked at the moment when drinking began (B1). During drinking, NAcc temperature after a certain delay began to decrease, but then stabilized and increased slightly at the end of drinking (A1). In contrast, muscle temperature increased within the first 30 s of drinking but then remained at this increased level to the end of drinking (A1). The NAcc-muscle differential decreased rapidly during drinking to baseline levels (B1). After Coca-Cola was fully consumed, NAcc and muscle temperatures began to increase again, with more rapid and strong acceleration in the brain vs. muscle, resulting in a second peak of NAcc-muscle differential at 2-3 min after the end of drinking (A1 and B1). Tight correlation between brain temperature

and behavioral events is especially evident when analyzed as a rate change (C1). NAcc temperatures rapidly and strongly increased following cup presentation and during searching behavior until the rat began drinking. Immediately after this event, the rate of change dropped and became negative ~30 s after. Inversion of brain temperature was quite rapid and evident within the first 5-10 s following onset of drinking. The second, more prolonged increase occurred after the end of drinking, when the rat again showed locomotor activation and numerous licks of an empty cup. This increase weakened gradually, returning to baseline ~5-8 min after Coca-Cola consumption. At this point, absolute temperatures peaked and then decreased slowly toward baseline (see Fig. 1A).

Removal of empty cup resulted in quite different pattern of temperature fluctuations (Fig. 2, right panel). In this case, NAcc and muscle temperatures increased monotonically, with a higher rise in the NAcc than in the muscle (A2) and weaker but more prolonged increase in the NAcc-muscle temperature differential (B2). The magnitude of temperature increases in both NAcc and muscle was significantly weaker than with full cup presentation (see also Table 2) and the rate of NAcc temperature changes showed the initial rapid increase (3-5 min post-stimulation) followed by a subsequent return toward baseline (C2).

2. Changes in basal temperatures and locomotion induced by DA receptor blockers and naloxone

Consistent with our previous data (Kiyatkin and Brown, 2005; Kiyatkin, 2008), a mixture of D1- and D-2 selective DA receptor blockers (SCH23390 and eticlopride 0.2 mg/kg each) strongly inhibited spontaneous locomotor activity, moderately increased brain and muscle temperatures, and slightly increased skin temperature (Fig. 3, left panel). These effects became evident from ~10-15 min after drugs administration and they were preceded by a typical arousal-related temperature and motor response induced by the injection procedure. As shown in Fig. 3 (right panel), sc saline injections induced a similarly rapid, transient locomotor activation, monotonic increase in brain and muscle temperatures, and rapid decrease in skin temperature. While the changes in NAcc-muscle differentials during DA receptor blockade were similar to those seen after saline injection (compare B1 and B3), there were differences in skin-muscle differentials. After the initial decrease similar in both groups, skin-muscle differentials slightly increased in control but significantly decreased in the DA antagonist group.

Table 1 shows changes in basal temperatures seen in rats under drug-free conditions and following administration of DA antagonists and naloxone. These values were assessed for 10-min intervals preceding the first and second Coca-Cola presentations and the first and the second cup removals from the cage, allowing comparisons between drug treatments and controls. In contrast to the slight decreases in basal NAcc and muscle temperatures seen during the session in controls, these parameters significantly increased during DA receptor blockade (~0.9°C at 40 min post-injection; $p < 0.05$ vs. control), suggesting that motor inhibition is associated with increased metabolic brain activity and heat accumulation in both the brain and body. These increases in brain and muscle temperature were associated with a slight increase in skin temperature, suggesting enhanced heat loss. Although between-group temperature differences decreased before the second cup removal (~ 4 hrs post-injection), spontaneous locomotor activity was still drastically lower in the DA antagonists group than in controls at this time point.

As shown in Fig. 3 (middle panel), the immediate effects of naloxone administration (locomotor activation, increases in NAcc and muscle temperatures, decrease in skin temperature, and transient rise in brain-muscle differential) were similar to those seen after injection of saline and DA antagonists. However, between-group differences emerged from 15-20 min post-injection (compare A2, B2 and A3, B3). While in both groups, NAcc and

muscle temperatures after the initial increase decreased below pre-injection baseline, the decrease after naloxone was stronger, especially in the NAcc ($p < 0.05$, see Table 1). After the initial injection-associated decrease in skin temperature seen in both groups, skin temperature and skin-muscle differentials significantly increased in naloxone group, while they returned to baseline in controls. These effects were weaker and shorter than those with DA antagonists and all temperatures were similar to controls at ~180 min after naloxone injection, before the second Coca-Cola test (see Table 1). As shown in Table 1, basal locomotor activity did not show evident changes after naloxone administration.

3. Effects of DA antagonists on Coca-Cola drinking behavior and associated changes in temperatures and motor activity

All animals injected with DA antagonists showed no interest in the presented cup and no Coca-Cola drinking in each of 10 tests. This behavior-blocking effect was evident for each of two drink presentations at ~40 and ~200 min post-injection, respectively. Because of this, data shown in Fig. 4 represent the average of two tests in each rat.

Despite a full blockade of drinking behavior and virtually no motor response to the presented cup (see Fig. 4D1; minimal changes could be, at least in part, related to the artifacts during cup's placement in the cage), there were clear and significant temperature responses to both the introduction of a cup and its removal from the cage during DA receptor blockade (Fig. 4A-C). Despite significantly higher brain and muscle temperatures before cup presentation (see Table 1), they both transiently increased and skin temperature decreased slightly after cup presentation and its removal. In each case, temperature response in the NAcc was more rapid and stronger than in the muscle, increasing brain-muscle differentials (C). In each case, skin temperature dropped rapidly but later slowly increased to the levels above baseline.

These responses had a similar time-course and, as shown in Table 2, they were virtually identical in strength. However, the mean magnitude of NAcc and muscle temperature elevations induced by cup presentation during DA receptor blockade was significantly weaker than in control, but the response associated with cup removal was similar in both groups. While skin temperatures on average decreased after cup presentation in controls, they changed very little during DA receptor blockade ($p < 0.05$).

Despite a general similarity of temperature responses seen at low temporal resolution, high-resolution temperature analysis revealed drastic differences in temperature responses induced by cup presentation during DA receptor blockade vs. control, drug-free conditions (see Fig. 5; left panel). While the initial component of NAcc temperature increase was identical in both conditions, temperature increased further after cup presentation during DA receptor blockade, but showed a transient, drinking-related drop in controls. While in controls, this drop was followed by the second temperature elevation, NAcc temperature still increased slowly and peaked at ~6 min during DA receptor blockade (A1). A similar difference was typical to muscle temperature (B1), which increased much stronger and monotonically during DA receptor blockade, when the rats were fully inactive compared to the control conditions, when rats showed licking behavior followed by strong increase in locomotor activity. In contrast to robust and prolonged decreases in skin temperature seen in controls, vasoconstrictive effect was minimal and transient during DA receptor blockade (C1).

Rapid brain and muscle temperature changes associated with cup removal were similar in both conditions, but the response was somewhat weaker during DA receptor blockade (Fig. 5, right panel; A2 and B2). Similarly, skin hypothermia during DA receptor blockade was much weaker and more transient than in control (C2).

4. Effects of naloxone on Coca-Cola drinking behavior and associated changes in temperatures and motor activity

While not as robust as with the DA antagonists, naloxone had a certain effect on motivated drinking behavior. While in 6/8 tests, rats made the first interaction with the Coca-Cola-containing cup with the latencies within the control range (20-75 s), in one test the latency was extremely long (372 s), and in other test the rat refused to try Coca-Cola within an hour after its presentation. More robust effects were seen with respect to Coca-Cola consumption, which was normal in duration and complete only in 3/8 tests (157-176 s). In four other tests, rats did several attempts to drink, and apparently consumed all the drink volume but in many interrupted licking episodes. In one test, drinking never occurred. Because of this behavioral variability it was impossible to average temperature changes related to drinking behavior *per se*.

The inhibiting effect of naloxone on Coca-Cola drinking seen with the first test, disappeared during the second test performed 3-hours after drug injection. In this case, latencies to drinking were within a control range in 6/7 tests (20-130 s) and duration of drinking was normal in 5/7 tests. In remaining two cases, latencies were unusually long and drinking was interrupted by poses, but Coca-Cola volume was fully consumed in all tests.

As shown in Fig. 6 (left panel) and Table 2, temperature responses to the first drink presentation after naloxone were attenuated strongly in each recording location ($p < 0.05$ for NAcc and muscle). On average, brain and muscle temperatures increased 0.25-0.30°C above baseline, with increases ~0.6°C in controls (compare Figs 1 and 6). The mean response magnitude (0.14 and 0.16°C for NAcc and muscle, respectively) was two- to three-fold lower than in controls (0.41 and 0.37°C, respectively). Naloxone-treated rats also showed a significantly weaker and more transient response in the skin and about two-fold weaker locomotor activation ($p < 0.01$; see Table 2). Despite a highly significant inhibiting effect of naloxone on temperature responses associated with motivated drinking behavior, naloxone-treated rats showed a similar to control responses to the removal of an empty cup (see Table 2). In both cases, NAcc temperatures increased slightly (0.22 and 0.29°C, respectively) and returned to baseline within ~20 min after cup removal.

As shown in Fig. 6 (right panel) and Table 2, temperature and locomotor responses following the second Coca-Cola presentation 3 hours after naloxone administration were significantly stronger in each recording location than those seen with the first presentation and similar to drug-free control.

Precise time-course of temperature differences in each recording location that occurred during motivated drinking between naloxone-treated and control conditions is shown in Fig. 7. As can be seen in A, mean basal NAcc temperature is lower than in control before the first Coca-Cola presentation after naloxone administration. In contrast to control, NAcc temperature increased weaker both at the first, searching-related temperature acceleration and the second post-consumption acceleration (B). While NAcc temperatures peaked (+0.7°C) at ~800 s after a cup presentation in controls, the peak was two fold-lower (~0.3°C) and occurred at ~420 s in naloxone-treated conditions. Basal NAcc temperature and its increase were clearly higher during the second Coca-Cola presentation in naloxone-treated conditions. In this case, the initial temperature increase was even larger than in control, but temperature still peaked at the earlier time (~560 s).

Similar differences were seen in muscle temperature dynamics. Muscle temperature increase during the first Coca-Cola presentation was weaker than in control, but became larger than in control during the second test. While, similarly to control, skin temperature decreased during the first post-naloxone Coca-Cola test, this effect was more transient. In contrast, decrease in

skin temperature became stronger and more pronounced than in control during the second Coca-Cola test.

Discussion

Similar to humans that widely consume sugar-containing beverages, rats rapidly learned to consume a Coca-Cola drink and maintained this consumption during multiple daily sessions without any food or water deprivation. After a couple of days of passive training, most rats developed a stereotyped behavioral pattern following the presentation of a Coca-Cola-containing cup. They initiated searching behavior, which with a certain variable latency (~50 s) resulted in drinking, a chain of uninterrupted licking movements until all volume of a drink was fully consumed. When the cup was empty, rats showed the second, more prolonged peak of motor activation, associated with periodic returns to the empty cup and its licking. Such a simple paradigm was used in this study to examine the effects of either DA or opioid receptor blockade on motivated appetitive behavior and behavior-associated temperature fluctuations in the brain, muscle and skin. Since the Coca-Cola-containing cup is not only an appetitive stimulus, but a novel object, which always induces locomotor activation and a distinct temperature response (Kiyatkin et al., 2002), we also examined temperature and motor responses following the removal of an empty cup, long after the rats finished drinking and were in quiet rest again. This test was important to differentiate between “motivational arousal” triggered by an appetitive stimulus and a non-specific arousal that is induced by any environmental change. Finally, two drinking tests, with about a 2.5-h intervals between them, were conducted during each daily session in both control and drug-administered conditions. While the changes associated with both tests were similar in controls, between-test differences after drug treatments were important for evaluating pharmacological specificity of the observed effects in the light of known duration of drug-induced receptor blockade.

1. What could be learned about motivated drinking behavior based on temperature recordings?

When the experienced rat was presented with a Coca-Cola-containing cup, it showed behavioral activation associated with rapid increases in NAcc and decreases in skin temperatures that continued until the rat initiated drinking. High-speed analysis revealed that these temperature changes are very rapid, being significant within 10-15 s following cup presentation. In contrast, temporal muscle temperature did not change significantly (even slightly decreased) at this stage despite increased locomotion. Since both the brain and temporal muscle receive arterial blood supply from the same source, more rapid and stronger temperature increase in the brain vs. muscle, with a rise in brain-muscle differential, suggests metabolic neural activation and intra-brain heat production as a primary cause of brain temperature elevation. Although skin temperature also depends upon temperature of arterial blood supply, its rapid fall suggests vasoconstriction, a known adaptive response triggered by different arousing and emotional stimuli (Altschule, 1951; Baker et al., 1976; Solomon et al., 1964) and aimed at heat retention under conditions of potential danger. Therefore, it appears that motivated search that is triggered by an appetitive stimulus is associated with metabolic brain activation and peripheral vasoconstriction.

However, temperature dynamics drastically changed immediately after drinking began. The rate of NAcc temperature increase rapidly dropped after the first lick, weakened progressively within the next 25-30 s of drinking, and then inverted into negativity. In contrast, muscle temperature increased slightly during licking behavior. These opposite changes resulted in rapid decrease in brain-muscle differential, which returned from its peak at the start of drinking to baseline at the end of drinking. Therefore, it appears that drinking *per se* (=consummatory behavior) is associated with rapid cessation of previous search-related neural activation.

Since the size of the presented drink could not fully satisfy rats and they are able to consume much larger drink volumes, they consistently showed another, more prolonged bout of motivated search, with repeated interactions with an empty cup, its periodic licking and a strong increase in locomotor activity. The re-appearance of motivated search following consumption was associated with the second increase in NAcc temperatures, which, in contrast to the initial increase, was maximal immediately after the end of drinking, decreasing gradually within the next 5-8 min. At this moment (10-15 min after cup presentation), brain temperatures reached their absolute peaks, returning slowly to baseline within the next 20-25 min. Although muscle temperature followed the same pattern, its changes were weaker and more delayed, resulting in a second, larger and more prolonged increase in the NAcc-muscle differential.

Although tightly correlating with key behavioral events, changes in central and peripheral temperatures seen in this study appear to reflect global, non-specific alterations in metabolic neural activity that could be triggered by other arousing stimuli and occur during different motivated behaviors. A similar pattern of temperature fluctuations coupled with locomotor activation was also observed following exposure to other arousing and reinforcing stimuli (i.e., tail-pinch, presentation of inaccessible food in food-deprived conditions, presentation of sexual partner, presentation or removal of novel environmental objects, etc.) (see Kiyatkin, 2005 for review). While the initial components of this response were similar for different stimuli and situations, obviously reflecting the detection of environmental change and triggering of behavioral activation, its further dynamics and strength differed dramatically, being dependent upon the specifics of animal behavior. In the case of environmental stimulation (i.e., tail-pinch and cup removal in this study), the activation response was always monophasic, with all parameters returning to baseline within a certain time, depending upon the stimulus strength and salience. However, in cases when relevant stimulation triggers motivated behavior, this activation persists. While in this study searching behavior quickly resulted in drinking (reward), sharply altering brain temperature dynamics, much stronger and prolonged brain temperature increases occurred during male copulatory behavior (Kiyatkin and Mitchum, 2003). In this case, motor activity and temperatures gradually increased following exposure to receptive female, and further increased during mounts and intromissions. Similar to that seen with Coca-Cola consumption, rapid, transient decreases in NAcc temperatures (albeit much stronger and more prolonged) also occurred immediately after ejaculation, the final, rewarding event of male copulatory behavior. Finally, a similar pattern of brain temperature dynamics was seen during intravenous cocaine self-administration, unnatural drug-motivated behavior (Kiyatkin and Brown, 2003). In this case, brain temperature rapidly increased following exposure to cocaine-related sensory cues, further grew during cocaine-seeking behavior and after the first self-injection, but transiently decreased with each subsequent cocaine injection. Therefore, despite the basic common features, each type of motivated behavior has some distinct differences in its physiological correlates.

2. DA receptor blockade and its behavioral and physiological consequences

Our previous neuronal studies suggest that the combination of SCH23390 and eticlopride at 0.2 mg/kg (0.7 and 0.6 μ M, respectively) provides an effective blockade of both D1- and D2-like DA receptors, fully antagonizing striatal neuronal responses to iontophoretic DA (Kiyatkin and Rebec, 1999). This treatment regimen was used in the present study to block DA transmission, differing from many behavioral studies, in which either D1- or D2-selective DA antagonists are used or the drugs are administered at smaller doses, which are unable to block most DA receptors.

Consistent with our previous work (Brown et al., 2007; Kiyatkin and Brown, 2005), DA receptor blockade strongly inhibited spontaneous locomotor activity and tonically increased brain and muscle temperatures. This association seems unusual in the light of a positive

correlation between behavioral activation and temperature increases seen under physiological conditions (see Kiyatkin, 2005 for review). It also seems unusual from a pharmacological point of view since drugs with sedative, behavior-inhibiting effects (i.e., general anesthetics, diazepam, THC) typically decrease brain and muscle temperatures, while the drugs that induce behavioral activation (i.e., amphetamine, methamphetamine, cocaine) increase these temperatures. Moderate brain and body hyperthermia during DA receptor blockade was associated with increased skin temperatures, pointing at enhanced heat dissipation. However, a decrease in skin-muscle differentials suggests that enhanced heat dissipation results from enhanced heat production (and warming of arterial blood inflowing to the skin) and is associated with peripheral vasoconstriction (see Fig. 3), which diminishes heat dissipation and contributes to heat accumulation in the body. Therefore, it appears that robust inhibition of motor activity following DA receptor blockade is associated with some kind of metabolic activation in the brain and body, with enhanced heat production and increased heat loss to the external environment.

During DA receptor blockade rats did not show any interest to the presented Coca-Cola-containing cup, no locomotor activation, no tasting of the drink, and no consumption. However, cup presentation induced consistent temperature responses in each recording location, indicating that rats detect the presented cup and show central activation response similar to that seen with cup presentation in controls in drug-naïve conditions. However, brain and muscle temperature increases were two-fold lower, and skin hypothermic response was greatly attenuated. Interestingly, removal of a cup from the cage induced a similar arousal-related temperature response, similar to that seen with cup presentation (see Table 2).

Our data are consistent with the idea that the DA system is essential for mediating the behavior-activating effects of appetitive stimuli or motivational (driving) aspects of appetitive behavior (Le Moal and Simon, 1989; Salamone, 1992; Bozarth and Wise, 1987). Although these stimuli are detected by an animal during full DA receptor blockade and induce a central arousal response, they are unable to trigger searching behavior, an essential stage preceding consumption. Based on our data, we cannot speculate on why the behavioral search was fully blocked and whether the rats do not “want” (lost motivation) or simply are unable to initiate the behavioral activation. It is also unclear to what extent this effect on drinking behavior could be generalized to other forms of motivated behavior. Although interruption of DA transmission in this study fully blocked behavioral activation and Coca-Cola consumption, the motor-inhibiting effect was strong but weaker with respect to tail-pinch (~11% of control) and social interaction (~6% of control) (Kiyatkin, 2008). While it is possible that rats will respond behaviorally to other, stronger positive (i.e. food after food deprivation) or negative reinforcing stimuli (i.e. aversive, noxious stimuli) and such a view has an experimental support (Blackburn et al., 1989; Salamone, 1992; Barbano and Cador, 2006), it is unclear whether or not pharmacological protocols used to block DA transmission were able to block all DA receptors efficiently and prevents interaction of endogenous DA with them completely. Animals with extensive neurotoxic damage of the DA neurons or genetically-engineered DA-deficient animals are adynamic, generally unable to be engaged in basic motivated behavior, and most die, if not artificially fed, due to starvation (Ungerstedt, 1971; see Palmiter, 2008 for review). Finally, movement activation (search) and motivation could be different sides of the same phenomenon, making questionable a distinct separation between them (unable to move – no desire to move). At an objective, physiological level, It could be also questionable a true distinction between so-called motivational and reinforcing properties of environmental stimuli (Wise and Bozarth, 1987), which could be different terms to define the ability of these stimuli to induce a relevant behavioral response.

The ability of DA antagonists to inhibit motor activation induced by behaviorally relevant stimuli may help to explain a critical role of the DA system in mechanisms of reinforcement.

If the behavior-activating effects of these stimuli are diminished during DA receptor blockade, both the consummatory behavior and subsequent learning of the stimulus-response association will be also be inhibited.

3. Blockade of μ -opioid receptors and its behavioral and physiological consequences

Consistent with previous observations (Wright and Katovich, 1996; Romanovsky et al., 1994; Stewart and Eikelboom, 1981), naloxone slightly decreased brain and muscle temperatures and increased skin temperature. The latter effect suggests vasodilatation and increased heat loss from skin surface. Since naloxone at different doses has no evident effects on glucose utilization (Fanelli et al., 1988), peripheral vasodilatation could be a primary contributor to drug-induced decreases in brain and body temperatures. Although several findings of this study (decrease in NAcc-muscle differential, weak hypolocomotion, diminished response to arousing stimuli) are consistent with inhibiting effect of naloxone on brain metabolic activity, this effect, if it exists, appears to be weak and incomparable to that seen with true sedative drugs [i.e. general anesthetics (Kiyatkin and Brown, 2005) and THC at high doses (Smirnov and Kiyatkin, 2008)]. To some extent, this action could be similar to that of diazepam, which at low doses (1 mg/kg) also slightly decreases brain and body temperatures and increases skin temperature (Kiyatkin and Bae, 2008).

Consistent with previous studies (Agmo et al., 1995; Bodnar, 2004; Levine and Billington, 2004), naloxone had a minimal effect on the latencies to initiate drinking but prolonged consummatory behavior that was interrupted by inactivity pauses and retreats. This effect resembles that seen with food consumption, when naloxone did not change the latencies of feeding following repeated small-size food presentations, but gradually increased durations of consumption (Wise and Raptis, 1986). Although behavior-inhibiting effects of naloxone were variable and evident only in some animals, both locomotor activation and temperature response were strongly attenuated compared to that seen in controls. In naloxone-treated rats, NAcc temperature increases were significantly weaker after both cup presentation and post-consumption period, resulting in almost three-fold reduction of mean response magnitude (see Table 2). The effect was weaker in the muscle, but also highly significant. Therefore, it appears that interruption of endogenous opioid transmission decreases the arousing or behavior-activating effects of palatable stimuli, thus making them less palatable and consumption less rewarding. Consistent with relatively short duration of naloxone's blocking action on opiate receptors (Kiyatkin, 1989), rats regained normal, pre-treatment temperature responses during the second Coca-Cola test conducted >3hrs after drug administration. However, locomotor activation during this test was still lower than in controls.

Based on our data we can conclude that endogenous opioid peptides are involved in mediating brain activation (arousing) effects of appetitive stimuli; because of this action appetitive stimuli during opioid receptor blockade induce weaker central excitatory responses, thus decreasing searching activity and subsequent consumption. This basic effect could be equally interpreted in terms of both motivation (decrease in motivational properties of appetitive stimuli) and reinforcement (decrease in reinforcing, i.e. behavior-activating effects of appetitive stimuli). If these stimuli elicit weaker central effects and diminish behavioral search with deactivated opioid system, their reinforcement will also be diminished, resulting in less efficient motivated behavior. While this conclusion appears to contradict the view that endogenous opioid system is primarily involved in evaluating the hedonic, pleasant properties of positive reinforcers (Barbano and Cador, 2007; Berridge, 1996; Hayward et al., 2002; Kelley et al., 2005) as well as pleasantness or taste of palatable foods and drinks (Yeomans and Grey, 1996; see however Arbis et al., 1999; Trechard and Silverstone, 1983), this contradiction could be mainly semantic or conceptual in nature. If central activating effects of positive reinforcers

became weaker during opioid receptor blockade, their rewarding (or satisfying) action will be also weaker.

While is unclear whether inactivation of endogenous opioid system has a similar inhibiting effect on central activational processes associated with other reinforcing stimuli, it seems likely because naloxone also attenuated body core hyperthermic responses associated with different arousing stimuli (Blasig et al., 1978; Stewart and Eikelboom, 1981) and inhibited different types of motivated behavior established by positive and negative reinforcers (Bolles and Fanselow, 1982; Holloway et al., 2004; Kim et al., 1997; Kiyatkin and Brown, 2003).

Conclusions

DA receptor blockade induced robust adynamia but increased brain, muscle and skin temperatures, suggesting metabolic activation, with enhanced heat production and heat loss. This treatment dramatically attenuated behavioral activation (but not central and peripheral temperature responses) induced by appetitive stimuli and fully blocked Coca-Cola consumption. In contrast, pharmacological interruption of opioid transmission decreased brain and muscle and increased skin temperatures, pointing at enhanced heat loss and possible minor inhibition of metabolic activity. This treatment attenuated behavioral activation as well as central and peripheral responses induced by appetitive stimuli, resulting in prolonged consumption.

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Fig. 1. Mean changes in absolute (A) and relative temperatures (B), temperature differentials (C), and locomotion (D) following presentation of a Coca-Cola-filled cup (left panel) and removal of empty cup (right panel) in drug-naive control conditions. *n* shows numbers of averaged tests and filled symbols show values significantly different from baseline. One-way ANOVA with repeated measures was used for statistical evaluation of temperatures and locomotion. *F* values for cup presentation are: NAcc – $F_{33,1053}=27.42$; Skin – 14.70; Muscle – 26.01; locomotion – 2.73; at least $p<0.01$ each. *F* values for cup removal are: NAcc – $F_{14,464}=6.36$; Skin – 3.87; Muscle – 4.67; locomotion – 1.64; at least $p<0.05$ each.

Fig. 2.

Phasic changes in NAcc and temporal muscle temperatures (A) and NAcc-muscle temperature differentials (B) associated with key events of motivated drinking behavior (left panel) and subsequent removal of empty case from the cage (right panel). The bottom graphs (C) show rates of NAcc temperature change (mean difference between each subsequent 5-s values). Three vertical lines on the left panel mark the moments of cup presentation, initiation of drinking behavior, and its end. Horizontal hatched lines show zero values or no change for each parameter. Statistical evaluation for cup presentation was done separately for each of three events, with plus (following) and minus (preceding) time comparisons. Filled symbols show values statistically significant from reference points (either a 5-s value immediately preceding the start and end of drinking or the first 5-s value after these events). F values for cup presentation are: NAcc $F_{32,164}=4.88$; $p<0.001$; Muscle=0.58, not significant, NAcc-muscle differential=5.64, $p<0.001$. F values for initiation of drinking: (1) preceding drinking onset: NAcc $F_{32,164}=36.46$, $p<0.001$; Muscle=1.73, not significant; NAcc-muscle differential=23.99, $p<0.001$; (2) following drinking onset: NAcc $F_{32,989}=2.36$; Muscle=15.05, NAcc-muscle differential=22.47, each $p<0.001$. F values for changes following the end of drinking: (1)

preceding end of drinking: NAcc $F_{32,956}=5.01$, $p<0.001$; Muscle =6.34, $p<0.001$, NAcc-muscle differential=0.59; (2) following drinking offset: NAcc $F_{32,1022}=57.13$, Muscle =20.17, NAcc-muscle differential=57.13, all are $p<0.001$. F values for empty cup removal: NAcc $F_{30,960}=24.54$; Muscle=8.00; NAcc-Muscle differential= 3.27 (all $p<0.01$). While most changes are significant, note quantitative differences in F values that show the strength of the effect.

Fig. 3.

Mean changes in relative temperatures (A), temperature differentials (B), and locomotion (C) induced by sc administration of a mixture of DA receptor blockers (SCH 23390 0.2 mg/kg and eticlopride 0.2 mg/kg; left panel; marked as A1, B2, and C1), naloxone (1 mg/kg; middle panel; marked as A2, B2, and C2), and saline (right panel; marked as A3, B3 and C3) in freely moving rats. Vertical hatched lines (0 min) show the moment of drug injections. *n* is number of averaged tests. The effects of each treatment were significant as evaluated with repeated measure ANOVA. *F* values for the effects of DA receptor blockers are: NAcc $F_{5,185}=4.06$; Muscle=3.13, Skin=5.19, locomotion=4.66 (all at least $p<0.01$). *F* values for the effects of naloxone are: NAcc $F_{7,247}=11.92$; Muscle=13.03, Skin=6.08, Locomotion=5.50 (all at least $p<0.01$). *F* values for the effects of saline are: NAcc $F_{11,371}=7.40$; Muscle=6.60, Skin=7.12, Locomotion=6.03 (all at least $p<0.01$). Values significantly different from baseline (the last pre-injection value) are shown by filled symbols. For clarity, standard errors are shown only for locomotion.

Fig. 4.

Mean changes in absolute (A) and relative temperatures (B), temperature differentials (C), and locomotion (D) induced by cup presentation (left panel) and cup removal (right panel) in rats during full DA receptor blockade. Vertical hatched lines show the moments of cup presentation or removal. Both procedures induced significant but quantitatively different effects on NAcc ($F_{9,309}=8.96$, $F_{7,191}=2.13$, respectively, for cup presentation and removal; $p<0.01$) and muscle temperatures (6.13 and 2.63; $p<0.01$), no effects on skin temperature (1.65 and 0.75) and locomotion. Values significantly different from baseline (the last pre-injection value) are shown by filled symbols. For clarity, standard errors are shown only for absolute temperatures and locomotion.

SCH 23390 + Eticlopride

Fig. 5.

Differences in high-speed temperature dynamics in the NAcc (A), temporal muscle (B), and skin (C) following cup presentation (left panel) and cup removal (right panel) in rats in drug-naïve, control conditions (circle) and during DA receptor blockade (triangle). As can be seen, temperatures in each location changed similarly in both conditions immediately after cup presentation, but then strongly diverged. While NAcc and muscle temperatures relatively decreased in control conditions during drinking behavior, both parameters monotonically increased during DA receptor blockade, when the rat was generally adynamic and showed no interest in presented cup. In control conditions, NAcc and muscle temperatures showed the second, stronger increase after cup was emptied and the rats resumed searching activity. Because of this, both temperatures increased much stronger and for a longer time in control than during DA receptor blockade. Changes in skin temperature during DA receptor blockade were greatly attenuated compared to control conditions. Solid, fat lanes with asterisks show significant ($p < 0.05$) between-condition differences.

Naloxone

Fig. 6. Mean changes in absolute (A) and relative temperatures (B), temperature differentials (C), and locomotion (D) following the first (left panel) and second (right panel) cup presentation at different times (+40 and +180 min) of naloxone administration. Vertical hatched lines show the moments of cup presentation. Both procedures induced significant but quantitatively different effects on NAcc ($F_{7,247}=2.59$, $F_{7,247}=5.66$, respectively, for first and second cup presentations $p<0.001$), muscle (1.62 and 4.00; $p<0.05$) and skin temperatures (1.76 and 10.37) as well as locomotion (2.31 and 2.12; $p<0.01$ both). Values significantly different from baseline are shown by filled symbols. For clarity, standard errors are shown only for absolute temperatures and locomotion.

Fig. 7.

High-speed temperature dynamics associated with Coca-Cola drinking behavior in rats during opioid receptor blockade (Naloxone; 40-min post-injection), in post-blockade period (Post-naloxone; 3 hrs post-injection) and in drug-free control conditions (Control). The changes are shown separately for each recording location (left, NAcc; middle, Muscle; right, Skin) and for absolute (top) and relative (bottom) temperatures. As can be seen, despite much lower NAcc temperatures in the naloxone group (A), its relative change associated with cup presentation and subsequent drinking behavior was much lower than in controls both at the initial and post-drinking periods (B). In contrast, these changes were even stronger than in controls during the second, post-naloxone test. Differences in muscle (C and D) and skin (E and F) temperatures were similar but less pronounced.

Table 1

Basal values of temperatures and locomotion preceding key behavioral events in rats in control, drug-free conditions and following administration of a mixture of DA receptor blockers and naloxone

	n	NAcc	Skin	Muscle	Locomotion
Control					
Pre-Coca-Cola 1	16	36.52±0.17	34.59±0.16	35.55±0.16	1.30±0.22
Pre-removal 1	15	36.40±0.18	34.38±0.14	35.46±0.20	3.10±0.78
Pre-Coca-Cola 2	18	36.24±0.10	34.55±0.13	35.32±0.13	1.48±0.31
Pre-Removal 2	15	36.33±0.17	34.27±0.14	35.29±0.20	3.92±0.83
Pre-Coca-Cola all	34	36.36±0.10	34.57±0.10	35.42±0.10	1.39±0.19
DA receptor blockers					
Pre-Coca-Cola 1	6	37.39±0.31*	34.88±0.33	36.49±0.37*	0.60±0.44
Pre-Removal 1	5	37.01±0.30	35.10±0.33	36.32±0.33*	0.16±0.16*
Pre-Coca-Cola 2	4	36.53±0.32	34.54±0.15	35.62±0.22	0.23±0.23*
Pre-removal 2	4	36.24±0.18	34.49±0.17	35.29±0.43	0.93±0.33*
Pre-Coca-Cola all	10	37.05±0.26*	34.78±0.21	36.13±0.27	0.45±0.15*
Naloxone					
Pre-Coca-Cola 1	8	35.90±0.20*	34.10±0.34	35.12±0.19	1.87±0.71
Pre-Removal 1	7	36.05±0.21	34.25±0.26	35.25±0.21	1.38±0.58
Pre-Coca-Cola 2	8	36.04±0.13	34.12±0.13	35.19±0.16	1.01±0.30
Pre-Removal 2	5	36.43±0.20	34.50±0.26	35.48±0.21	3.20±1.52

Basal values are means (±sem) of ten 1-min values preceding the event of interest. Values for locomotion are averaged activity per min. Asterisks show significant differences between each treatment condition and controls for the same comparison groups. Each number represents basal parameters, which were determined before the first and the second cup presentations (pre-Coca-Cola 1, Pre-Coca-Cola 2) and before the first and second cup removals (Pre-removal 1 and Pre-removal 2). Basal values for both cup presentations in control and DA receptor blockade groups were also combined in one group (pre-Coca-Cola all).

Table 2

Mean changes in NAcc, muscle and skin temperatures and locomotion following presentation of a Coca-Cola-containing cup and its removal in rats under drug-free control conditions and after administration of a mixture of DA antagonists and naloxone.

Group	Parameter			
	n	NAcc	Muscle	Skin Locomotion
Control				
Cup In	34	0.41±0.08	0.37±0.08	-0.26±0.08 11.96±1.06
Cup Out	15	0.22±0.04*	0.18±0.09	-0.09±0.08 5.38±0.55*
DA antagonists				
Cup In	10	0.12±0.06 ^{oo}	0.17±0.04 ^o	0.02±0.03 ^o 0.57±0.22 ^{ooo}
Cup Out	8	0.14±0.04	0.04±0.04	-0.02±0.02 0.16±0.13 ^{ooo}
Naloxone				
Cup 1 In	8	0.14±0.04 ^{oo}	0.16±0.04 ^o	-0.09±0.08 6.50±1.35 ^{oo}
Cup 1 Out	7	0.29±0.04*	0.27±0.04*	0.23±0.08 ^{*,oo} 3.11±0.46 ^{*,o}
Cup 2 In	8	0.35±0.08*	0.38±0.04*	0.05±0.14 8.94±1.43

Temperature and locomotion values represent means (±sem) for 25 1-min values following the event of interest. Statistical comparisons were conducted both within each group (cup removal vs. cup presentation; *, p<0.05) and between groups (the same events in drug-treated vs. drug-naïve conditions, o, p<0.05; oo, p<0.01 and ooo, p<0.001). "Cup In" and "Cup Out" represent responses to cup presentation and its removal from the case, respectively. For control and DA antagonist groups, data from both daily tests were averaged. For naloxone group, data for the first and second Coca-Cola tests as well as first cup removal are shown separately. Data for second cup removal in this group are not shown because of small number of tests and recording artifacts.