Pharmacology, Biochemistry and Behavior 91 (2008) 233–242

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Brain temperature responses to salient stimuli persist during dopamine receptor blockade despite a blockade of locomotor responses

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, United States

ARTICLE INFO

Article history:
Received 18 April 2008
Received in revised form 1 August 2008
Accepted 4 August 2008
Available online 8 August 2008

Keywords:
Brain metabolism
Brain and body temperature
Dopamine systems
Dopamine antagonists,
Natural arousing stimuli
Intravenous cocaine

ABSTRACT

We examined how an acute dopamine (DA) receptor blockade affects locomotor and brain (nucleus accumbens or NAcc), muscle and skin temperature responses to three arousing stimuli (procedure of sc injection, tail-pinch and social interaction with another male rat) and intravenous cocaine (1 mg/kg). DA receptor blockade was induced by mixture of D1- (SCH23390) and D-2 selective (eticlopride) DA antagonists at 0.2 mg/kg doses. Each arousing stimulus and cocaine caused locomotor activation, prolonged increase in NAcc and muscle temperature (0.6–1.0 °C for 20–50 min) and transient skin hypothermia (−0.6 °C for 1–3 min) in drug-naive conditions. DA receptor blockade strongly decreased basal locomotor activity, but moderately increased brain, muscle and skin temperatures. Therefore, selective interruption of DA transmission does not inhibit the brain, making it more metabolically active and warmer despite skin vasodilatation and the enhanced heat loss to the body and the external environment. DA antagonists strongly decreased locomotor responses to all stimuli and cocaine, had no effects on acute skin vasoconstriction, but differentially affected stimuli- and drug-induced changes in NAcc and muscle temperatures. While brain and muscle temperatures induced by cocaine were fully blocked and both temperatures slightly decreased, temperature increases induced by tail-pinch and social interaction, despite a significant attenuation, persisted during DA receptor blockade. These data are discussed to define the role of the DA system in regulating the central activation processes and behavioral responsiveness to natural arousing and drug stimuli.

Published by Elsevier Inc.

1. Introduction

Intravenous (iv) cocaine used passively at low, self-administering doses (0.5–1 mg/kg) mimics salient somato-sensory stimuli in their ability to induce behavioral and autonomic activation, manifesting in hyperlocomotion, arterial blood pressure increase and peripheral vasoconstriction (Kiyatkin and Brown, 2005; Poon and van den Buuse, 1998). Iv cocaine and salient stimuli such as tail-pinch and social interaction also induce a similar pattern of changes in central and peripheral temperatures (Kiyatkin and Bae, 2008). In each case, brain and muscle temperatures increased, with more rapid and stronger changes in brain structures than in the muscle, suggesting metabolic neural activation and intra-brain heat production as the primary cause underlying brain hyperthermia. In contrast, skin temperature rapidly decreased with all stimuli, suggesting a similar peripheral vasoconstriction and decreased heat loss as a contributor to hyperthermia. Previously we showed that behavioral and temperature effects of iv cocaine are dramatically altered during dopamine (DA) receptor blockade (Kiyatkin and Brown, 2005). Under this condition, cocaine-induced locomotor activation is fully blocked and brain and muscle temperatures considerably decreased. However, transient skin hypothermia remained virtually intact.

To examine the role of the DA system in mediating common arousing effects of natural and pharmacological stimuli, in this study we evaluated how DA receptor blockade affects behavioral and temperature responses to various somato-sensory stimuli and compared these effects with those induced by cocaine. Using a within-animal design, we compared changes in locomotion and brain (nucleus accumbens or NAcc), muscle and skin temperatures induced by three stimuli (tail-pinch, social interaction, procedure of sc injection) and iv cocaine at a typical reinforcing dose (1 mg/kg) in male rats in intact, drug-free conditions and during DA receptor blockade, induced by a mixture of selective D1-like (SCH23390) and D2-like (eticlopride) DA antagonists. By effective and relatively long-term blockade of all types of DA receptors, this treatment prevents the interaction of endogenously released DA with these receptors, thus allowing the study of centrally mediated physiological and behavioral responses without functional DA.

2. Materials and methods

2.1. Subjects and surgeries

Eight Long-Evans male rats (Taconic, Germantown, NY), weighing 420–480 g and housed under a 12 h light cycle (lights on at 0700) with

* Tel.: +1 410 550 5551; fax: +1 410 550 5553.
E-mail address: ekiyatki@intra.nida.nih.gov.

0091-3057/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2008.08.004

ad libitum food and water, were used. 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.

All animals were implanted with three thermocouple electrodes as previously described (Kiyatkin and Brown, 2005). Animals were anesthetized with Equithesin (3.3 ml/kg intraperitoneally or ip; dose of sodium pentobarbital, 32.5 mg/kg and chlorale hydrate, 145 mg/kg) and mounted in a stereotaxic apparatus. Holes were drilled through the skull over the nucleus accumbens, shell (NAcc; 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 a thermocouple probe was slowly lowered to the desired target depth (7.4 mm, measured from the skull surface). NAcc was chosen as a representative ventrally located brain structure, which is known to be essential in sensory-motor integration, behavioral regulation, and mediation of the reinforcing properties of natural stimuli and addictive drugs (Wise and Bozarth, 1987). A second thermocouple probe was implanted subcutaneously along the nasal ridge with the tip approximately 15 mm anterior to bregma. This location is instrumental in assessing fluctuations in skin temperature, an important measure of peripheral vasoconstriction (Baker et al., 1976). A third thermocouple probe was implanted in the deep temporal muscle (*musculus temporalis*) — a nonlocomotor head muscle that receives the same blood supply as the brain, thus allowing evaluation of the contribution of arterial blood supply to alterations in brain temperature. The probes were secured with dental cement to three stainless steel screws threaded into the skull. During the same surgery session, all animals were implanted with a jugular iv catheter. 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. The catheter was run subcutaneously to the head mount and secured with dental cement. Rats were allowed three days recovery and two more days of habituation (6 h session) to the testing environment before the start of testing.

2.2. Experimental protocol

All tests occurred inside a Plexiglas chamber (32×32×32 cm) equipped with four infrared motion detectors (Med Associates, Burlington, VT, USA), placed inside of a sound attenuation chamber. Rats were brought to the testing chamber at ~09:00 and attached via a flexible cord and electrical commutator to thermal recording hardware (Thermes 16, Physitemp, Clifton, NJ, USA). A catheter extension was also attached to the internal catheter, thereby allowing remote, unsignalled iv injections. Temperatures were recorded with a time resolution of 10 s and movement was recorded as the number of infrared beam breaks per 1 min. Room temperature was maintained at 23–24 °C and controlled by another thermocouple located in the recording chamber.

Each of the 8 rats underwent 4 recording sessions. During each session, after habituation to the testing chamber (at least 2 h), rats were injected sc with either saline (1 ml/kg) or a mixture of DA antagonists (SCH at 0.2 mg/kg and ETI at 0.2 mg/kg in 0.3 ml saline). The order of these injections was counter-balanced and each rat received two injections of both saline and DA antagonists (Day 1: saline or SCH+ETI; Day 2: SCH+ETI or saline, and so on). 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 1:51,4000; Neve and Neve, 1997). Both these drugs were obtained from Sigma (St. Louis, MO), dissolved in saline immediately before use, mixed, and injected as one bolus. 30 min post-injection, rats were exposed to the first stimulus (either tail-pinch or social interaction). One hour later, rats were exposed to the second stimulus. The order of stimulus presentation was again counter-balanced between rats and sessions; thus rats experienced one tail-pinch and one social interaction procedure each session. To maintain the effective blockade of DA transmission, at ~2.5 h post-injection rats received the second injection of either saline or DA antagonists (at a half dose). Lastly, at ~180 min after the first injection (and ~30 min after an additional injection), rats received an iv cocaine injection (1 mg/kg in 0.15 ml saline). This dose is optimally effective in the self-administration paradigm (Pettit and Justice 1991). Recordings continued two hours after the cocaine injection, after which the cable was disconnected from the rats' head and they were returned to their home cages. In total, for each of the 4 stimuli, 16 tests were performed in 8 rats for both saline and DA antagonists treatment.

Sc injections were made in non-restrained animals by pinching the skin on the upper back. The tail-pinch procedure was performed by placing a wooden clothes pin at the base of the rat's tail for one minute. The social interaction procedure (sometimes defined as "intruder stress paradigm") was executed by placing an unfamiliar, conspecific male rat matched for age and weight in the testing chamber for one minute. Since our motion detection equipment cannot distinguish between two animals, locomotor activity during this minute (social interaction *per se*) was not included in our data analysis.

2.3. Histology and data analysis

When recording was 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 slices and placed on glass slides. All probes were located within the medial portion of ventral striatum (NAcc shell), as described in Paxinos and Watson (1998).

Temperature and movement data were analyzed with 1 min time bins and were presented as both absolute and relative changes with respect to the moment of stimulus presentation or drug administration. One-way ANOVA with repeated measures, followed by post-hoc Fisher tests, was used for statistical evaluation of temperature and movement responses induced by arousing stimuli and cocaine. Student's *t*-test was used for comparisons of between-site and between-condition differences in temperature and locomotion. Basal temperature and basal locomotion were determined by calculating mean values for the 6 min preceding each stimulus presentation. Correlation (Pearson's *r*) and regression analyses were also used to assess the relationships between temperatures recorded from different sites and the dependence of stimulus- and drug-induced temperature change upon basal temperatures. Between-treatment differences were evaluated based on statistical comparisons of basal brain temperatures, absolute and relative temperature changes, and mean values of locomotor responses. The use of the words "increase" or "decrease" as well as "significant" means 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.

3. Results

3.1. Basal temperatures, procedure of injection and influence of DA receptor blockade on basal locomotion and temperatures

The sc injection of either a mixture of DA antagonists or saline was done 2–3 h after the rat was placed in the recording chamber, when locomotion and temperatures stabilized at basal levels (Fig. 1). At this moment (time=0 min), there were differences in basal temperatures in each recording location, with the highest values in the NAcc (range 36.31–37.66, mean 36.94±0.06, SD 0.33 °C), lower values in the muscle (range 35.04–36.49, mean 35.99±0.07, SD 0.39 °C;

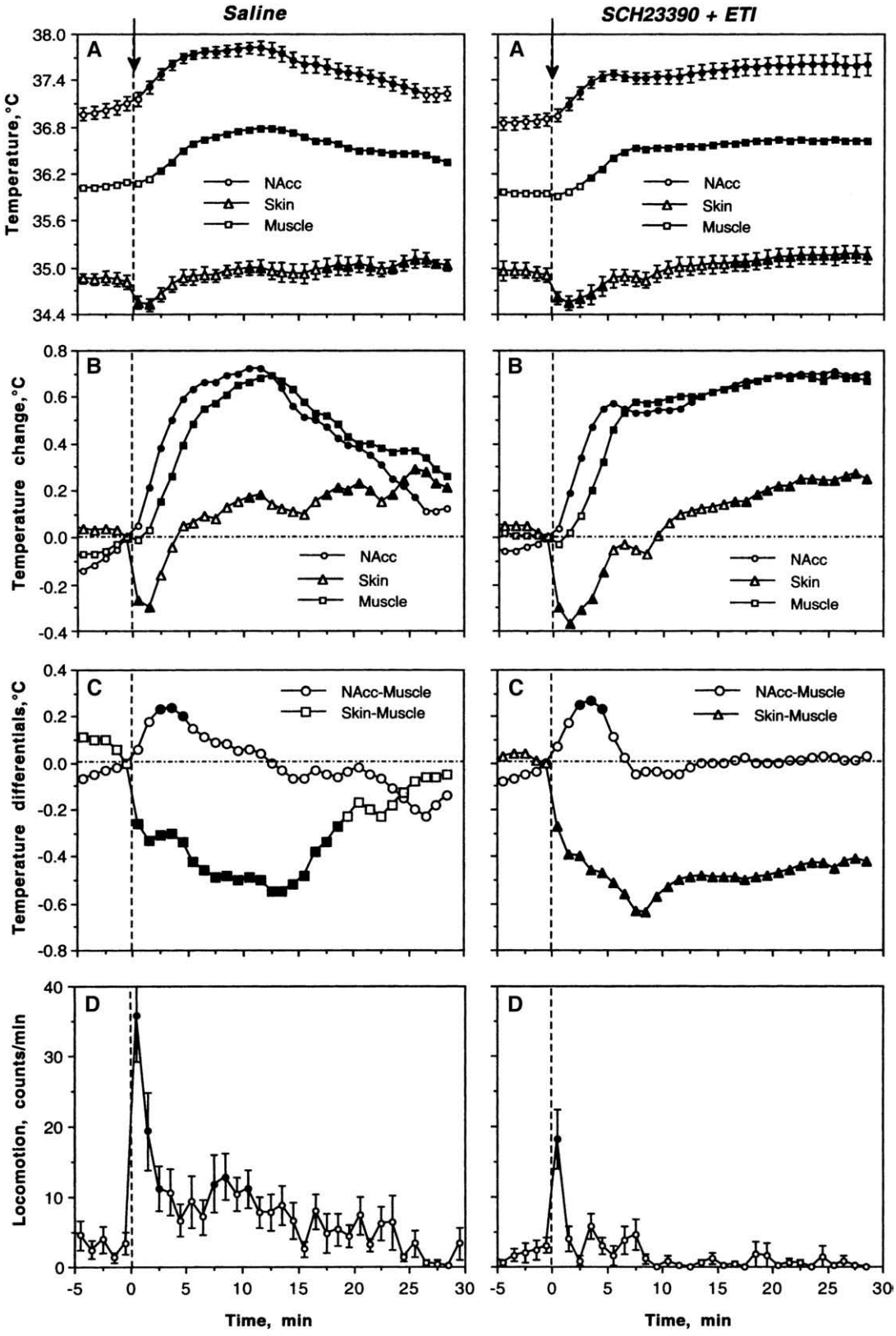

Fig. 1. Changes in temperature (A, B and C) and locomotion (D) following injection of saline (left column) and a mixture of D-1 and D-2 selective dopamine antagonists (SCH23390 and eticlopride 0.2 mg/kg each; right column). A shows absolute temperatures; B shows changes in temperature relative to pre-injection baseline and C shows relative changes in NAcc-muscle and skin-muscle temperature differential. For clarity, standard errors are shown only for movements (D). The effect of time, evaluated for temperature and locomotor activity, was significant and similar for both injections (saline $n=16$: NAcc – $F_{15,495}=18.3$, muscle = 14.4, skin = 5.5, locomotion = 6.0; DA antagonists $n=15$: NAcc $F_{14,464}=16.2$, muscle = 25.6, skin = 13.6, locomotion = 7.1; $p<0.001$ each). Filled symbols show values significantly different from baseline ($p<0.05$; post hoc Fisher test).

$p<0.05$ vs. NAcc), and minimal values in the skin (range 34.21–35.5 °C, mean 34.91±0.06, SD 0.36 °C; $p<0.05$ vs. NAcc and Muscle). Basal NAcc and muscle temperatures significantly correlated ($r=0.481$, $p<0.01$), while there were no correlations between NAcc and skin ($r=0.022$) and between muscle and skin ($r=0.164$). Therefore, if basal temperatures in the NAcc were higher, they were also higher in the muscle and *vice versa*.

As shown in Fig. 1, the injection procedure (either saline or DA antagonists) induced locomotor activation and significant temperature changes in each location. In both cases, temperatures in the NAcc and temporal muscle increased, while skin temperature decreased (A, B). In each case, the temperature increase in the NAcc was stronger than in muscle, resulting in a significant increase in the NAcc-muscle temperature differential for several minutes after the injection (C). Due to the opposite temperature responses in the muscle and skin, skin-muscle temperature differential significantly decreased after the injection (C). Locomotion peaked immediately after the injection, with a subsequent slow return toward baseline.

While the initial changes in temperatures and locomotion following the injection were similar for both saline and DA antagonists, there were major between-group differences from ~10 min post-injection. While NAcc and muscle temperatures peaked at ~10 min and then decreased toward baseline in the saline group, both temperatures remained elevated in the DA antagonists group, resulting in about a 0.5 °C between-group difference ($p<0.01$) at 30th min — the time, at which the first stimulus was presented. After the initial, injection-related bout of movement activity, which was similar in both groups, locomotion was strongly inhibited in the DA antagonists group (Fig. 1D), resulting in significant between-group differences at the time when the first stimulus was presented.

Fig. 2 shows changes in temperatures and locomotion during the session in both groups. Mean values of each parameter were determined for 0, 30, 90 and 180 min, corresponding to basal values preceding each stimulus presentation. As can be seen, mean values of temperature in each location gradually decreased in control group, while both NAcc and muscle temperatures in the SCH+ETI group remained higher, resulting in significant between-group differences at 30, 90 and 180 min. In contrast to the decrease in skin temperature observed in the saline group, skin was warmer in the SCH-ETI group, resulting in significant between-group differences in each time point. Similarly, hypoactivity during DA receptor blockade was evident at each time point. Although spontaneous locomotion decreased within the session in the saline group, this effect was much stronger with DA antagonists, resulting in significant between-group differences in this parameter at each time point of the session.

3.2. Changes in temperature and locomotion induced by tail-pinch and social interaction

Both tail-pinch and social interaction in the saline group induced powerful locomotor activation, a robust increase in NAcc and muscle temperatures, and a biphasic, down-up fluctuation in skin temperature (Figs. 3 and 4, right panels). Although the duration of stimulation was only 1 min in both cases, all parameters changed for a prolonged period of time. Locomotion peaked at the first post-stimulus minute but was significantly higher than baseline for about 12 min, and temperature increases in NAcc and temporal muscle were observed for about 30 min. With both stimuli, temperature increases in the NAcc were more rapid and stronger than in muscle, resulting in a significant increase in the brain-muscle differential for ~8 min (Figs. 3 and 4, B). In both cases, skin-muscle differentials rapidly decreased after the start of stimulation but at ~16 min they both became inverted, showing a rebound-like increase. Finally, locomotor activation and skin hypothermia had the shortest onset latencies in both cases; these changes were more acute and transient than changes in NAcc and muscle temperatures.

Under conditions of DA receptor blockade, tail-pinch and social interaction induced virtually no locomotor responses and much weaker temperature responses than in control conditions (Figs. 3 and 4 and Table 1). While latency and duration of brain and muscle hyperthermia were about the same in both groups, their amplitudes were about two- (tail-pinch) or three-fold (social interaction) lower in the drug-treated group than in the control. Skin hypothermia induced by each stimulus was equally rapid in both groups but weaker (see Table 1; $p<0.05$ for tail-pinch and no differences for social interaction) and lacking a subsequent rebound-like hyperthermia in drug-treated conditions.

3.3. Changes in temperature and locomotion induced by iv cocaine

Consistent with our previous data (Kiyatkin and Brown, 2005), iv cocaine induced powerful hyperlocomotion, strong NAcc and muscle hyperthermia and a biphasic, down-up fluctuation in skin temperature in control conditions (Fig. 5, right panel). While this response pattern mimicked those occurring during tail-pinch and social interaction, the changes were stronger and more prolonged (see Table 1). Similar to sensory stimuli, NAcc-muscle differentials significantly increased and skin-muscle differentials significantly decreased after iv cocaine (Fig. 5B); these changes were also stronger and more prolonged than those seen after tail-press and social interaction.

When cocaine was administered during the DA receptor blockade, cocaine-induced locomotion was completely blocked, while NAcc and muscle temperatures strongly decreased (see Fig. 5). In contrast, acute skin hypothermia was minimally affected, but its magnitude was significantly lower than in the control (see Table 1).

3.4. Dependence of brain temperature responses upon basal brain temperatures in control and drug-treated conditions

Brain temperature increases induced by sensory stimuli and cocaine were highly variable in different animals and tests, and they were dependent upon basal brain temperatures in control conditions. As can be seen in Fig. 6, a significant and relatively strong correlation was evident for each of four stimuli ($r=-0.60$, -0.70, -0.72 and -0.45 for saline injection, tail-pinch, social interaction and cocaine, respectively). When basal temperatures were lower, their elevations induced by each stimulus were larger and *vice versa*. In each case, regression lines crossed the line of no effect at about 38.5 °C, suggesting that the response should disappear at high basal brain temperatures.

During DA receptor blockade, these correlations for tail-pinch and social interaction became weaker, but the correlation remained the same in strength for cocaine (Fig. 6). However, the regression line moved downward in the DA antagonist group, suggesting that cocaine induces stronger decreases in brain temperature when basal temperatures are high and weaker decreases when temperatures are low.

4. Discussion

While this study confirms that DA receptor blockade strongly decreases spontaneous locomotion and locomotor responses to somato-sensory stimuli, it compliments the behavioral measure with monitoring of central and peripheral temperatures and compares the effects of three different stimuli (sc injection, tail-pinch and social interaction) and iv cocaine — a pharmacological stimulus with a positive reinforcing value.

4.1. Methodological considerations

Our previous neuronal studies suggest that the combination of SCH23390 and eticlopride at 0.2 mg/kg (0.7 and 0.6 μM, respectively) provides an effective blockade of DA transmission for about three hours as tested by the antagonism of striatal neuronal responses to

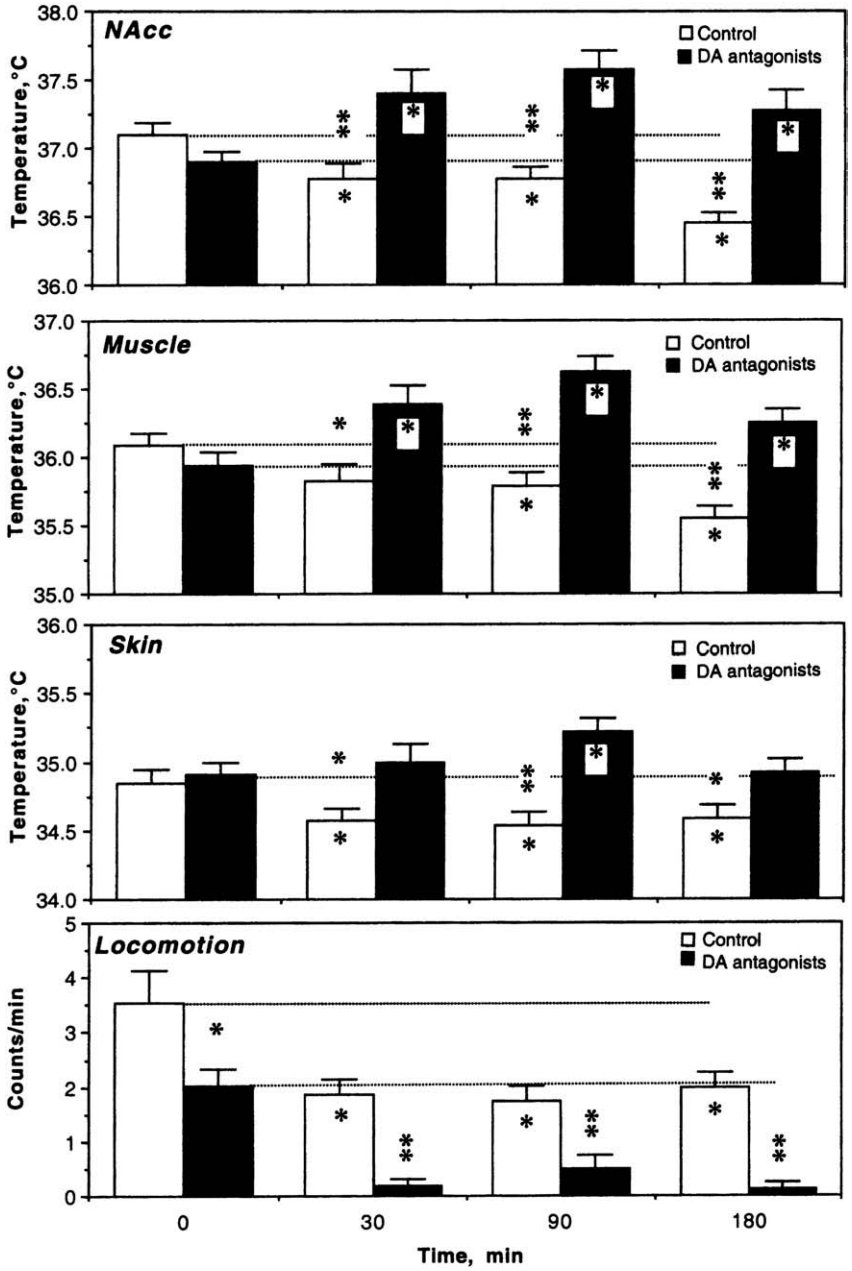

Fig. 2. Changes in basal temperatures (mean ± standard errors for NAcc, muscle and skin) and locomotion at different time points after DA antagonists (or saline) injection. Hatched horizontal lines show the initial values. Asterisks inside of bars show significance of difference vs. the initial values (*$p<0.05$; Student's *t*-test) and asterisks above bars show significance of between-treatment differences (o, $p<0.05$; Student's *t*-test).

iontophoretic DA (Kiyatkin and Rebec, 1999). It also has been shown that the effects of SCH23390 (at 0.25 mg/kg or higher) on body temperature are evident within a ~3 h duration (Faunt and Crocker, 1987; Schlenker, 2008). Since our goal was to maintain DA receptor blockade within a 4–5 h period, our initial treatment was supplemented by an additional half-dose drug administration ~2.5 h after the first injection. Since DA receptors are localized not only on central neurons but in a number of organs and tissues, including the peripheral nervous system, various vascular beds, the heart, the gastrointestinal tract, and the kidney (Amenta et al., 2002), the blockade of both centrally and peripherally located DA receptors should also be taken into account as possible contributors to the observed behavioral and temperature effects of DA antagonists. Since the presentations of tail-pinch and social interaction were equally balanced between rats and sessions and cocaine was always injected ~30 min after the second drug administration, we have reason to believe that the observed changes reflect the consequences of the drug-induced DA receptor blockade. Although most receptor-selective drugs used at higher doses could act on other receptors and SCH23390 has some affinity to 5-HT2 serotonin receptors (Bischoff et al., 1986), it is unlikely that the observed effects reflect significant "non-specific" interaction of ETI and SCH23390 with other receptors because the drug doses were relatively low. Since the behavioral and temperature responses to arousing stimuli and cocaine greatly vary in different rats and sessions and depend upon basal brain temperatures (see Fig. 6), for accurate evaluation of between-group differences we used a within-animal design (the same animals received both drug and saline treatment in different days) and control data were equally balanced with respect to sample size and session numbers. Finally, each rat underwent two sessions with DA receptor blockade, with 48 h and one saline session in-between, thus controlling for the possible long-term consequences of DA antagonists treatment.

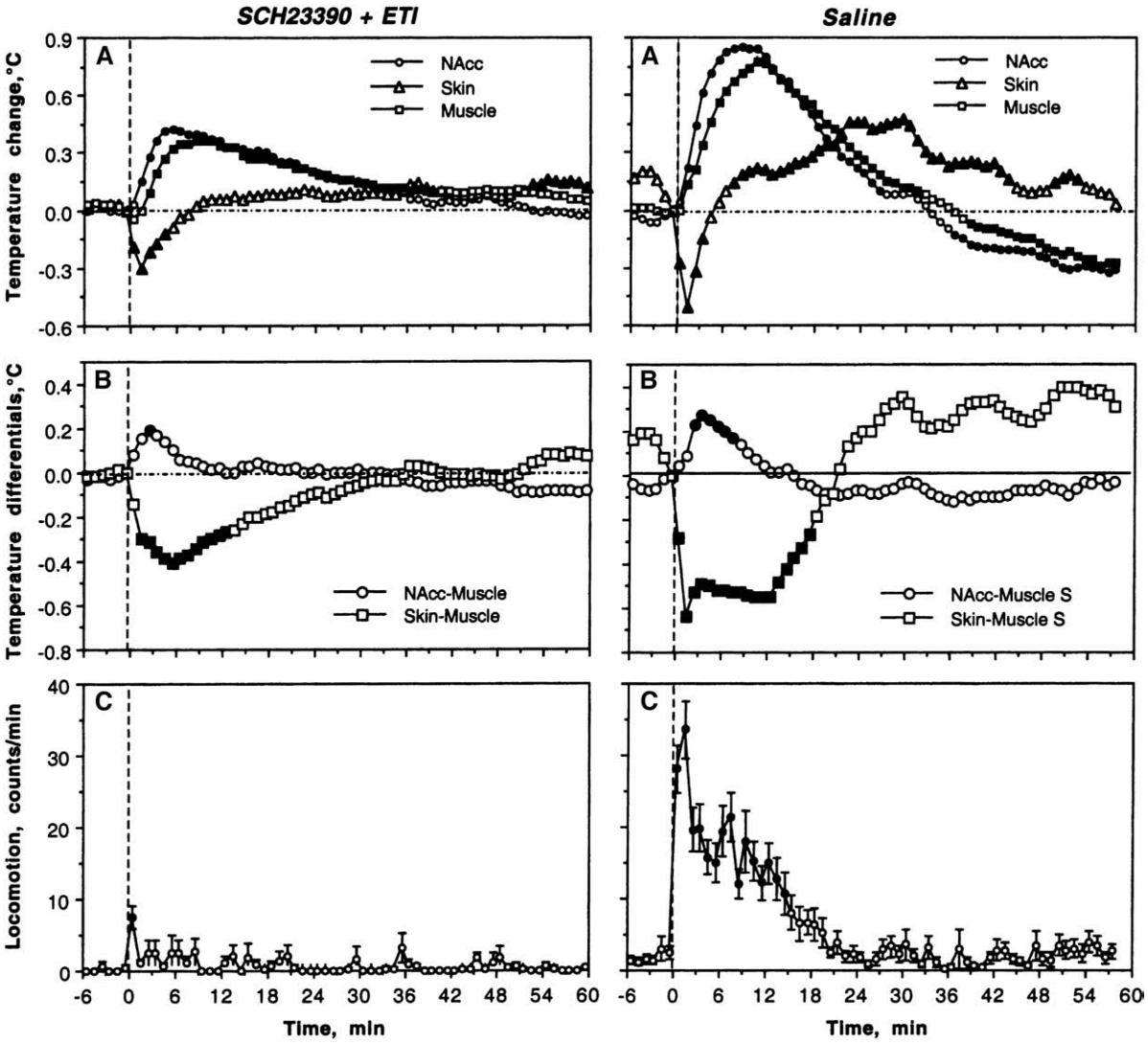

Fig. 3. Mean changes in temperature (A, relative; B, NAcc-muscle and skin-muscle temperature differentials) and locomotion (C) induced by tail-pinch in rats in drug-naive state (after saline) and during DA receptor blockade (SCH23390+eticlopride). The effect of time, evaluated for temperature and locomotor activity was significant for both conditions (saline $n=15$: NAcc – $F_{14,464}=27.9$, muscle = 21.2, skin = 16.7, locomotion = 12.1; DA antagonists $n=15$: NAcc $F_{14,464}=13.2$, muscle = 14.2, skin = 7.1, locomotion = 2.1; $p<0.001$ each). Filled symbols show values significantly different from baseline ($p<0.05$; post hoc Fisher test).

4.2. Hypoactivity and hyporesponsiveness during DA receptor blockade

While hypodynamia is a known effect of DA antagonists, it was associated with tonic increases (0.9–0.5 °C) in brain and muscle temperatures. This association seems unusual in the light of a positive correlation between behavioral activity and brain temperature (see Kiyatkin, 2005 for review). In addition, mild brain and body hyperthermia was coupled with increased skin temperature (Figs. 1C and 2), pointing at vasodilatation (or decreased vascular tone) and increased heat dissipation. Therefore, this dissociation between increased heat production and increased heat loss to the external environment suggests that DA receptor blockade results in some kind of metabolic activation in the brain and body.

DA receptor blockade also resulted in greatly diminished locomotor responses to tail-pinch and social interaction, suggesting that the DA system is essential for mediating the behaviorally activating effects of natural stimuli. In contrast, temperature responses to these stimuli had the same pattern as in control but were dramatically decreased (see Figs. 3 and 4). The attenuating effect was evident with respect to response amplitude (see Table 1) but not duration, which was quite similar in both groups. In contrast to brain and muscle, the attenuating effect was less evident in the skin. Therefore, it appears that other, non-DA mechanisms are involved in triggering metabolic brain activation and associated changes in physiological functions. These mechanisms, however, are supplemented by associated activation of the DA system, which is a significant contributor in shaping natural adaptive responses to salient environmental stimuli.

Since brain temperature responses induced by all tested stimuli inversely correlate with basal temperatures (see Fig. 6), weaker increases in NAcc and muscle temperatures during DA receptor blockade could reflect, at least in part, higher basal temperatures during this condition. Because of higher baselines and smaller stimulus-induced temperature increases, correlation between these parameters typical of normal, drug-free conditions was consistently weaker or even absent during DA receptor blockade.

4.3. Effects of cocaine during DA receptor blockade

Although cocaine acts on different neural substrates both in the brain and periphery, the pattern of its behavioral and temperature effects was surprisingly similar, albeit somewhat stronger and more prolonged, to that induced by somato-sensory stimuli (Fig. 5).

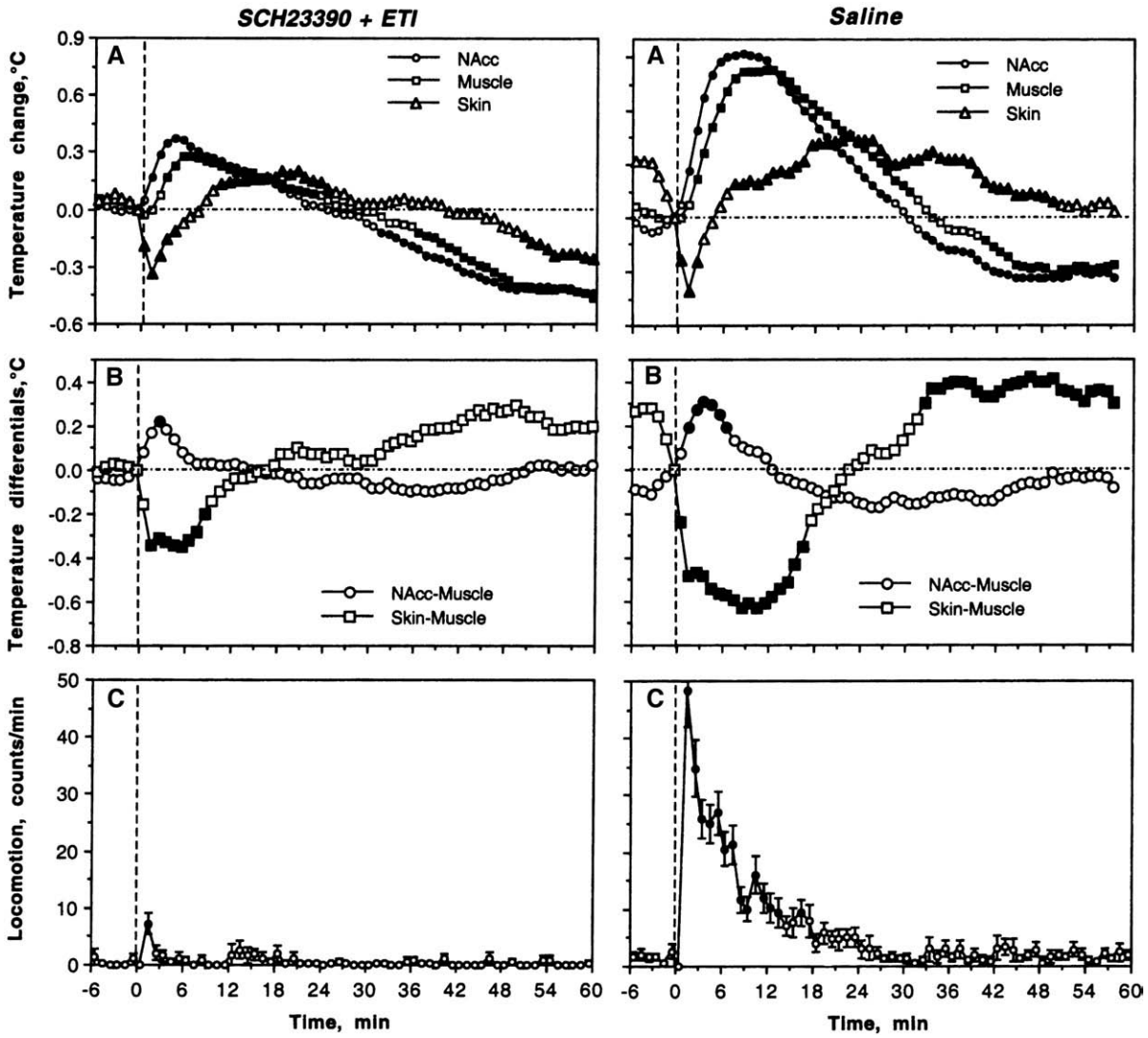

Fig. 4. Mean changes in temperature (A, relative; B, NAcc-muscle and skin-muscle temperature differentials) and locomotion (C) induced by social interaction in rats in a drug-naive state (after saline) and during DA receptor blockade (SCH23390 + eticlopride). The effect of time, evaluated for temperature and locomotor activity, was significant for both conditions (saline: $n=15$: NAcc – $F_{14,464}=22.0$, muscle = 15.3, skin = 7.1, locomotion = 11.4; DA antagonists, $n=15$: NAcc $F_{14,464}=27.7$, muscle = 9.6, skin = 13.8, locomotion = 8.9; $p<0.001$ each; $n=15$ for both groups). Filled symbols show values significantly different from baseline ($p<0.05$; post hoc Fisher test). Note that the value of locomotion corresponding to social interaction *per se* in both conditions (0.5 min) was excluded because of its "contamination" by the movements of another rat.

Similarly, the hyperthermic effects of cocaine were also dependent upon basal brain temperature, being strong at low, basal temperatures and weak or absent at higher temperatures. Iv cocaine also induced rapid skin hyperthermia, reflecting its well-known centrally-mediated vasoconstrictive action (Gillis et al., 1995; Knuepfer and Branch, 1992; Williams and Wasserberger 2006). It is more surprising, however, that this drug-induced vasoconstriction was qualitatively and quantitatively similar (but more prolonged) to that occurring with natural stimuli. On the other hand, peripheral vasoconstriction is a known phenomenon occurring in animals and humans after various arousing and stressful stimuli (Altschule 1951; Baker et al., 1976; Solomon et al., 1964). Therefore, based on the pattern of behavioral and temperature responses, iv cocaine at a typical reinforcing dose can be viewed as an arousing stimulus.

DA receptor blockade fully blocked locomotor-stimulatory and temperature-increasing effects of cocaine, pointing at an exclusive role of DA mechanisms in their mediation. Instead of temperature increases, both NAcc and muscle temperature slowly decreased, clearly exceeding "normal" decreases typically occurring in intact animals kept undisturbed in quiet, resting conditions (see Fig. 5). Therefore, it

Table 1
Amplitude of temperature responses induced by natural arousing stimuli and cocaine in rats in control, drug-free conditions and during DA receptor blockade induced by a mixture of SCH23390 (0.2 mg/kg) and eticlopride (0.2 mg/kg)

Parameters	Control	DA receptor blockade
Tail-pinch		
NAcc	0.829 ± 0.09	0.395 ± 0.06**
Muscle	0.703 ± 0.09	0.355 ± 0.07**
Skin	−0.511 ± 0.06	−0.299 ± 0.06*
Social interaction		
NAcc	0.924 ± 0.10	0.281 ± 0.05***
Muscle	0.813 ± 0.10	0.235 ± 0.06***
Skin	−0.274 ± 0.09	−0.341 ± 0.06
Cocaine		
NAcc	1.215 ± 0.09	−0.185 ± 0.05***
Muscle	1.020 ± 0.10	−0.183 ± 0.06***
Skin	−0.577 ± 0.05	−0.333 ± 0.06**

Numbers represent mean values (±SEM) of relative changes in peak temperature (in °C). Asterisks show the degree of statistical difference between control and DA antagonist conditions (*$p<0.5$, **$p<0.01$ and ***$p<0.001$).

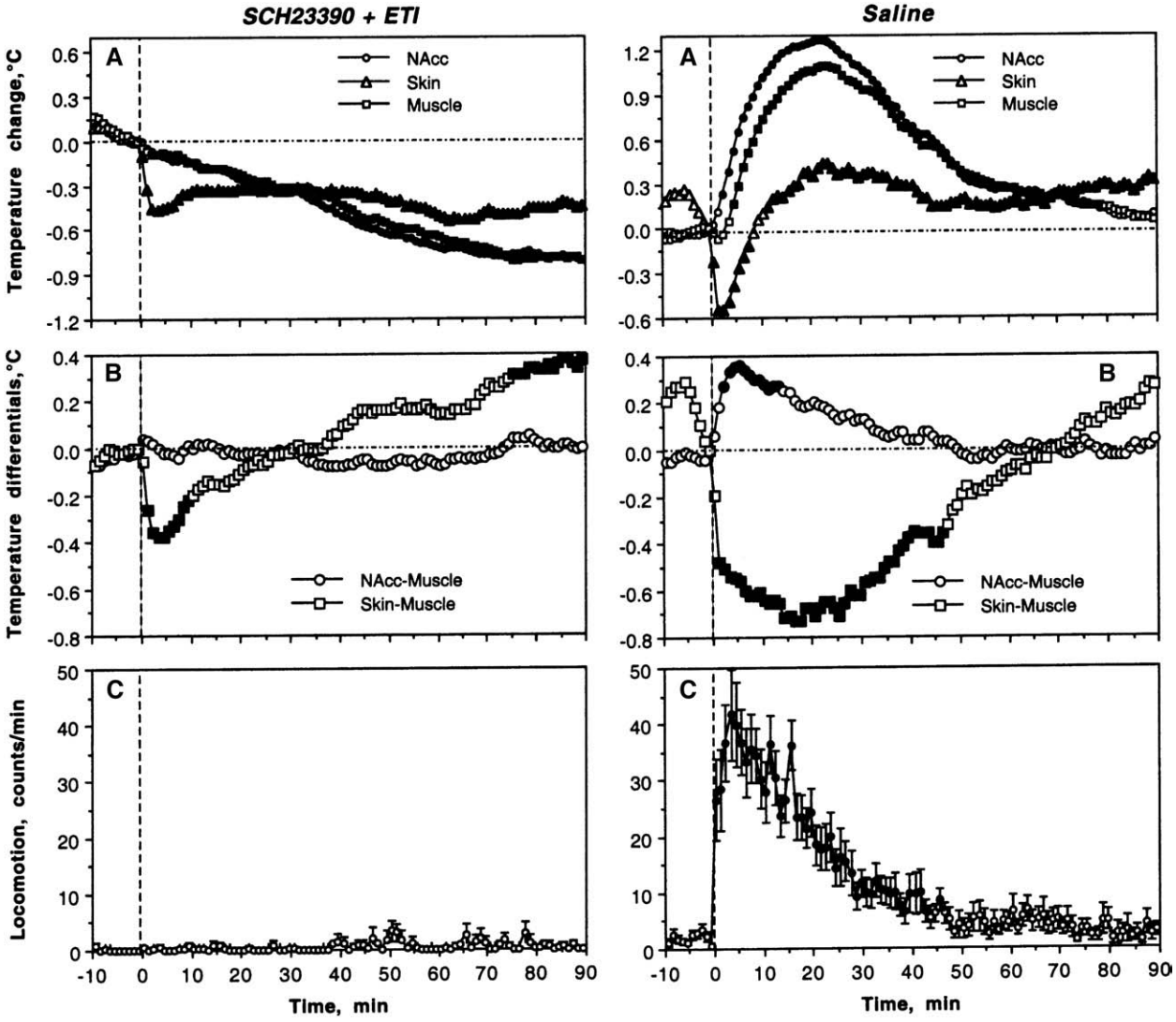

Fig. 5. Mean changes in temperature (A, relative; B, NAcc-muscle and skin-muscle temperature differentials) and locomotion (C) induced by iv injection of cocaine (1 mg/kg) in rats in drug-naive state (after saline) and during DA receptor blockade (SCH23390 + eticlopride). The effect of time, evaluated for temperature and locomotor activity, was significant for both conditions (saline, $n = 15$: NAcc – $F_{14,464} = 62.4$, muscle = 61.2, skin = 26.1, locomotion = 5.1; DA antagonists, $n = 15$: NAcc $F_{14,464} = 19.5$, muscle = 10.8, skin = 5.7, locomotion = 0.74; $p < 0.001$ each except locomotion; $n = 15$ for both groups). Filled symbols show values significantly different from baseline ($p < 0.05$; post hoc Fisher test).

appears that DA receptor blockade, by blocking central excitatory effects of cocaine, reveals its DA-independent inhibitory effects. In contrast to the brain and muscle, DA receptor blockade did not affect cocaine-induced decreases in skin temperature. Therefore, cocaine-induced peripheral vasoconstriction, similar to that induced by somatosensory stimuli, appears to be largely mediated via non-DA systems. This finding agrees with previous data that the acute hypertensive and vasoconstrictive effects of iv cocaine are resistant to DA receptor blockade (Kiritsy-Roy et al., 1990; Poon and van den Buuse, 1998).

In contrast to a transient decrease in control conditions, skin temperature after cocaine injection during DA receptor blockade remained lower than baseline during the entire observation period. Since skin temperature depends not only on vascular tone but arterial blood temperature, this tonic skin hypothermia could reflect decreased heat supply by incoming arterial blood due to decreased body temperature.

4.4. Functional implications

Our data suggest that selective interruption of DA transmission, despite its powerful inhibiting effects on spontaneous locomotion and locomotor responses to salient environmental stimuli, does not inhibit the brain. In contrast, the brain without functional DA appears to be metabolically more active and warmer despite the enhanced heat loss to the body and the external environment. This change parallels electrophysiological findings, suggesting that the effects of DA on its target cells are generally inhibitory (Bloom et al., 1989; Kiyatkin and Rebec, 1996, 1999; Mercuri et al., 1985; Nicola and Malenka, 1997; Siggins, 1977; Windels and Kiyatkin, 2006), while most DA-sensitive central neurons become more active, albeit disorganized, without DA input (Calabresi et al., 2000; Kiyatkin and Rebec, 1999). Because most cells receiving DA input are GABA-ergic, interruption of this restraining influence of DA and subsequent hyperactivity of these cells may be a factor determining behavioral inhibition following DA deficit independent of its cause. Our present data also support multiple behavioral and neurochemical evidence, suggesting the importance of DA in motor-activating effects of salient environmental stimuli and central activational processes (Bloom et al., 1989; Le Moal and Simon, 1991). However, they also demonstrate that other than DA mechanisms are involved in triggering metabolic neural activation and inducing transient increases in brain and body temperatures induced by environmental stimuli. These data are consistent with primarily regulatory or

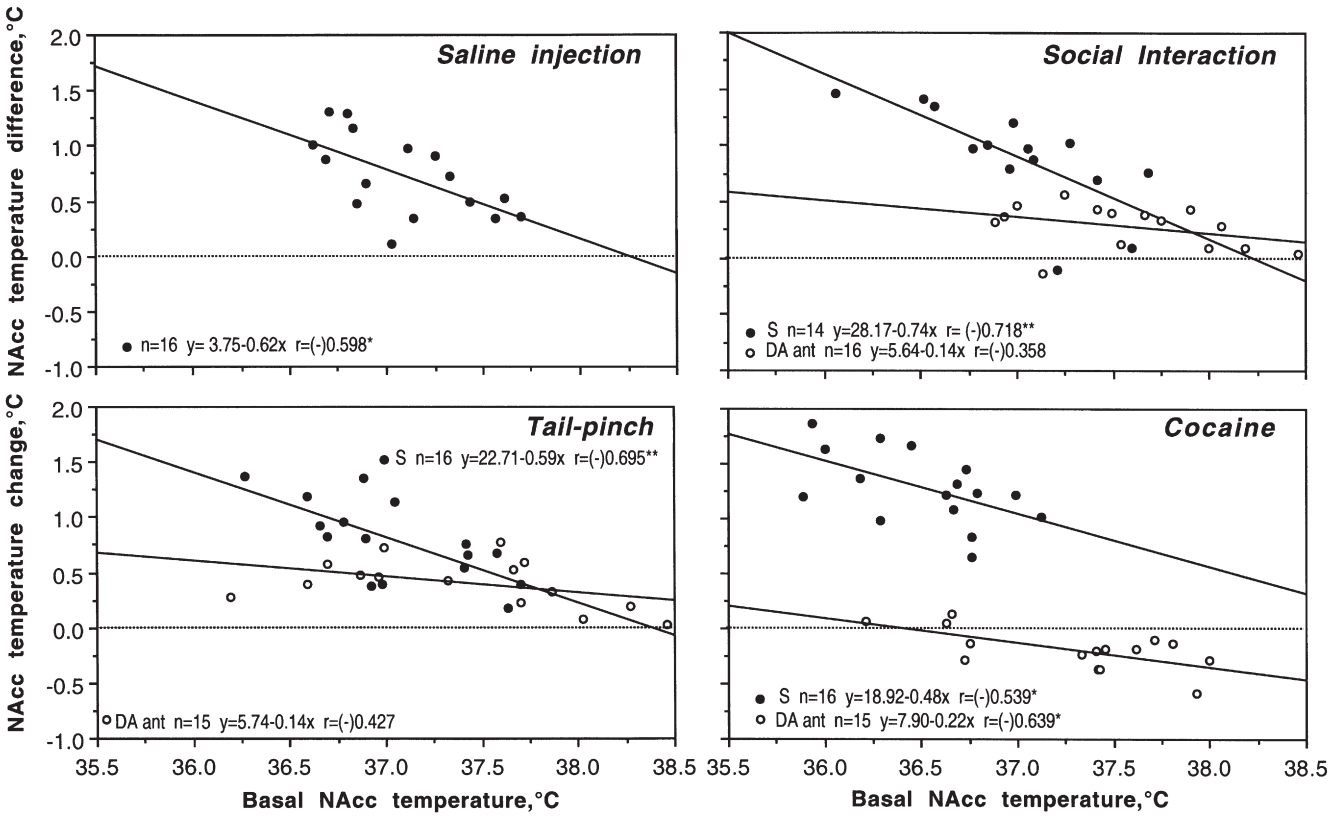

Fig. 6. Correlative relationships between basal NAcc temperature and its changes induced by the procedure of injection (ip saline), tail-pinch, social interaction, and iv cocaine in the saline and DA antagonists conditions. Each point represents basal and peak values (for cocaine — 16 min, for social interaction — 9 min; for tail-pinch and IP injection 10 min) and shows two regression lines, two regression equations, and two correlation coefficients for two groups.

modulatory functions of DA with respect to other neurochemical systems involved in brain activational processes. While our data confirm the importance of DA in mediating the locomotor-stimulatory and temperature-increasing effects of iv cocaine, they also suggest that cocaine also has other, non-DA mediated inhibitory physiological effects that are masked in normal conditions.

Our data support the view that the tight association between locomotor activation and increased brain metabolism that appears to exist under physiological and behavioral conditions (see Kiyatkin, 2005 for review) does not hold for drug-induced behavioral activation. Strong locomotor inhibition following DA receptor blockade is accompanied by increases in brain, muscle and skin temperature, suggesting metabolic brain activation, despite enhanced heat loss from skin surfaces. While this uncoupling looks surprising, apomorphine, which strongly increases locomotor activity, decreases brain and muscle temperatures despite vasoconstriction that limits heat dissipation to the external environment (Brown et al., 2007). Therefore, it appears that naturally occurring locomotor activation (searching, grooming, rearing) and inhibition (rest, sleep) differ in its basic underlying mechanisms from "similar" behaviors induced by drugs.

Finally, our data could provide a better understanding of why DA antagonists powerfully inhibit the development of motivated behavior. Not only cocaine but all natural reinforcers have four important and related features: they induce metabolic brain activation, arousal, brain hyperthermia and behavioral activation. All these features are strongly attenuated by DA receptor antagonists. By removing these features, reinforcers loose their reinforcing qualities and behavior does not develop. Therefore, consistent with earlier views (Le Moal and Simon, 1991), the DA system may be viewed as an important component of a general activation (arousal) system, which is stimulated by various salient stimuli and is essential for the development and performance of motivated behavior.

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, NIDA. I wish to thank P. Leon Brown and David Bae for valuable technical assistance in conducting the experiments described in this paper and Michael Smirnov for editorial assistance.

References

Altschule MD. Emotion and circulation. Circulation 1951;3:444–54.
Amenta F, Ricci A, Tayebati SK, Zaccheo D. The peripheral dopaminergic system: morphological analysis, functional and clinical applications. Ital J Anat Embryol 2002;107:145–67.
Baker M, Cronin M, Mountjoy D. Variability of skin temperature in the waking monkey. Am J Physiol 1976;230:449–55.
Bischoff S, Heinrich M, Sonntag HM, Krauss J. The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT_2) receptors. Eur J Pharmacol 1986;129:367–70.
Bloom FE, Schulman JA, Koob GF. Catecholamines and behavior. In: Trendelenburg, U, Weiner N, editors. Handbook of Experimental Pharmacology, vol. 90/II. Springer: Berlin; 1989. p. 27–88.
Brown PL, Bae D, Kiyatkin EA. Relationships between locomotor activation and alterations in brain temperature during selective blockade and stimulation of dopamine transmission. Neuroscience 2007;145:335–43.
Calabresi P, Centonze D, Bernardi G. Electrophysiology of dopamine in normal and denervated striatal neurons. Trends Neurosci 2000;23:S57–63.
Faunt JE, Crocker AD. The effects of selective dopamine receptor agonists and antagonists on body temperature in rats. Eur J Pharmacol 1987;133:243–7.
Gillis RA, Hernandez YM, Erzouki HK, Raczkowski VF, Mandal AK, Kuhn FE, et al. Sympathetic nervous system mediated cardiovascular effects of cocaine are primarily due to a peripheral site of action of the drug. Drug Alcohol Depend 1995;37:217–30.
Kiritsy-Roy JA, Halter JB, Gordon SM, Smith MJ, Terry LC. Role of the central nervous system in hemodynamic and sympathoadrenal responses to cocaine in rats. J Pharmacol Exp Ther 1990;256:154–60.
Kiyatkin E. Brain hyperthermia as physiological and pathological phenomena. Brain Res Rev 2005;50:27–56.
Kiyatkin EA, Bae D. Behavioral and brain temperature responses to salient environmental stimuli and intravenous cocaine in rats: effects of diazepam. Psychopharmacology 2008;196:343–56.

Kiyatkin EA, Brown PL. Dopamine-dependent and dopamine-independent actions of cocaine as revealed by brain thermorecording in freely moving rats. Eur J Neurosci 2005;22:930–8.
Kiyatkin EA, Rebec GV. Dopaminergic modulation of glutamate-induced excitations of neurons in the neostriatum and nucleus accumbens of awake, unrestrained rats. J Neurophysiol 1996;75:142–53.
Kiyatkin EA, Rebec GV. Striatal neuronal activity and responsiveness to dopamine and glutamate after selective blockade of D1 and D2 dopamine receptors in freely moving rats. J Neurosci 1999;19:3594–609.
Knuepfer MM, Branch CA. Cardiovascular responses to cocaine are initially mediated by the central nervous system in rats. J Pharmacol Exp Ther 1992;263:734–41.
Le Moal M, Simon H. Mesocorticolimbic dopaminergic network-functional and regulatory roles. Physiol Rev 1991;71:155–234.
Mercuri N, Bernardi G, Calabresi P, Cotugno A, Levi G, Stanzione P. Dopamine decreases cell excitability in rat striatal neurons by pre- and postsynaptic mechanisms. Brain Res 1985;358:110–21.
Neve KA, Neve RL. Molecular biology of dopamine receptors. In: Neve, KA, Neve, RL, editors. The Dopamine Receptors. Totowa, NJ: Humana Press; 1997. p. 27–76.
Nicola SM, Malenka RC. Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens. J Neurosci 1997;17:5697–710.
Paxinos J, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1998.
Pettit HO, Justice JB. Effect of dose on cocaine self-administration behavior and dopamine levels in the nucleus accumbens. Brain Res 1991;539:94–102.
Poon J, van den Buuse M. Autonomic mechanisms in the acute cardiovascular effects of cocaine in conscious rats. Eur J Pharmacol 1998;363:147–52.
Schlenker EH. In hamsters the D1 receptor antagonist SCH23390 depresses ventilation during hypoxia. Brain Res 2008;1187:146–53.
Siggins GR. Electrophysiological role of dopamine in striatum: excitatory or inhibitory? In: Lipton, MA, DiMascio, A, Killam, KF, editors. Psychopharmacology: A Generation of Progress. New York: Raven Press; 1977. p. 143–57.
Solomon GF, Moos RH, Stone GC, Fessel WJ. Peripheral vasoconstriction induced by emotional stress in rats. Angiology 1964;15:362–5.
Williams J, Wasserberger J. Crack cocaine causing fatal vosoconstriction of the aorta. J Emerg Med 2006;31:181–4.
Windels F, Kiyatkin EA. Dopamine action in the substantia nigra pars reticulata: iontophoretic studies in awake, unrestrained rats. Eur J Neurosci 2006;24:1385–94.
Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. Psychol Rev 1987;94:469–92.