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# State-dependent action of cocaine on brain temperature and movement activity: implications for movement sensitization

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**Abstract**

Because neural activity is highly energy consuming and heat producing, brain temperature offers a reliable, real-time measure of an animal's activity state and its changes induced by environmental and drug challenges. Therefore, it allows evaluation of the activity state of an animal preceding drug administration and its relation to subsequent drug-induced neural effects. This approach was used to explore the state dependency of cocaine's effects. Brain and body temperatures, as well as locomotion were measured simultaneously in rats during repeated, daily administration of cocaine (15 mg/kg ip, daily for 5 days) under different experimental conditions. The drug was administered via (a) a chronically implanted catheter in quiet resting conditions, (b) an injection made under quiet rest or (c) an injection under activated conditions associated with placement in the cage. Although brain temperature and movement increased after cocaine administration in each condition, cocaine's action (evaluated as cocaine–saline difference for both parameters) was situational. Catheter-administered cocaine induced the strongest movement activation and robust, monophasic temperature increase, which remained relatively stable following each subsequent drug infusion. Cocaine injected during quiet and, especially, activated conditions, induced a weaker locomotor activation, while the temperature response (evaluated as drug–saline difference) had a biphasic pattern. Cocaine initially inhibited the temperature increases seen in saline-treated animals (0–20 min) and then induced a more prolonged hyperthermia, which was about twofold weaker than that seen after catheter-administered drug. Although movement activation gradually increased following repeated treatment in activated conditions, the magnitude of this sensitized motor response barely reached the levels induced by the initial cocaine administration via catheter. These data suggest that both the acute effects of cocaine in the brain and their change following repeated drug administration are dependent upon the ongoing neural activity state of the animal. Cocaine's interaction with this activity state is a crucial factor determining the behavioral effects of this drug, including state-dependent motor sensitization.


*Keywords:* Cocaine; Behavioral sensitization; Brain temperature; Neural activation; Rats

## 1. Introduction

State dependency is a feature of most neuroactive and psychoactive therapeutic drugs. For example, opiate analgesics relieve pain in patients experiencing pain, but induce dose-dependent somnogenic, sedative and depressive effects in pain-free individuals (Melzack, 1973). Similarly, antipsychotic drugs, antidepressants and tranquilizers have their specific therapeutic effects in patients with psychosis, depression and anxiety, respectively. Addictive drugs also have characteristic state-dependent patterns of action, a fact reflected in the overwhelming prevalence of specific circumstances, under which these drugs are self-administered. For example, psychomotor stimulants, including cocaine, are usually taken during high-activity states, during various forms of social interaction and alongside other sensory stimuli (Jaffe, 1990; Kalant, 2001).

The effects of most drugs are also modulated by repeated administration, typically decreasing their potency (tolerance) following repeated administration (e.g., the pain-suppressive effects of opiate analgesics). While some effects of cocaine (e.g., cardiovascular) show rapid tolerance with repeated administration (Tella et al., 1991), cocaine-induced movement activation may increase over time (Castellani and Ellinwood, 1985; Downs and Eddy, 1932; Kalivas and Stewart, 1991; Robinson, 1988). This enhancement of cocaine's movement stimulation often called behavioral sensitization, depends on environmental conditions associ-

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ated with drug use. It typically occurs when the drug is injected repeatedly in a novel environment and is absent or minimal in the case of repeated drug exposure in the home environment (Badiani et al., 1995; Browman et al., 1998; Crombag et al., 2000; Hinson and Poulos, 1981; Post et al., 1981, 1987; Weiss et al., 1989).

To explore the state dependency of cocaine's action, we monitored drug-induced changes in movement and brain temperatures following the same protocol of repeated drug administration [15 mg/kg ip for 5 days] under different conditions. Because neural activity is highly energy consuming and accompanied by heat release (Laughlin et al., 1998; Ritchie, 1973; Siesjo, 1978), brain temperature offers a reliable, real-time measure of both the animal's activity state and the drug's central action (Kiyatkin and Wise, 2001, 2002; Kiyatkin et al., 2002; Kiyatkin and Brown, 2003), thus allowing a study of the relationship between these parameters. First, we administered cocaine via a chronically implanted intraperitoneal catheter to rats that were well habituated to the testing cage. This type of drug administration excluded all possible sources of tonic, state-dependent, and phasic, injection-dependent activation that may influence cocaine's pharmacological effects, thus allowing the pure drug action to be evaluated. Second, we injected cocaine after a 3-h habituation to the test cage, when the rats were in a quiet state. Third, we injected cocaine immediately after the rat was placed in the experimental cage; association of cocaine administration with placement in the test cage is often used in sensitization studies and is usually referred to as treatment under novel or activated conditions (Badiani et al., 1995; Crombag et al., 2000; Kiyatkin, 1992). Control animals were injected with saline twice to control for both activity state and procedure of injection. Finally, to verify the development of conditioned neural activation and conditioned movement activation induced by stimuli and the conditions associated with repeated drug exposure, animals of the second and third groups were injected with saline on Day 6.

As brain recording sites, we chose the nucleus accumbens (NAcc) and ventral tegmental area (VTA), the reciprocally connected, integrative brain structures that are involved in cocaine locomotor sensitization (Kalivas and Stewart, 1991; Roninson and Berridge, 1993). Body temperature was evaluated by recording from deep temporal muscle, a nonlocomotor head muscle that is supplied, as is the brain, with arterial blood from the common carotid artery. Measurements in both brain and muscle enable us to determine brain–muscle temperature gradients, a means by which metabolic brain activation can be represented (Kiyatkin et al., 2002).

## 2. Methods

### 2.1. Animals and surgery

Thirty-two male Long–Evans rats, weighing 440 ± 40 g (Charles River Laboratories, Greensboro, NC, USA), were used. They were housed individually under a normal light–dark cycle (lights on 0700–1900 h) with free access to food and water. All protocols were performed in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* (NIH, Publication 865-25) and were approved by the NIDA-IRP Animal Care and Use Committee.

On the day of surgery, each rat was anesthetized with Equithesin (0.33 ml/100 g ip) and implanted with four thermocouple probes as described in detail elsewhere (Kiyatkin and Wise, 2001). Two electrodes were aimed at the right NAcc (1.4 mm anterior from the bregma, 1.0 mm lateral from midline, and 7.3 mm depth from skull surface) and left VTA (5.5 mm posterior from the bregma, 2.0 mm lateral from midline, and 8.4 mm depth from skull surface at 10° angle) and two other electrodes were implanted in the right and left deep temporal muscles. After implantation of temperature probes, animals receiving catheter treatment were incised to allow insertion of a polyethylene catheter via a small ( ~ 2–4 mm) hole in the peritoneum, posterior to the spleen. With 30–40 mm of the catheter remaining inside the peritoneal cavity, the opening was tightly sutured to restore the integrity of the muscle wall and fix the catheter in place. The rest of the catheter was then guided subcutaneously to exit at the neck, where it was secured onto a filed 23-gauge needle attached to its plastic adapter. The adapter was plugged with a rubber plug and fixed onto the headpiece with dental acrylic, and the catheter was flushed with saline. During the experiment, the catheter exit on the rat's head was connected with an extension tube fastened onto the electric cord, so that the end with the exposed needle was inside the cage (and could be inserted into the catheter adapter) and the end with the adapter was outside the cage. This setup allowed for remote catheter drug administration from outside the cage. Experimentation began after a 3- to 4-day recovery period and continued for the next 6–8 days.

### 2.2. Thermocouple probes and recording instruments

Thermocouple probes were made of insulated copper and constantin wires (diameter ≈ 125 μm), which were welded together at the tip ( ≈ 0.4 mm) and insulated with polyester microshrink tubing and epoxy. The wires were connected to copper and constantin pins and were fixed in a plastic connector with epoxy. During experiments, the probes were connected to the recording instrument (Thermes-16, Physitemp, Clifton, NJ, USA) via individual sockets, a common cord and a nine-channel electric swivel. During the session, temperatures were continuously recorded and stored in computer memory at 10-s intervals. Temperature in the room was maintained automatically at 22 °C and stability of temperature in the chamber throughout the sessions (fluctuations between 22 and 23 °C) was confirmed by continuous monitoring of the cage temperature by an additional thermosensor.

### 2.3. Movement activity recording

Movement was monitored by an infrared photobeam array (Med-PC IV, Med Associates, St. Albans, VT, USA). Four pairs of individual sensors (four receivers and four transmitters) were installed. Two were placed at regular intervals on each outer wall of the test chambers at 2.0 cm above the cage floor level. The resulting grid consisted of four beams. A count was recorded whenever a beam was tripped, and measurements were stored as cumulative values for each subsequent 5-min bin.

### 2.4. Experimental protocol and treatment groups

Daily recordings took place between 0800 and 1600 h, during the light phase of the animals' light–dark cycle, in a Plexiglas chamber ($35 \times 35 \times 40$ cm) housed inside of a large wooden box ($64 \times 66 \times 65$ cm) with no transparent doors. For each session, the rats were brought individually from their housing facility, placed in individual chambers, connected to the recording instruments, and were treated according to one of the stated regimens. The first temperature and movement measurements were made within 4–5 min after the rat was taken from a home cage and recordings continued for 7–8 h before the animals were returned to their home cages.

The rats were divided into four equal groups according to treatment regimen. Rats in Group I ("catheter cocaine") were habituated to the recording environment for two daily sessions and received one daily intraperitoneal cocaine infusion (15 mg/kg in 0.5 ml saline for ~10 s) during the next 5 days. Drug administrations were made after a 2- to 3-h habituation when the rat was in a quiet, resting state and temperatures were stable and low. During the habituation session, these rats were administered once daily with saline (0.5 ml); these administrations were also done after at least a 2-h habituation to the cage, when the animal was in a quiet, resting state. Although all infusions in this group were made from outside of the chamber and animals could not detect the moment of the infusion, animals of this group were exposed to white noise (~60 dB) during each session to minimize possible interference.

Rats in Groups II and III received two daily intraperitoneal injections (cocaine and saline) for 5 days and one saline injection on Day 6. Rats in Group II ("quiet state") received a saline injection immediately after placement in the cage and a cocaine injection 3 h later, when the rat was in a quiet state. Rats in Group III ("activated state"), by contrast, received the first cocaine injection within 2 min after placement in the cage and the start of temperature recording and the second saline injection 4 h later under quiet conditions. On Day 6, the rats received only one saline injection, either upon placement in the cage (Group III) or 3 h later under quiet conditions (Group II). Rats in control Group IV received two daily injections of saline (after placement in the cage and 3 h later) for six consecutive days. All intraperitoneal injections were made by lifting the hindquarters of the rat by the tail to access the abdomen. By using this method, it was unnecessary to lift, restraint or otherwise hold the rat.

### 2.5. Histology

After completion of the experiments, each rat was deeply anesthetized and its brain was removed for subsequent histological verification of probe location. The location of the brain recording sites was determined from 45-μm slices mounted on glass slides according to the atlas of Paxonos and Watson (1998).

### 2.6. Data analyses

Changes in temperature associated with administration of cocaine and saline were analyzed using one-way ANOVA with repeated measures followed by post-hoc Fisher tests (10-min quantification bins) and were presented as absolute changes, changes relative to preadministration baseline and brain–muscle differentials. Temperature and movement activity were also analyzed as cocaine–saline difference. By subtracting changes occurring after saline injection in quiet and activated conditions, one can see the pure effect of the drug under each tested condition. Significance of cocaine–saline differences was evaluated using Student's *t* test. The subtraction technique (cocaine − saline) was also used to compare movement activation and temperature response induced by cocaine on different treatment days. The mean of eighteen 10-min values (i.e., 180 min post-cocaine) was used for day-to-day and state-to-state comparisons of magnitudes of cocaine-induced movement activation and temperature change.

## 3. Results

The present results were obtained from 24 rats, which had artifact-free recordings from all three sites and electrodes were verified histologically in the target areas (Fig. 1).

Because the effects of cocaine in our procedure may have depended upon two factors, the animal's state and the effect of successive administrations, two consecutive analyses were performed. First, changes in temperature and movement were analyzed with respect to the state (all five drug injections within the "state" category were combined). Second, changes depending on the order of administrations were analyzed within each of the four groups.

### 3.1. Conditions associated with repeated cocaine treatment

Because three procedures associated with cocaine administration may have an impact on the action of cocaine, we first analyzed changes in movement and temperature induced by (a) saline administration via intraperitoneal

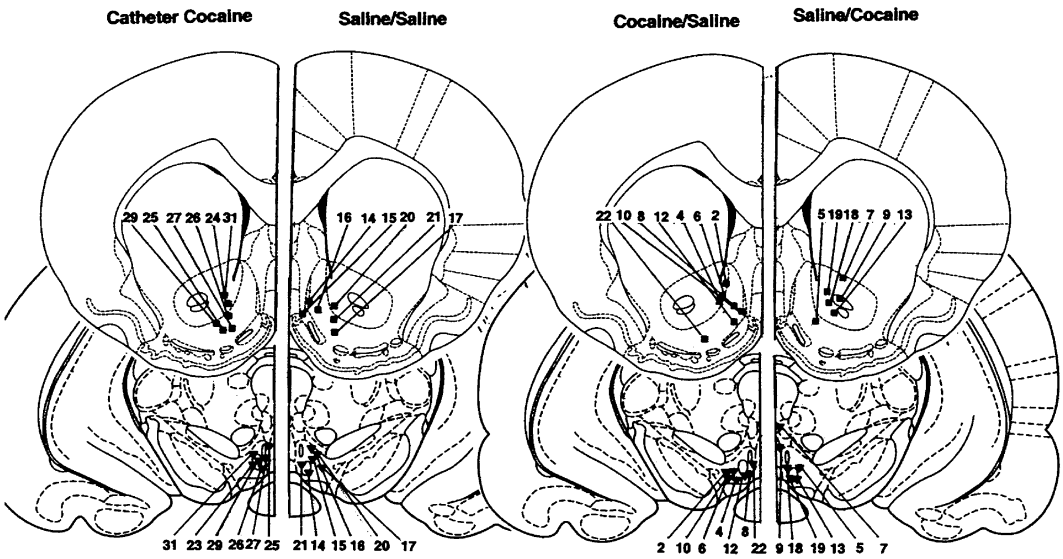



Fig. 1. Coronal sections of the brain illustrating reconstructed locations of thermocouple tips in four groups of rats used in this study. Lower graphs represent midbrain sections, with triangles showing electrode locations within the VTA, and upper graphs show forebrain sections, with squares showing electrode locations within the NAcc. Atlas of Paxinos and Watson (1998) was used to prepare drawings.

catheter in the quiet state, (b) saline injection in the quiet state and (c) saline injection in the activated state (Fig. 2).

As shown in Fig. 2, saline administration via intraperitoneal catheter did not result in any evident changes in movement or temperature (left panel; A, B and C), while robust and distinct changes in both parameters were seen after saline injection under both quiet and activated conditions. The procedure of injection under quiet conditions caused significant movement activation and temperature increase (0.4–0.5 °C), lasting about 30 min, and was followed by decreases in both parameters (middle panel; A, B and C). Increases in VTA and NAcc temperatures were stronger than that in the muscle (C), resulting in a significant rise in brain–muscle differential during the first 10 min after the injection (D). Robust movement activation and temperature elevation also occurred when the injection was performed immediately following the rat's placement in the cage (right panel). Because it was impossible to determine real baseline temperature values immediately before the rat was relocated from its home cage, minimal values within the session (different in each rat and in each daily session) were used as the session baseline. With respect to this point, temperature increased on average 1.6–1.9 °C with virtually identical changes in the VTA and NAcc, which were significantly greater than in the muscle (C), resulting in a strong but transient (40–60 min) elevation in brain–muscle differentials (D).

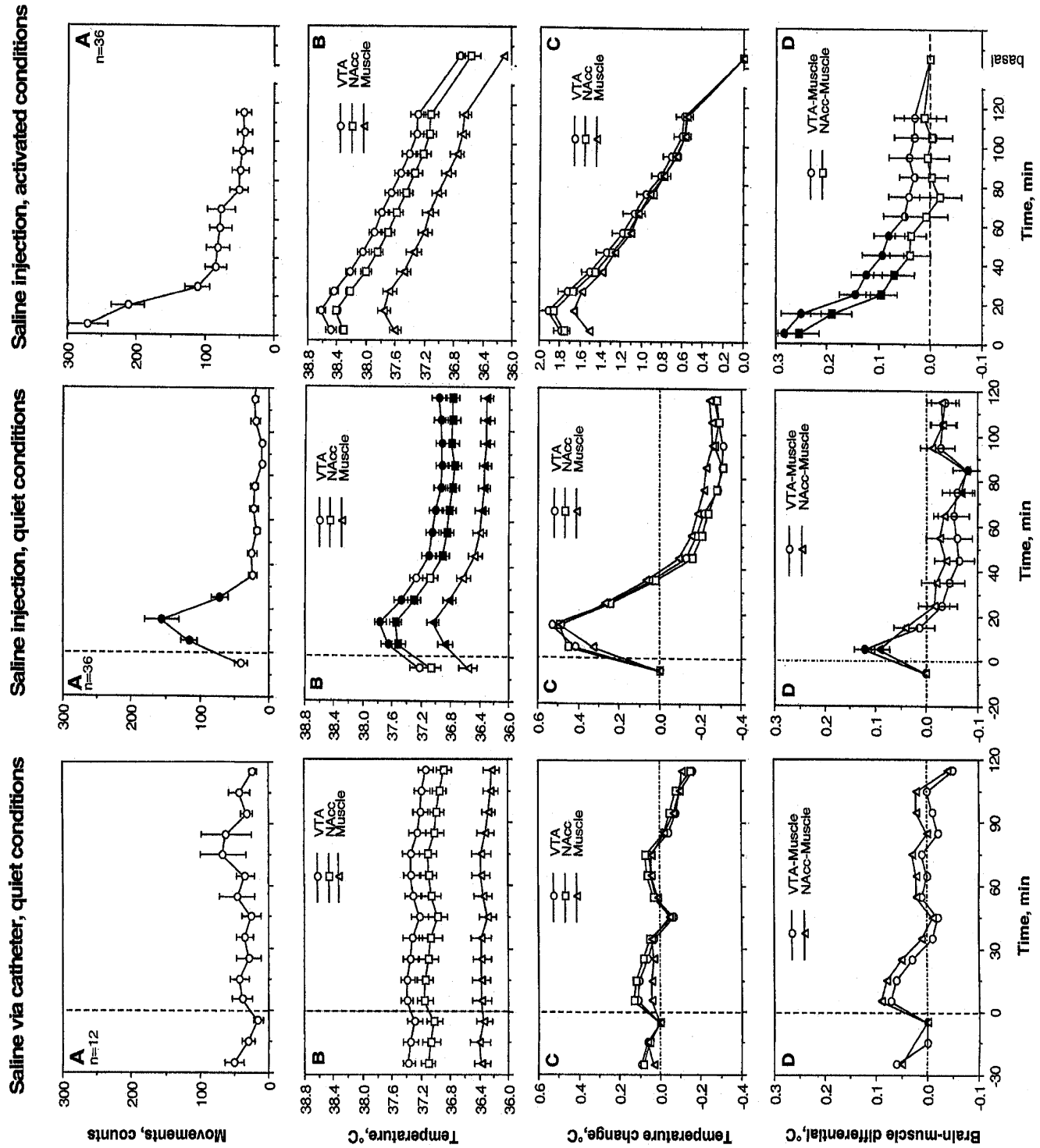
As shown in Fig. 2B, there were significant differences in basal temperatures between recording sites. VTA in each animal group had the highest temperatures and NAcc had slightly lower temperatures (no significant differences), while the muscle had the lowest temperatures, which was significantly lower than those of both VTA and NAcc ($P < .05$; Student's $t$ test). These differences were consistent in each animal group and maintained during repeated testing.

### 3.2. Cocaine administration via catheter under quiet conditions

Fig. 3 shows mean changes in movement activity and temperature for 30 cocaine administrations via intraperitoneal catheter under quiet conditions (six rats × five drug injections). Because saline administration via catheter had no effect on the recorded parameters (see Fig. 2), these changes represent cocaine's untainted action on locomotion and temperature.

As shown in Fig. 3, cocaine caused robust and long-term movement activation (A) and temperature elevation (peak ~ 1.3 °C) in each recording location (B). Movement values

Fig. 2. Changes in movements and temperature (VTA, NAcc and muscle) after saline administration via intraperitoneal catheter in quiet resting conditions (left panel), via intraperitoneal injection in quiet resting conditions (central panel) and via intraperitoneal injection in activated conditions (right panel). A = movement activity, B = absolute temperatures ± S.E.M.; C = relative temperature change; D = brain–muscle differentials. Filled symbols show values significantly different from the last preadministration (for saline administration via catheter or injection under quiet conditions) or basal (for saline administration under activated conditions) value (one-way ANOVA with repeated measurements followed by Fisher test; at least $P < .05$). Effect of time on temperature and movement after saline infusion via catheter was nonsignificant for each parameter [$F(12,168) = 0.97$, 0.91, 0.51 and 0.62 for VTA, NAcc, muscle and movements, respectively], but highly significant for saline injection under quiet, resting conditions [$F(39,774) = 68.89$, 70.48, 51.34 and 69.87 for VTA, NAcc, muscle and movement, respectively; each $P < .001$]. $n$ shows numbers of averaged responses (six rats exposed to either two or six tests). Vertical hatched line (0 min) indicates the moment of saline injection.


Saline via catheter, quiet conditions
Saline injection, quiet conditions
Saline injection, activated conditions
Movements, counts
Temperature,°C
Temperature change,°C
Brain-muscle differential,°C
A
B
C
D
n=12
n=36
VTA
NAcc
Muscle
VTA-Muscle
NAcc-Muscle
Time, min
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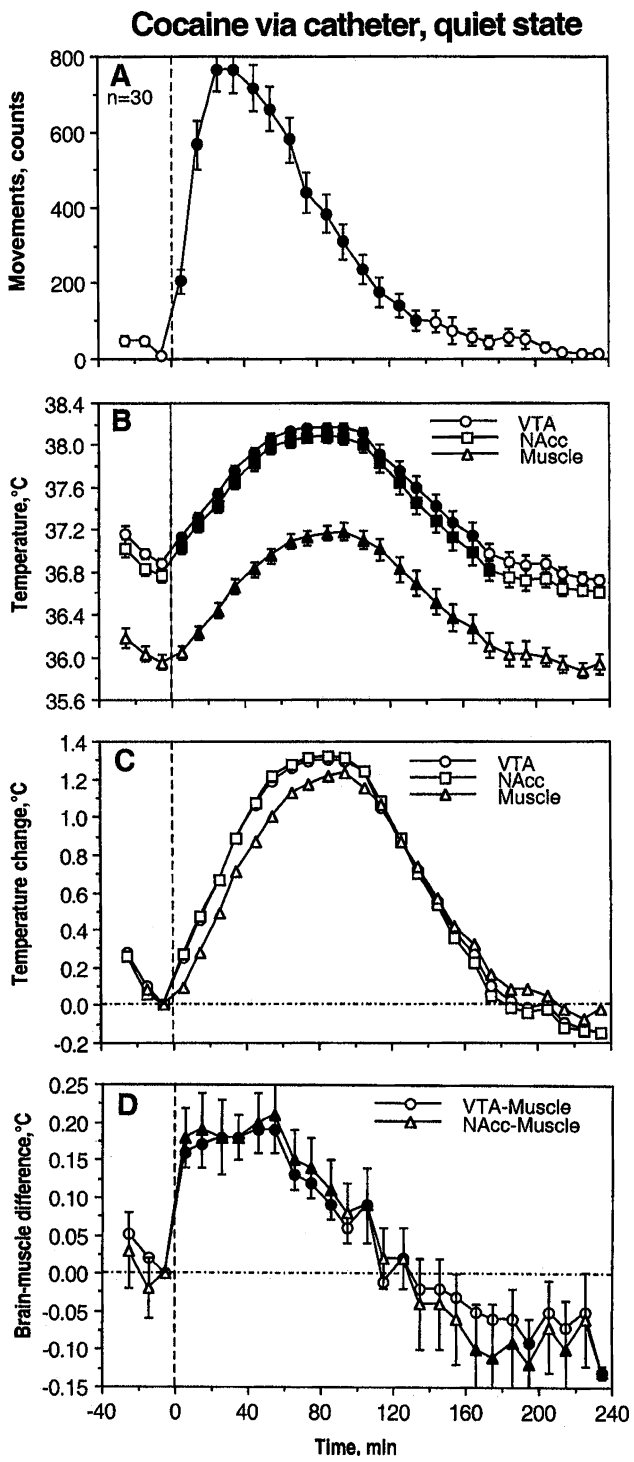


Fig. 3. Changes in movement (A) and temperature (B, absolute; C, relative change; and D, brain–muscle differentials) induced by cocaine (15 mg/kg ip; 30 injections in six rats) administered via intraperitoneal catheter to well-habituated rats under quiet resting conditions. Filled symbols indicate values significantly larger than the last predrug value (one-way ANOVA with repeated tests; $P<.05$; Fisher test). Effect of time on temperature change was significant for each brain structure [$F(24,749)=72.05$, 73.28, 52.23 and 69.87 for VTA, NAcc, muscle and movements, respectively; each $P<.001$] and for VTA- and NAcc-muscle differentials [$F(24,749)=17.72$ and 15.62, respectively]. *n* shows numbers of averaged responses (five tests in six rats).

peaked at 30–40 min and the increase was significant for a 140-min period, while temperature values peaked at 70–90 min but were significantly increased for approximately the same (160-min) duration. Brain temperature increased more rapidly and stronger in the VTA and NAcc than in the muscle (C), resulting in a significant rise in brain–muscle differentials. VTA-muscle and NAcc-muscle differentials sharply increased during the first 10 min after cocaine infusion, remained at a plateau level for the next 50 min, and declined below baseline from ~ 120 min. Changes in both movement and temperature were consistent within the group as shown in small values of standard errors of the mean (A and B).

### 3.3. Cocaine injection under quiet conditions

Fig. 4 shows mean changes in movement activity and temperature after cocaine and saline injections in the quiet state as well as cocaine–saline differences that represent the pattern of cocaine's action under this condition. Although the injection of cocaine significantly increased locomotor activity (5–105 min) and temperatures (15–115 min), saline injection also had a significant effect on both parameters (middle panel). Interestingly, peak temperature increase after saline injection ( ~ 0.55 °C) was even slightly larger than that after cocaine injection ( ~ 0.45 °C, compare C graphs). The subtraction procedure revealed (see right panel, A and B) that cocaine's action on temperature under these conditions is biphasic, with an initial inhibition of the injection-related increase (0–20 min) and a subsequent robust and more prolonged temperature elevation. While similar or even longer in duration, this temperature elevation was weaker than that occurring after catheter-delivered cocaine ( ~ 0.7 vs. ~ 1.3 °C at peak). In contrast, movement activation after saline subtraction (A) showed a monophasic change; this change was also smaller than that induced by catheter-delivered cocaine ( ~ 400 counts/10 min vs. ~ 750 counts/10 min at peak). Statistical comparison of cocaine-induced movement and temperature response (mean of eighteen 10-min values, i.e., area under curve) revealed that increases in both parameters are weaker after intraperitoneal cocaine injection than after catheter-delivered drug ($206.7 \pm 32.5$ vs. $334.4 \pm 57.5$ counts/10 min; $P<.05$; $0.396 \pm 0.071$ vs. $0.805 \pm 0.097$ °C; $P<.01$; Student's *t* test).

### 3.4. Cocaine injection under activated conditions

Fig. 5 shows mean changes in movement activity and temperature after cocaine (left column) and saline (middle column) injections performed immediately after the rat was placed from its home cage to experimental cage (environmental change). Comparing these data, one can see movement activation and temperature change in both groups with stronger movement and weaker temperature responses in the cocaine than in the saline group. Analysis of cocaine–saline

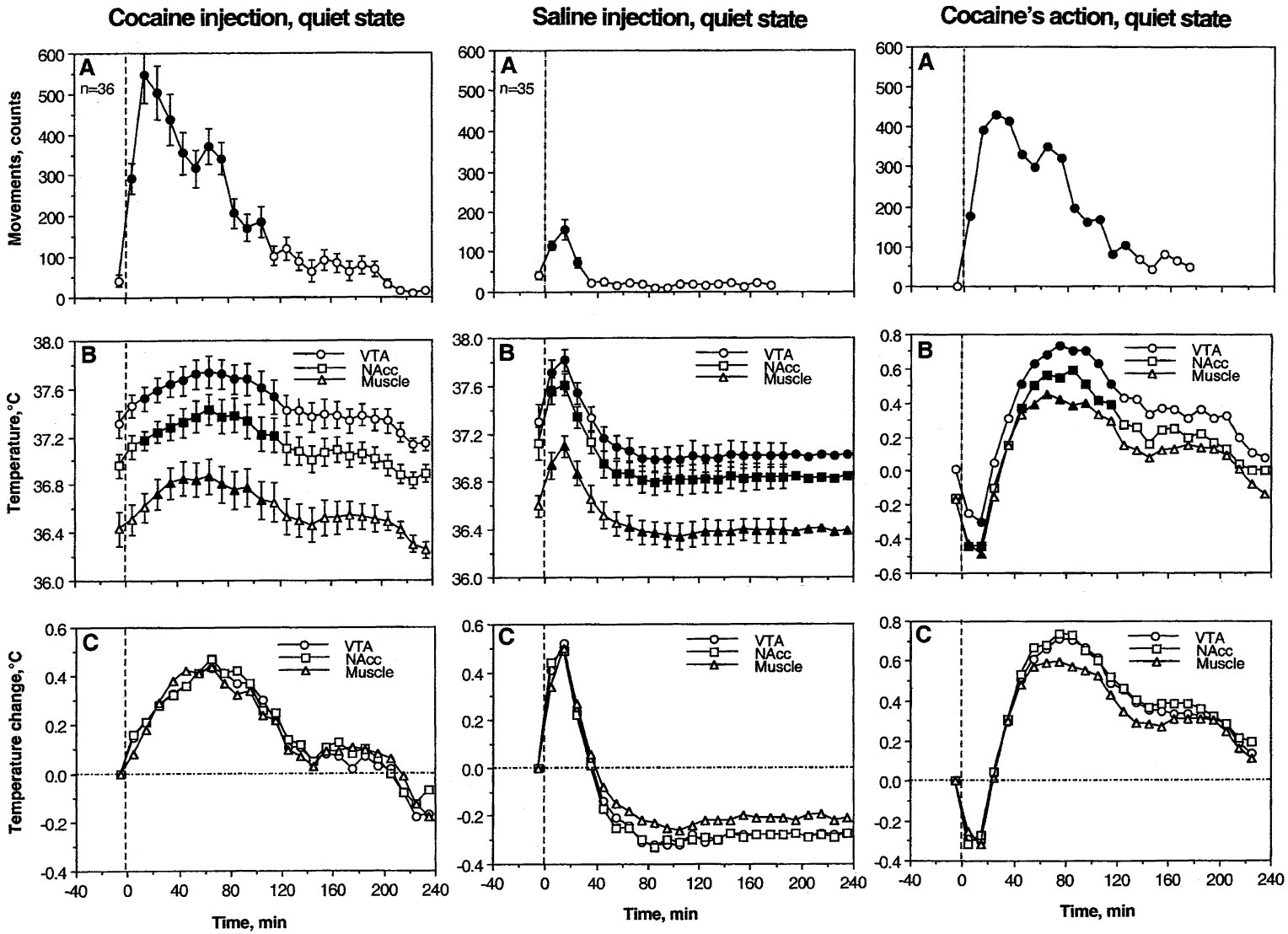


Fig. 4. Changes in movement (A) and temperature (B, absolute values and C, relative values) induced by cocaine (left panel) and saline (central panel) injected in quiet resting conditions. Filled symbols indicate values significantly larger than the last preinjection value ( $P < .05$; Fisher test). Effect of time on temperature change was highly significant for each brain structure in both groups [cocaine: $F(34,804) = 4.93$, 5.19, 3.64 and 24.59 for VTA, NAcc, muscle and movements, respectively; each $P < .001$; saline: $F(39,774) = 68.89$, 70.48, 51.34 and 69.87 for VTA, NAcc, muscle and movements, respectively; each $P < .001$). $n$ shows numbers of averaged responses. Right panel shows cocaine–saline difference in temperature and movements. Filled symbols in this case indicate difference ( $P < .05$; Student's $t$ test) between cocaine and saline groups.

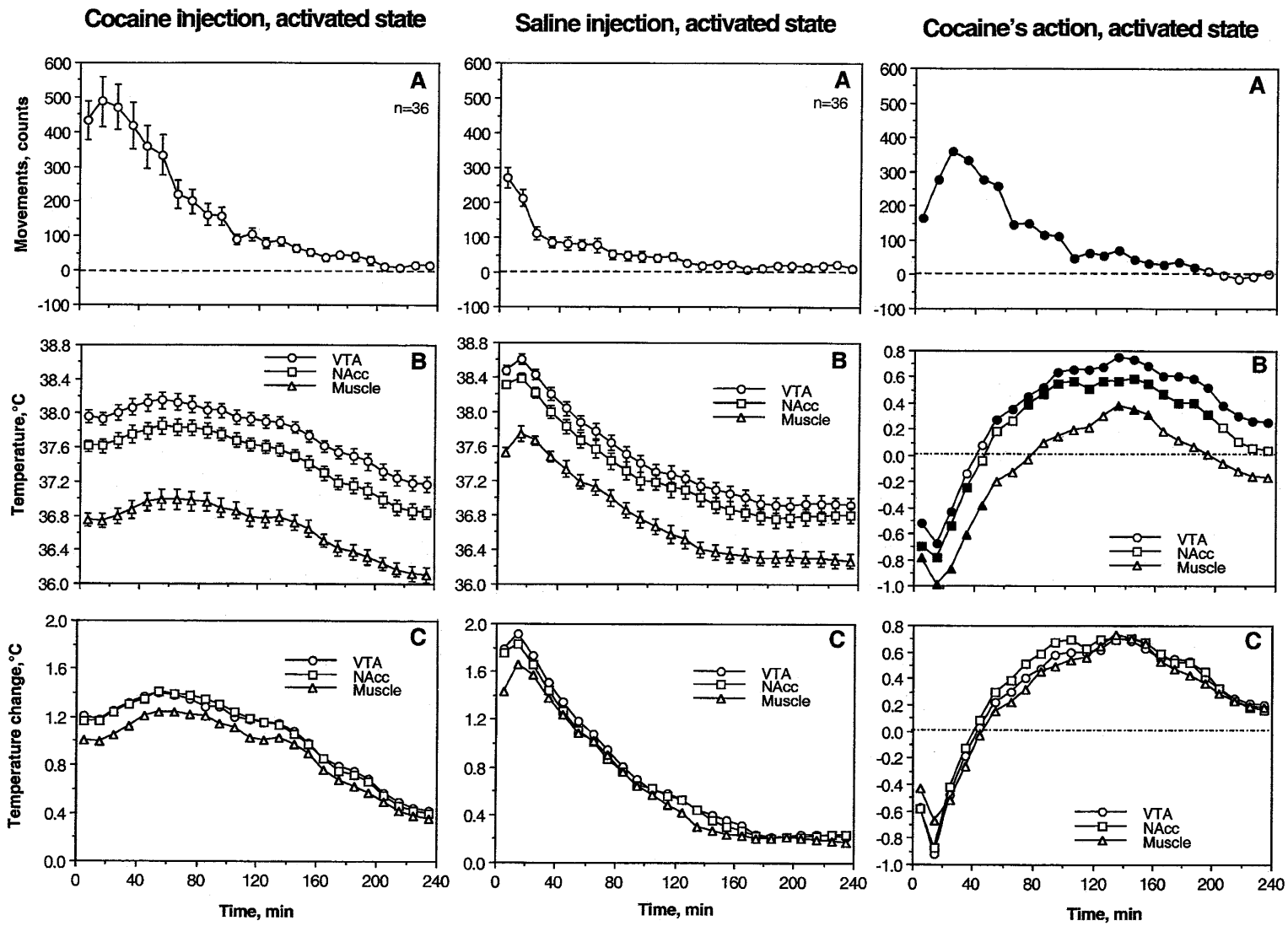


Fig. 5. Changes in movement (A) and temperature (B, absolute values and C, relative values) induced by cocaine (left panel) and saline (central panel) injected in activated conditions (zero time, the moment of placement in the cage). Right panel shows cocaine–saline difference in temperature and movements. Filled symbols indicate significant difference (Student's *t* test) between cocaine and saline.

difference (right column, A) revealed that cocaine's "pure" action on locomotion under activated conditions is monophasic, but smaller than that following cocaine administration via catheter and its injection under quiet conditions. In contrast, cocaine's action on temperatures is clearly biphasic (B and C), with the initial, relatively powerful inhibition of hyperthermia associated with environmental change (0–20 min) and subsequent more prolonged hyperthermia. While relatively long term (20–200 min), the magnitude of this temperature elevation is clearly weaker than that seen after catheter-administered cocaine ( ~ 0.7 vs. ~ 1.3 °C) but similar to that seen after cocaine injection in quiet, resting conditions ( ~ 0.7 °C). Although mean values of cocaine-induced movement activation (141.5 ±26.2 counts/10 min) and temperature response (0.308 ±0.111 °C) under activated conditions were lower than those under quiet conditions (206.7 ± 32.5 and 0.396 ± 0.071 °C), the differences were not statistically significant. In contrast, differences vs. catheter-delivered drug were highly significant ($P < .001$; Student's $t$ test) for both parameters.

### 3.5. Repeated saline injections under quiet and activated conditions

Consistent changes in movement and temperature occurred with repeated saline injections in the quiet state (Fig. 6A and B). For clarity, only temperature values for the VTA are presented. On Day 1, basal temperature was maximal and temperature elevation was minimal (0.3 °C), and basal temperatures decreased but the injection-associated elevation increased ( ~ 0.7 °C) and was relatively stable during each subsequent day. Movement activation, following injection in quiet state, was less subject to habituation and was consistent throughout the 6 days. Nevertheless, basal movement activity was maximal and relative change in movement induced by the injection was minimal on Day 1. It is important to note that the postinjection temperature increases were tightly linked to the preinjection basal temperatures. When the preinjection values were low (i.e., the animal is in the lower activity state), the injection-induced temperature increases were stronger. Conversely, when preinjection values were high (Day 1), they were followed by mild increases in both movement and temperature.

Similar changes in temperature and movement activity also occurred with repeated placement in the test cage (Fig. 6C and D). Although both temperature and movement values were maximal on Day 1, they became lower on Day 2, and fluctuated little through Day 5. Temperature elevations induced by the environmental change during each repeated session were similar in magnitude but temperature on consecutive days decreased more rapidly to a lower baseline. Similarly, movement activation was maximal during the first session and became shorter on subsequent sessions.

### 3.6. Repeated cocaine administration under different conditions

Fig. 7 shows changes in temperature and movement activity after each of five daily cocaine administrations

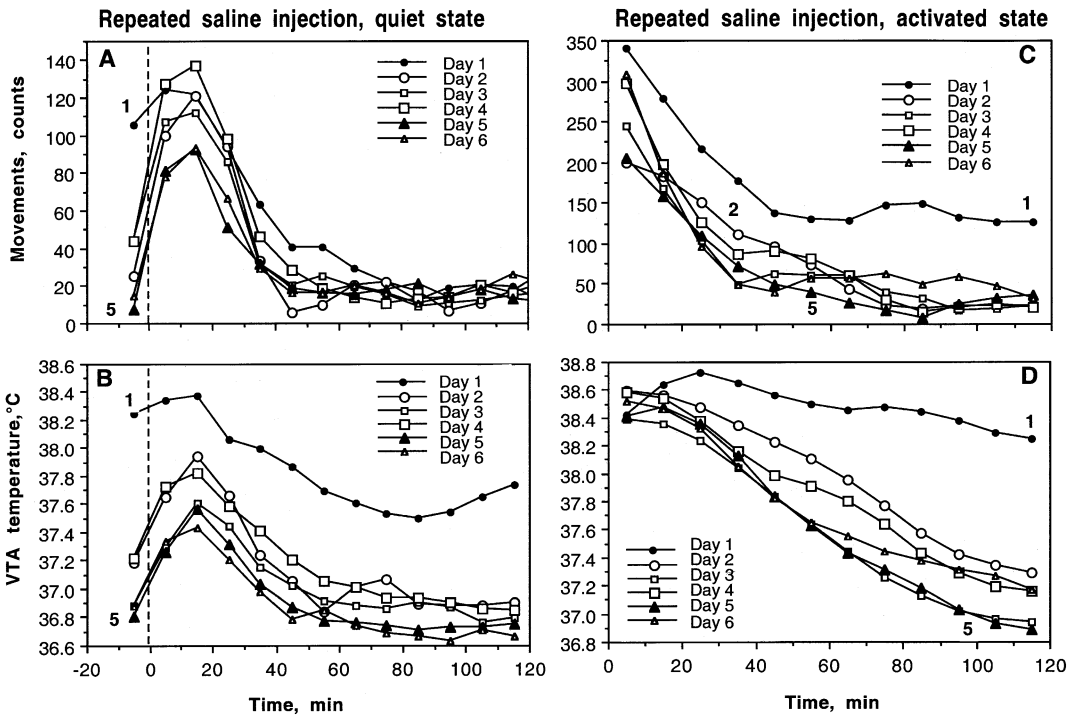


Fig. 6. Changes in movement (A and C) and VTA temperature (B and D) after each repeated saline injection made under quiet (A and B) and activated (C and D) conditions.

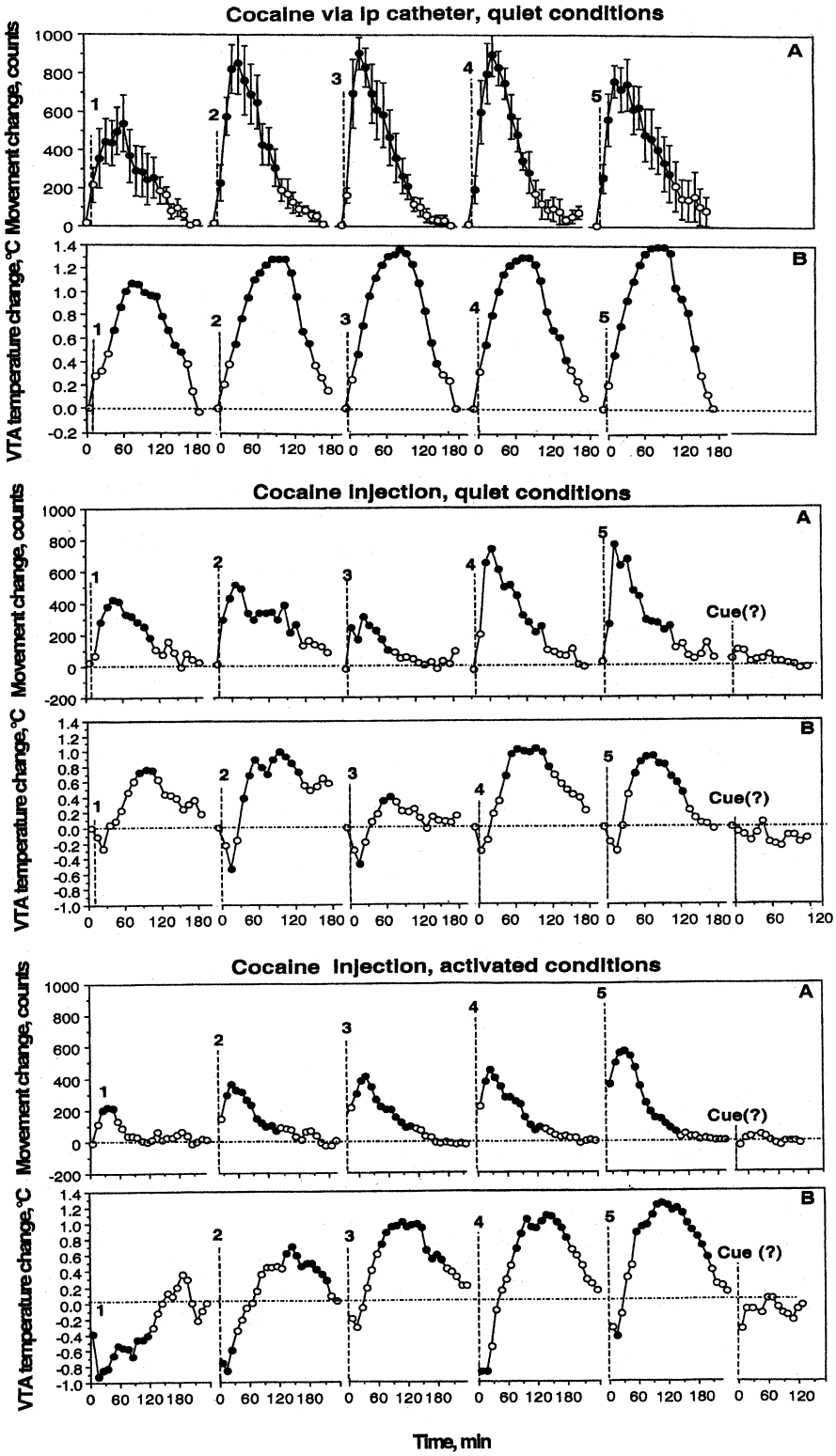


Fig. 7. Changes in movement activity (A) and VTA temperature (B) induced by each subsequent cocaine administration (1, 2, 3, 4 and 5) under three tested conditions (top, via intraperitoneal catheter to well-habituated rats under quiet conditions; middle, via intraperitoneal injection under quiet conditions; bottom, via intraperitoneal injection under activated conditions). For cocaine injections under quiet and activated conditions, each graph represents the difference between cocaine- and saline-treated animals with filled symbols showing significantly different values ($P < .05$; Student's $t$ test). The last injection in these graphs (cue?) shows the difference in the effect of saline between cocaine- and saline-treated rats. Filled symbols in these two graphs show values significantly larger than baseline.

repeatedly performed under three tested conditions. Following repeated administration via intraperitoneal catheter (top), both movement activity and temperature slightly increased (movement more so than temperature) from Day 1 to Day 2 but then stabilized and remained very similar following subsequent drug administrations. ANOVA with repeated measures confirmed no significant effect of repeated treatment on either movement [$F(5,29) = 0.61$; $P = .67$] or VTA temperature change [$F(5,29) = 0.54$; $P = .72$].

In contrast, there were day-to-day changes in movement and VTA temperatures following two other conditions of drug administration (Fig. 7, middle and bottom graphs). In both cases, movement activation (analyzed as cocaine–saline difference) was evident after the first cocaine administration, became larger with repeated administrations, but was completely absent when saline was substituted for cocaine on Day 6. The increase was gradual and consistent in activated conditions but less evident in quiet conditions. Changes in temperature were more complex. Under both conditions of drug administration, the biphasic pattern of temperature change was evident in each treatment day and the temperature response was completely abolished on Day 6. Under activated conditions (bottom graphs), the initial inhibition was maximal and subsequent temperature increase was minimal on Day 1. Following subsequent cocaine administrations, the initial decrease became weaker and subsequent increase became stronger with the largest hyperthermia on Day 5. Although less evident, the same pattern was seen following repeated cocaine injection under quiet conditions.

Fig. 8 shows differences in movement activation and VTA temperature change associated with cocaine administration under the three tested conditions. These data represent a mean movement activity and relative temperature change (for eighteen 10-min values) analyzed as cocaine–saline difference for each condition and each treatment day. As can be seen, movement activation and VTA temperature increase were maximal and relatively stable following each repeated cocaine administration via catheter (a weak increase in movement, however, occurred between Days 1 and 5). In contrast, both these parameters gradually grew following repeated cocaine administration under activated conditions. On Day 1, movement activation was very weak and mean temperature response was negative; however, they significantly increased on Day 2 and additionally grew on subsequent days. Both movement and VTA showed no change after saline injection on Day 6. Despite this day-to-day increase, both values on the last treatment day were similar to those after the initial cocaine administration via catheter (hatched lines on the graphs). In fact, movement activation induced by catheter-delivered cocaine on Day 2 (361.6 ± 69.13 counts/10 min) exceeds the levels of move-

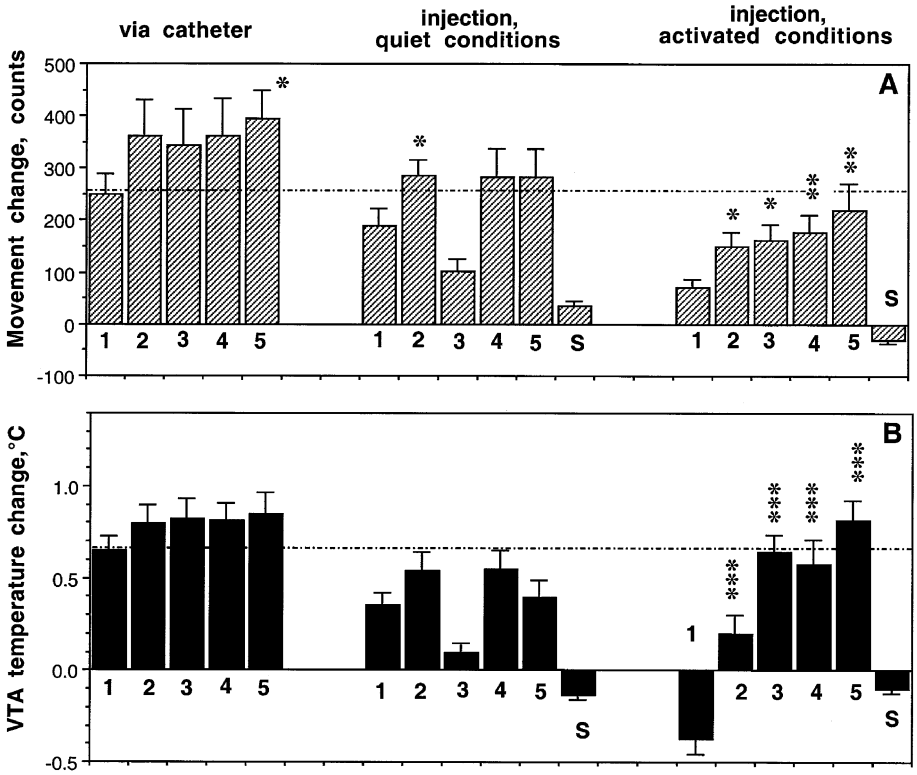


Fig. 8. Changes in movement and VTA temperature (the mean ± S.E.M. of eighteen 10-min values shown in Fig. 7, i.e., for 180 min postcocaine) associated with each cocaine administration in three conditions. S is saline administration on Day 6. Asterisks indicate values significantly different from Day 1 ($P < .05$, $P < .01$ and $P < .001$; Student's $t$ test). Horizontal hatched line shows levels of movement activation and VTA temperature change after the first cocaine administration via catheter.

ment activation evoked by the injected cocaine on Day 5 in either quiet (284.7 ± 52.6) or activated (221.3 ± 49.2) conditions. Movement response to cocaine injected in the quiet conditions was most variable, showing an increase on Day 2 vs. Day 1 ($P < .05$), decrease on Day 3, increase again on Days 4 and 5, and no change when saline was used instead of cocaine on Day 6 (A). Changes in VTA temperature were equally variable and they generally paralleled changes in movement activity.

## 4. Discussion

The primary goal of this study was to identify the neural effects of cocaine under different conditions of repeated administration and to establish the relationships between these neural effects and associated locomotor activation. While locomotion is a traditional measure of cocaine-induced behavioral activation, we supplemented this measure with brain temperature monitoring, a technique which allows assessment of both the activity state of an animal and a direct action of cocaine in the brain. Using this approach, it was also possible to assess the relationships between the animals' neural activity state at the time of drug administration and subsequent drug effects; this assessment is crucial for studying the state dependency of cocaine's action. Our goal, therefore, was twofold. First, we wanted to characterize how the neural action of cocaine depends on conditions of its administration. Second, we wanted to determine how the effects of cocaine change during repeated administration under different conditions.

Along with the use of physiological and behavioral parameters and a careful comparison of drug–saline pairs, our experimental design allowed us to examine the "pure" pharmacological action of cocaine. When given via chronic intraperitoneal catheter to rats well habituated to their environment and in a quiet, sleeplike state, the influences of handling and needle prick, strong arousing stimuli, and of a resting activity state were eliminated. This procedure also eliminated any possible visual and auditory stimuli, which can act during repeated administration as drug-related cues.

### 4.1. State-dependent action of cocaine and its mechanisms

Although cocaine was administered at the same dose and route, obviously resulting in similar pharmacokinetic changes, its effects on brain temperature and movement were drastically different in each of three tested conditions. Unexpectedly, cocaine-induced brain hyperthermia and motor activation were maximal when the drug was administered via intraperitoneal catheter to well-habituated animals under quiet resting conditions (Fig. 3). While NAcc and VTA showed similar dynamics, temperature increases in both brain structures had shorter latencies and were stronger than those of the muscle, suggesting brain activation as a primary cause of brain hyperthermia and a factor triggering more delayed and weaker body hyperthermia. Both temperature and movement activity increased, but their changes did not correlate, suggesting that different mechanisms are involved in their mediation. While the rate of temperature elevation was more gradual than locomotion, peaking at ~ 80 min after cocaine infusion, NAcc- and VTA-muscle temperature differentials, a true measure of brain activation (Kiyatkin et al., 2002), sharply increased at 5 min (i.e., 0–10 min) and remained at an elevated plateau for ~ 60 min postcocaine. In contrast, movement activation began later and peaked at ~ 30 min after drug administration. Cocaine-induced neural activation, therefore, is the force that determines movement activation.

When cocaine was injected during either activated or quiet conditions, cocaine-induced motor stimulation, evaluated as cocaine–saline difference, was lower and brain temperature changes were different from those induced by the catheter-administered drug. In contrast to the monophasic and relatively strong ( ~ 1.3 °C) temperature increase induced by the catheter-administered drug, cocaine's action in these cases was clearly biphasic (inhibition followed by activation) and drug-induced hyperthermia was weaker ( ~ 0.7 °C). While these data indicate that the conditions of cocaine administration determine the pattern of its effects, they also suggest that cocaine used under conditions of associated neural activation is able to inhibit this activation. This inhibiting action of cocaine was evident for the first 20 min and was stronger when the drug was injected in activated conditions, which were associated with greater and longer brain temperature increases ( ~ 1.8 °C for >120 min) than those induced by the injection procedure ( ~ 0.5 °C for 30 min). Because of this initial inhibiting action, the total temperature increase induced by cocaine under both these conditions was smaller than that induced by the drug administered via catheter. Despite varying magnitudes, temperature elevations peaked at the same time (80–90 min) in all three tested conditions. Importantly, the biphasic pattern of cocaine's action was clearly evident at the level of temperature, a biphasically fluctuating physiological parameter, but it was not evident for motor stimulation, which is quantified only in one direction. Nevertheless, drug-induced motor activation, evaluated as cocaine–saline difference, was smaller when the drug was injected under quiet and especially activated conditions than after its infusion during quiet resting conditions. Therefore, cocaine has a clear state-dependent pattern of action; its central and behavioral effects are different at different activity states of an organism, and these effects are different from a simple summation of the "pure" effects of cocaine and state.

Although surprising, the state dependence of cocaine's central action seen in dynamics of brain temperature is supported by numerous data. Acute administration of cocaine in drug-naive animals, for example, increased regional cerebral metabolic rate for glucose (London et al., 1986) and cerebral blood flow (Howell et al., 2002), while in experienced drug users expecting drug administration, cocaine at a

dose producing euphoria reduced glucose utilization (London et al., 1990) and decreased cerebral blood flow (Pearlson et al., 1993; Wallace et al., 1996). Cocaine is known to induce EEG desynchronization, suggesting widespread neural activation, but only when injected during synchronization (Yabase et al., 1990). Finally, in our previous studies, we found that the first in-session intravenous cocaine self-administration induced a rapid temperature increase, but all subsequent self-administrations were accompanied by biphasic (decrease followed by increase) temperature fluctuations (Kiyatkin and Brown, 2003).

The state-dependent action of cocaine seen in our experiments is also in accordance with the drug's known neuromodulatory action on neural functions, particularly its interaction with monoamine transporters and $Na^+$ channels (local anesthetic action). While cocaine in vitro has no effect on dopamine (DA) release, it effectively increases DA concentrations by potentiation of drug-independent DA release (Hekkila et al., 1975). Acting via $Na^+$ channel inhibition, cocaine has weak effects on endogenous pacemaker activity of neural cells maintained via activation of $Ca^{++}$ and $K^+$ conductancies (for example, on DA cells in vitro; Brodie and Dunwiddie, 1990; Johnson and North, 1992), but the action is much stronger when spontaneous or evoked activity is maintained with participation of $Na^+$ channels. Iontophoretic cocaine, for example, is highly effective in inhibiting striatal neurons and these effects are resistant to DA receptor blockade (Kiyatkin and Rebec, 2000). On these cells, the effects of iontophoretic cocaine greatly mimic those of procaine; the inhibiting action of both drugs is directly related to spontaneous activity rate, and is stronger on glutamate-induced high-rate activity than on low-rate spontaneous activity (Kiyatkin and Rebec, 2000). Clearly, further analytical studies are necessary to clarify the neural mechanisms underlying the state-dependent action of cocaine and its ability to strongly inhibit associated neural activation.

### *4.2. State dependency of changes in motor-activating effects of cocaine following repeated administration*

In sharp contrast to continuous cocaine infusion that results in rapid tolerance of its locomotor effects (King et al., 1994; Reith et al., 1987), repeated intermittent cocaine administration may result in reversed tolerance or sensitization of these effects (Kalivas et al., 1988; Post and Rose, 1976; Robinson, 1988). While the source and the underlying mechanisms of this phenomenon are under discussion, a large body of evidence suggests the importance of environmental factors in development of "sensitized" behavioral effects of cocaine (Badiani et al., 1995; Browman et al., 1998; Crombag et al., 2000; Post et al., 1987; Weiss et al., 1989).

Our data indicate that the activity state of an individual is not only a crucial factor determining the behavioral effects of cocaine, but is also a factor determining the changes in these behavioral effects following repeated drug administration. While cocaine-induced motor stimulation was generally stable following repeated drug administration via catheter, it was gradually enhanced following drug treatment in activated conditions. The enhanced movement effect of cocaine seen on Day 5, however, was not greater than that seen after the initial unstressed and unsignalled cocaine administration via catheter. Thus, an augmented motor response to cocaine repeatedly used under conditions of activation is not a "locomotor enhancement" per se, but rather the result of a gradual habituation to the conditions associated with repeated drug administration. Both locomotor activation and brain temperature increase consistently changed with repeated saline injections made upon placement in the cage, and the cocaine–saline difference in both parameters became larger with repeated tests. In parallel with brain temperature habituation seen in control animals following repeated placement in the cage, the initial inhibiting action of cocaine on temperature became shorter and weaker with repeated administration, while the subsequent hyperthermia became stronger and more prolonged (see Fig. 7). Such an explanation of behavioral sensitization as the result of habituation in control and blocked habituation in cocaine-treated animals is consistent with previous work (Damianopoulos and Carey, 1992; Gold et al., 1988).

Although a similar pattern occurred in rats repeatedly injected with cocaine and saline in quiet conditions, cocaine–saline difference in movement and temperature remained relatively stable at levels lower than those seen after cocaine administration via catheter. This parallels a relative stability of movement activation and phasic hyperthermia consistently seen during each repeated injection in control animals. Cocaine-induced movement activation in this case, however, had clearly shorter onset latencies and was sharper than that seen after the initial drug administration. This change in the pattern of motor activation can be viewed as further evidence of a sensitized motor response (Carey and Gui, 1998; Yen and Haertzen, 1991). The change in the behavioral effects of cocaine is therefore not a simple change in cocaine's effect on an unchanging organism, but the result of consistent change in the organism's state following repeated administration.

### *4.3. Conditioning and lack of conditioning*

Like natural reinforcers, which after pairing with specific sensory stimuli may change the activating effects of those stimuli (Pavlov, 1927), conditioned responses can be induced by sensory stimuli paired with cocaine. It is hypothesized that conditioning may significantly contribute to cocaine-induced behavioral sensitization (Stewart and Eikelboom, 1987; Stewart, 1987). The present study, however, produces no evidence of conditioned locomotion or conditioned hyperthermia for either condition of cocaine injection. Movement activation and brain temperature increase induced by saline injection after five previous cocaine

injections in both activated and quiet conditions did not differ from those induced by a sixth saline injection in control animals.

The nature of the associated factors used in our study (procedure of injection and placement in the cage) may determine this lack of conditioning. If a sensory stimulus is paired with cocaine, its repeated presentation alone results in progressive habituation of its activating effects, as shown with movement (Damianopoulos and Carey, 1992; Gold et al., 1988) and temperature recordings (Kiyatkin et al., 2002). In contrast, habituation to the activating effect of repeated stimulus presentation is blocked when it is repeatedly paired with cocaine. Because of these differential changes in cocaine- and saline-treated animals, a sensory stimulus previously paired with cocaine (conditioned stimulus) may induce a stronger activation (i.e., conditioned response) than that induced by the stimulus repeatedly used alone. In the present experiment, repeated saline injections and repeated placement in the cage did not result in the clear habituation of motor stimulation and brain temperature response. Thus, it is unrealistic to expect a greater response to these two "stimuli" presented alone after their repeated pairing with cocaine. Recent discussions on the growth of incentive motivation (Robinson and Berridge, 2001) and reward anticipation (Schultz, 1997; Schultz and Dickinson, 2000) might lead one to expect stronger effects of the arousing sensory stimuli and the related temperature and movement response as this cue became associated with cocaine administration. This view, however, appears to be inconsistent with Pavlov's (1927) teachings, which suggest that the initial response to the sensory stimulus (to-be-cue) during pairing with the reinforcer fails to undergo normal habituation, rather than increasing with training. When a compound sensory stimulus (light + sound) was paired with intravenous cocaine injections, this stimulus presented alone induced conditioned locomotion and conditioned hyperthermia (Kiyatkin and Brown, 2004). These effects, however, were not stronger than those induced by the initial presentation of this stimulus before drug association.

### 4.4. Conclusions

Our results suggest that both the acute behavioral effects of cocaine and the emerging patterns of their change following repeated drug administration are greatly dependent on the ongoing neural activity state. Although cocaine may induce robust neural activation, it also has an ability to transiently inhibit state- and activity-dependent (i.e., drug-independent) neural activation. While further analytical studies are necessary to investigate neural mechanisms underlying state dependency of cocaine's central action, drug–state interaction appears to be crucial for changes in the behavioral effects of cocaine during its repeated administration, particularly for the development of state-dependent motor sensitization. This drug–state interaction may be also important for cocaine-induced learning and the development of drug-taking behavior.

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