Published in final edited form as:

Brain Res. 2008 September 4; 1228: 145–160. doi:10.1016/j.brainres.2008.06.069.

Behavioral and temperature effects of delta 9-tetrahydrocannabinol in human-relevant doses in rats

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Abstract

Marijuana smoking dramatically alters responses to various environmental stimuli. To study this phenomenon, we assessed how delta-9-tetrahydrocannabinol (THC), a primary psychoactive ingredient of marijuana, affects locomotor and brain (nucleus accumbens or NAcc), muscle and skin temperature responses to natural arousing stimuli (one-min tail-pinch and one-min social interaction with another male rat) and iv cocaine (1 mg/kg) in male rats. THC was administered at three widely varying doses (0.5, 2.0 and 8.0 mg/kg, ip), and the drug-induced changes in basal values and responses to stimuli were compared to those occurring following ip vehicle injections (control). Each stimulus in control conditions caused acute locomotor activation, a prolonged increase in brain and muscle temperature (0.6–1.0°C for 20–50 min) and transient decrease in skin temperature (–0.6°C for 1–3 min). While THC at any dose had a tendency to decrease spontaneous locomotion as well as brain and muscle temperatures, true hypothermia and hypoactivity as well as clearly diminished locomotor and temperature responses to all stimuli were only seen following the largest dose. In this case, temperature decreases in the NAcc were stronger than in the muscle, suggesting metabolic brain inhibition as the primary cause of hypoactivity, hypothermia and hyporesponsiveness. While weaker in strength and without associated vasodilatation, this response pattern is mimicked by general anesthetics, questioning to what extent the hypothermic action of THC is specific (i.e., mediated via endogenous cannabinoid receptors) or non-specific, reflecting drug interaction with membrane lipids or other receptors. In contrast, weaker behavioral and temperature effects of THC at lower doses resemble those of diazepam, whose locomotion- and temperature-decreasing effects are evident only in activated conditions, when rats are moving and basal temperatures are elevated.

Keywords

brain metabolism; brain and body temperature; cannabinoids natural arousing stimuli; intravenous cocaine; vasoconstriction

1. Introduction

The cannabis plant, commonly known as marijuana, has been used over many centuries for medicinal, religious, and recreational purposes. The primary psychoactive ingredient in marijuana is Δ^9 -tetrahydrocannabinol (THC), which in humans induces mild euphoria,

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relaxation and profound changes in perception (Dewey, 1986; Martin, 1986), all obviously contributing to its abuse potential. Similar to anxiolytics, marijuana and THC are also able to attenuate the subjective, generally negative emotional states associated with various arousing and stressful stimuli; this stress-attenuating effect could be another possible contributor to its abuse. Even though some animal studies have shown a conditioned place preference for THC (Lepore et al., 1995; Tanda et al., 2000) and its self-administration (Bairda et al., 2004; Panlilio et al., 2007), there is little evidence of its potential to induce physical dependence. Along with its psycho-emotional effects, THC induces numerous physiological effects such as analgesia, hypoactivity, hypotension, and hypothermia (Malone and Taylor, 1998; Varvel et al., 2005).

While the behavioral, physiological and psychoemotional effects of THC are generally attributed to its binding to centrally located CB1 receptors (Lake et al., 1997; Compton et al., 1996), THC has been known to interact with other receptors (Hejazi et al., 2006; Oz, 2006; Tiburu et al., 2007) and lipid layers of neuronal membranes, thus indirectly affecting other neuroreceptors. Therefore, some of the effects of THC, especially at high doses, could be non-specific or mediated via other neurochemical systems (Athanasίου et al., 2007). This issue could be especially important for correlating human and animal studies. While humans usually receive THC through either smoke inhalation or the ingestion of THC-infused oils, in animal studies this drug is administered intravenously (iv) or intraperitoneally (ip) and usually at much higher doses. Humans have reported feeling the effects of THC for up to 8 hours (peak at 2–4 hours) after a dose of 0.1 and 0.2 mg/kg even though plasma levels drop off 2 hours after the injection (Curran et al., 2002). Rats, on the other hand, are usually given much higher doses of the drug to allow for the observation of clear physiological and behavioral effects. For example, hypothermia in rats was evident at 2–5 mg/kg iv THC dose (Malone and Taylor, 1998) and the animals have been able to distinguish THC from vehicle at 3 mg/kg ip dose (Wiley et al., 1995).

Although it is known that THC causes acute, dose-dependent body hypothermia in animals (Hardman et al., 1971; Holtzman et al., 1969; Malone and Taylor, 1998), the mechanisms underlying this effect and its relevance for human conditions remain unclear. Since the body temperature balance is determined by two variables, heat production and heat dissipation, and THC decreases oxygen consumption (Athanasίου et al., 2007; Fitton and Pertwee, 1982) without evident effects on vascular tone (O'Sullivan et al., 2006), metabolic inhibition with diminished heat production, but not increased heat loss, appears to be the primary mechanism underlying body hypothermia. Although it is shown that the inhibiting action of THC on metabolism and body temperature in animals occurs via its action on centrally located substrates (Fitton and Pertwee, 1982), presumably CB1 receptors (Campton et al., 1996), it is unclear whether or not these effects could be induced by THC at much lower, human-relevant doses. The specificity of these effects could be questioned since both serotonergic and dopaminergic (via D2 receptors) mechanisms have been implicated as their crucial mediators (Davies and Graham, 1980; Nava et al., 2000).

Since brain temperature is a sensitive index of brain metabolism, showing consistent fluctuations in response to various arousing and stressful stimuli as well as psychoactive drugs (see Kiyatkin, 2005 for review), in this study we examined how THC affects locomotor activity along with brain, muscle and skin temperatures as well as behavioral and temperature responses to various arousing stimuli in male rats. We used both natural somatosensory stimuli (procedure of ip injection, tail-pinch, social interaction with another male) and iv cocaine (as a representative stimulant drug which is often used together with marijuana). THC was administered via an ip injection at a wide range of doses (0.5, 2.0 and 8.0 mg/kg), varying from relatively low (corresponding to effective human doses) to high, typically used in pharmacological studies. To represent brain temperature we chose the ventral striatum (nucleus accumbens or NAcc), a deep brain structure implicated in sensory-motor integration,

behavioral regulation and mediating the reinforcing effects of addictive drugs (Wise and Bozarth, 1987). While the brain hyperthermic response primarily reflects alterations in metabolic neural activity, fluctuations in skin temperature provide a valuable measure of peripheral vasoconstriction—another sensitive, centrally mediated response to arousing stimuli and psychoactive drugs (Baker et al., 1976). The temporal muscle is a non-locomotor head muscle that receives the same blood supply as the brain (via the carotid artery), thus allowing evaluation of the contribution of arterial blood supply to alterations in brain temperature. Simultaneous temperature monitoring from different locations, moreover, allows one to investigate the direction of heat flow within the organism, while additional recording of locomotion provides a measure of behavioral activation to correlate with temperature responses.

2. Results

2.1. Data sample

Each rat used in this study was exposed to four stimuli (ip injection, tail-pinch, social interaction, iv cocaine injection) during six daily sessions; three times with THC and three times with vehicle. Since basal temperatures and spontaneous locomotion show consistent changes during repeated sessions and within each individual session, we first analyzed behavioral and temperature responses in control conditions, after vehicle administration. Second, we analyzed changes in basal temperatures and locomotion induced by THC at different doses. These values were obtained for 6 min preceding each stimulus presentation. In each case, these effects were compared with those occurring after vehicle administration on the same days. Finally, we examined the effects of THC in each dose on changes in temperatures and locomotion induced by tail-pinch, social interaction and iv cocaine. These effects were also compared to those occurring after vehicle administration in the same rats and same sessions. Therefore, each dose of THC had its own unique control. While all animals were exposed to the same protocol, the quantity of tests in each data group is lower than maximally possible ($n=36$ for vehicle and $n=12$ for each drug dose/corresponding vehicle) due to missing data, failed recordings and failed iv catheters.

2.2. Basal temperatures and responses to natural arousing stimuli and iv cocaine in drug-naive conditions (control)

Figure 1 shows mean changes in locomotion and temperatures in each recording location induced by each of the four stimuli in control conditions. Despite the different nature of each stimulus, they all induce a similar pattern of locomotor and temperature responses. In each case, there was a locomotor activation (D), an increase in brain and muscle temperatures, and a transient decrease in skin temperature (A and B). With each stimulus, temperature increases in the NAcc were more rapid and of a higher amplitude than in the muscle, resulting in a significant increase in the NAcc-muscle temperature differential within several minutes after the stimulus onset (C). Opposite changes in skin and muscle temperatures resulted in strong decreases in skin-muscle differentials, which slowly returned to baseline within 20–30 min (C). While changes in brain and muscle temperatures occurred with the longest latencies (2–3 and 3–5 min, respectively) and were prolonged (20–40 min), locomotor activation and decrease in skin temperature occurred rapidly (within the time of stimulation) and were shorter in duration. As shown in Fig. 1A, there were consistent differences in basal temperatures at each recording location, with the highest values in NAcc (range 35.45–38.49°C, mean 36.63 ± 0.07, SD 0.62°C), lower values in the muscle (range 34.43–37.45, mean 35.68 ± 0.08, SD 0.68°C; $p < 0.05$ vs. NAcc), and lowest values in the skin (range 33.04–36.81°C, mean 34.79 ± 0.07, SD 0.60°C; $p < 0.05$ vs. NAcc and muscle).

As shown in Fig. 2A, basal NAcc and muscle temperatures correlated strongly, directly and linearly ($r=0.92$, $p<0.001$). Temperatures in the NAcc were consistently lower than in the muscle, and the regression line was parallel to the line of equality. Therefore, if basal temperatures in the NAcc are higher, they are also higher in the muscle and *vice versa*. Basal skin temperature was also directly related to basal NAcc temperatures (B), but the correlation was weaker ($r=0.57$, $p<0.05$). The difference was larger at high temperatures and lower at low temperatures, but was evident within the entire range of statistical variability ($\text{mean} \pm 3 \text{ SD}$). Muscle and skin temperatures also significantly correlated in the baseline (see C; $r=0.56$, $p<0.05$) and their difference was also greater at high temperatures. In this case, however, the regression line crossed the line of equality exactly at the lower border of statistical variability for muscle temperature ($\text{mean}-3\text{SD}$). Therefore, at very low temperatures ($\sim 33.7^\circ\text{C}$), skin and muscle temperatures will theoretically become equal.

Brain temperature increases induced by sensory stimuli and cocaine were highly variable among different animals and tests (Fig. 3) and were dependent upon baseline brain temperatures ($r = -0.43$, -0.49 , -0.40 and -0.54 for ip vehicle injection, tail-pinch, social interaction and cocaine, respectively). When NAcc basal temperatures were lower, their elevation induced by each stimulus was larger. In each case, regression lines approached the line of no effect at ~ 39 – 39.5°C , suggesting that the hyperthermic response should disappear at these high basal brain temperatures.

2.3. Procedure of injection and influence of THC on basal locomotion and basal temperatures

Figure 4 shows changes in temperature associated with administration of vehicle and THC at different doses analyzed at high temporal resolution. Figure 5 shows changes in spontaneous locomotion and temperatures in each recording location after THC administration at different doses evaluated at 60, 120 and 180 min post-injection. Since different doses were tested in different days, they were always compared with respective vehicle controls obtained on the same days. As can be seen in Fig. 4, ip injection of THC at 0.5 mg/kg did not differ from its respective control in each tested parameter. In each case, brain and muscle temperature transiently increased immediately after the injection and slowly decreased below baseline from ~ 20 min post-injection. Skin temperature acutely decreased in both cases but never restored to baseline levels. As shown in Fig. 5, basal temperatures decreased during the session in both drug and vehicle groups, but this decrease was somewhat stronger for each location and each time point in rats that received THC. The difference was significant for both NAcc and muscle at 60 min post-injection.

Similar and somewhat stronger differences were seen with THC at 2 mg/kg as compared to 0.5 mg/kg (Fig. 4 and 5). While brain and muscle temperatures increased and skin temperature decreased after administration of either THC or vehicle, the initial increase was weaker and the subsequent decrease stronger in the drug-treated group (see Fig. 4). Although the immediate decrease in skin temperature was similar in both groups, it was slightly more visible in THC-treated conditions. Even though locomotion and temperatures in each location decreased in the corresponding vehicle groups, the decrease was always stronger in drug-treated conditions. Similarly to the 0.5 mg/kg dose, the difference was maximal and significant at 60 min post-injection (Fig. 5).

Strong and significant decreases in temperature were seen following THC at the highest dose (Fig. 4 and 5). In this case, between-group differences were seen within several minutes following the injection, were maximal at 60 min, but still evident at other times during the three-hour period of analysis. In contrast to the clearly hyperthermic response to the injection procedure in control conditions, all temperature robustly decreased in the THC group. Despite somewhat parallel changes in each recording location, temperature decrease in the NAcc was significantly stronger than in the muscle from ~ 20 min post-injection, and the decrease in skin-

muscle differential was stronger and more prolonged than in the control (see Fig. 4). Importantly, changes occurring after THC administration at 8.0 mg/kg were highly variable with both strong and weak responses in individual animals. Because of this, mean values in this group had high standard errors and between-group differences were significant only at 60 min post-injection. In contrast to the lower doses, rats were hypoactive and temperature differences maintained up to 180 min post-injection (see Fig. 5).

2.4. Changes in locomotor and temperature responses to arousing stimuli and iv cocaine after THC treatment

To evaluate the effects of THC on responsiveness, we compared temperature and locomotor responses to each of four stimuli in three pairs of groups (THC 0.5 mg/kg-vehicle; THC 2.0 mg/kg-vehicle and THC 8.0 mg/kg-vehicle). These data are shown both in tables, which report mean pre-stimulus values along with absolute and relative changes, and in figures, which show the time course of relative temperature changes and the time-course of locomotion after drug/vehicle treatment.

As shown in Table 1 and Fig. 6, changes in temperature and locomotion induced by all stimuli were virtually identical after THC treatment at 0.5 mg/kg and its respective control. While between-group differences were absent in relative temperature changes and locomotor response to each of three stimuli (Fig. 6), absolute increases in NAcc and muscle temperatures during social interaction were smaller in the THC group due to lower baselines (Table 1). This effect was also evident for tail-pinch but changes did not reach the level of statistical significance. The effects of cocaine was virtually identical in both conditions.

As shown in Table 2 and Fig. 7, THC at 2 mg/kg induced a similar effect, but changes were somewhat stronger. In this case, both the changes in locomotion and NAcc temperature increases induced by each stimulus were weaker than in the control. This effect, however, also failed to reach a level of statistical significance. However, due to lower baseline temperatures in the NAcc, absolute increases induced by both tail-pinch and social interaction were significantly lower than in control conditions.

As shown in Table 3 and Fig. 8, a significantly weaker locomotor activation and lower increases in NAcc and muscle temperatures occurred in rats following THC at 8.0 mg/kg. The difference was absent for skin temperature, which showed even slightly stronger responses in the THC group. Since THC also induced significant decreases in baseline, absolute temperature increases were also significantly lower than in the control for tail-pinch and social interaction. Similar to the baseline (see above), high variability in temperature response was seen after THC 8.0 mg/kg. Because of high standard errors, between-group differences were not as pronounced and not significant for cocaine despite large differences in mean values. Importantly, although rats were clearly hypoactive after THC at 8.0 mg/kg, they showed a similarly rapid and strong locomotor activation following stimulus presentation. This locomotion, however, was shorter in duration than in control and rats became hypoactive again from ~10 min after presentation of sensory stimuli. While similar in pattern, the effect was weaker for cocaine.

Using visual observation, it was difficult to notice any differences in the animal activity following 0.5 and 2.0 mg/kg doses of THC, but a decreased motility and hyporesponsiveness to stimuli were typically evident with 8.0 mg/kg dose. When a tail-pinch occurred, vehicle-injected rats usually immediately began chewing vigorously on the clothespin. As doses of THC were increased, it often took the rats longer to react to the clothespin, and at 8 mg/kg, chewing was almost always replaced by docile squirming. The highest dose of THC caused the rats to be much less interested in the introduced companion during social interaction, typically showing less sniffing behavior than usual.

3. Discussion

Cannabis smoking appears to be the most relevant method of THC delivery to its centrally located receptor sites in order to induce desired psycho-emotional effects. The situation, however, becomes more complex when the effects of THC are studied in animals. For effective dose control, the drug is administered through an iv or ip injection, altering the drug's pharmacokinetic properties typical of inhalation. Since THC is hydrophobic, it should be delivered from either oil- or ethyl alcohol-containing stock solutions with the addition of other substances as solvents. These other substances (i.e., Tween) act on various ionic channels (Oz et al., 2004) and might induce additional physiological and behavioral effects by acting on the terminals of sensory nerves abundantly innervating both the abdominal cavity (ip) and peripheral vessels (iv). In our pilot studies we observed that Tween-containing solvent (which is used to dissolve both THC and Ribonabant) induces behavioral effects and temperature changes when injected via a chronically implanted ip catheter. This makes additional control tests with vehicle injection critically important. In addition, THC is a highly oxidizable substance and could lose its biological activity within 7–8 days, even in a cold (+4°C) and dark environment (preliminary studies in this work). This factor could contribute, at least to some extent, to the well-known tolerance of various physiological effects of THC described in animals (Uran, 1980) but not evident in humans with marijuana smoking. Finally, drug action in humans is typically defined by its subjective effects, but in animals it relies exclusively on alterations in physiological and behavioral parameters. This principal difference could explain why the effective doses of psychoactive drugs in humans are usually much lower than those in animals. Higher metabolism in rats could also be a contributor, but this factor alone fails to explain the magnitude of dose increase. We tried to resolve all these methodological issues, but several important questions regarding brain and body temperature, its physiological fluctuations, and relations with activity state should all be considered to interpret the effects of THC seen in this study.

3.1. Brain temperature as a sensitive index of brain metabolic activity

The present study confirms and extends our previous observations, which suggest that various environmental challenges and iv cocaine induce brain and body hyperthermia. Although tail-pinch, social interaction and the procedure of ip injection are different stimuli, they all induce a rapid increase in NAcc and muscle temperatures (0.6–0.8°C) along with locomotor activation. These changes all greatly exceed the duration of stimulation but had varying time-courses (see Fig. 1). Interestingly, iv cocaine, which acts on different neural substrates both in the brain and periphery, induced a similar pattern of its behavioral and temperature effects (but with a slightly stronger locomotion and larger temperature changes) as seen with somatosensory arousing stimuli.

Although basal temperatures in the temporal muscle were consistently lower than those in the NAcc, changes in both locations generally paralleled each other with each stimulus presentation (Fig. 1). These two temperatures, moreover, strongly positively correlated at the baseline ($r=0.92$), being equally different within the entire range of statistical variability (Fig. 2). While brain temperature fluctuations depend primarily on metabolic neural activity (see Kiyatkin 2005 for review), *musculus temporalis* is a non-locomotor muscle and its temperature depends upon two simultaneously acting but opposing factors: heat inflow by arterial blood and change in vessel tone. The increase in arterial blood temperature tends to increase muscle temperature, but vasoconstriction has the opposite effect, tending to decrease muscle temperature. There are, however, important differences in the time-course of temperature changes between the brain and muscle seen when the recordings are performed with high temporal resolution. With each stimulation, temperature increases in the NAcc are always more rapid and stronger than in muscle, resulting in a significant increase in NAcc-muscle

temperature differentials during the first 4–6 min after stimulus onset (Fig. 1C). This relative change correlates more tightly with locomotor activation. This difference appears to be important, suggesting metabolic brain activation as a primary cause of intra-brain heat production and a factor that determines, via activation of effector mechanisms, subsequent body hyperthermia.

Each stimulus also induced a rapid and robust decrease in skin temperature, suggesting acute vasoconstriction (Baker et al. 1976). This effect was always evident within the first 20–30 s after stimulus onset, resulting in a significant temperature fall during the first minute (Fig. 1C). In contrast to slower and more prolonged increases in brain and muscle temperature, this effect was brief, peaking at the first 1–2 min, and followed by a rebound-like increase in skin temperature. While acute 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), it also suggests diminished heat dissipation as a common feature of hyperthermic responses induced by arousing stimuli. This diminished heat dissipation was especially evident in skin-muscle differentials, which robustly decreased following each stimulus presentation and slowly returned to baseline at 20–30 min. A further increase in skin temperature points to a post-stimulation increase in heat dissipation. In light of the known vasoconstrictive action of cocaine (Gillis et al. 1995; Williams and Wasserberger 2006), it is surprising that acute decrease in skin temperature following its iv administration was quantitatively similar (but more prolonged) to that occurring with natural arousing stimuli. In contrast to the very tight correlation between basal brain and muscle temperatures, such a correlation was much weaker for skin temperature (Fig. 2B and Fig. 2C). Although skin temperatures were also directly related to NAcc and muscle temperatures at basal conditions, differences were larger at high temperatures and became smaller at lower temperatures. Interestingly, muscle-skin temperature difference disappeared at very low values ($\sim 33.5^{\circ}\text{C}$), which represented the lower limits of statistical variability (mean-3SD). Skin also showed an initial opposite correlation with brain and body temperatures following stimulation, and inversely mirrored locomotor activation, which also peaked within the first 1–3 min after the stimulus presentation.

Consistent with our previous studies (see Kiyatkin, 2005 for review), the magnitude of brain temperature elevation with each stimulus widely varied and was dependent upon basal brain temperatures ($[r=(-)0.40-0.55]$ see Fig. 3). This correlation appears to be valid for any arousing stimuli, reflecting some basic relationships between basal activity state (basal arousal) and its changes induced by environmental stimuli. These observations may be viewed as examples of the *law of initial values*, which postulates that the magnitude and even direction of autonomic response to an «activating» stimulus is related to the pre-stimulus basal values (Wilder, 1958). These relationships between basal levels and the response had important implications for evaluating the effects of any drug, which could differ depending on the time of injection within a session and the session number. When the rat is carried from the animal facility and placed in an experimental box, its brain and body temperatures are about 2°C higher than the «quiet rest» levels which are established following several days of habituation and in several hours after cage transfer. To minimize the influence of these factors, our rats underwent at least 3 habituation sessions before any drug injection and it was performed at least two hours after the rat was placed in the chamber. However, there was a gradual decrease in basal NAcc and muscle temperatures ($\sim 0.5^{\circ}\text{C}$) within the session and, despite previous habituation, mean basal temperatures during the last treatment session were about 0.5°C lower than during the first treatment session. Therefore, the effects of THC were always compared with those of the vehicle observed on the same day.

3.2. Effects of THC on temperature and locomotion

At the lowest dose (0.5 mg/kg), THC has a tendency to decrease temperatures in each recording location without any evident effects on spontaneous locomotion as well as temperature and locomotor responses to all stimuli used. At the moderate dose (2 mg/kg), THC had a slightly stronger decreasing effect on basal temperatures and spontaneous locomotion while also slightly abating temperature responses induced by tail-pinch, social interaction and cocaine. The latter effect was evident only as the decreased absolute response magnitude, mainly due to the lower pre-stimulation baseline. Finally, THC at the highest dose (8 mg/kg) strongly decreased spontaneous locomotion, lowered basal temperatures in each location, and attenuated locomotor and temperature responses to all stimuli. In this case, both absolute and relative response magnitudes were lower than in the control. The effects of THC at any dose were highly variable, especially with the 8 mg/kg dose, with both robust decreases and weak changes seen in different animals. We cannot exclude the possibility that this high variability could be partially due to the injection procedure. THC injected into the peritoneal cavity must enter into the open space in order to diffuse appropriately and consistently into the animal. If some of the THC is accidentally injected elsewhere, such as abdominal cavity fat tissue, the hypothermic effects will most likely be diminished. We could only speculate on whether an injection was always successful, and therefore could not eliminate data that we suspected to be erroneous.

3.2.1. Diazepam-like effects of THC at low doses—Although body hypothermia is known to be one of the primary physiological effects of THC, based on our data it is difficult to interpret a weak negative temperature difference between THC (0.5 and 2.0 mg/kg) and vehicle-treated conditions as true *hypothermia*, i.e., a temperature decrease below baseline. Although both NAcc and muscle temperatures were significantly lower than the starting baseline for most points following THC administration, they also decreased within a session in the vehicle group, thus minimizing the between-group differences (Fig. 5). This effect was also mimicked by locomotion, which at 2.0 mg/kg was consistently lower than in control, but, again, well within “normal” spontaneous activity in control habituated animals. Although THC at 0.5 mg/kg did not have significant effects on temperature and locomotor responses to stimuli, absolute increases in brain and muscle temperatures were lower than in control, especially during social interaction, due to lower pre-stimulation baselines. This pattern was strengthened with THC at 2 mg/kg, which decreased both temperature baselines and relative temperature increases, resulting in significantly lower absolute increases. Interestingly, diazepam at a low, human-relevant dose (1.0 mg/kg) had a similar pattern of action, slightly decreasing basal brain and muscle temperatures and slightly attenuating temperature and locomotor responses to natural arousing stimuli and cocaine (Kiyatkin and Bae, 2008). Because of these two effects, absolute temperature increases induced by all arousing stimuli were diminished in rats treated with diazepam. Similarly to THC (0.5 and 2.0 mg/kg), diazepam induced neither hypothermia nor hypoactivity *per se*, despite significantly lower values with respect to control. Similarly to THC, this difference virtually disappeared when locomotion was low and basal temperatures stabilized at low levels in habituated conditions of quiet rest. Therefore, THC at lower doses appears to exert state-dependent, diazepam-like effects, decreasing activity levels when they are naturally increased. To verify this action, we tried to administer THC to highly habituated rats via a chronically implanted ip catheter (to exclude activating influence of the injection procedure). This experiment, however, failed since control rats showed locomotor and temperature effects after vehicle administration, suggesting that substances used to dissolve THC are able to act on abdominal sensory receptors. Since Rimonabant, a CB1 receptor antagonist, is dissolved in the same vehicle, this complication also prevented us from testing the receptor selectivity of THC-induced effects. This test, moreover, could be complex because THC at lower doses has weak effects, which are statistically insignificant when compared to proper controls.

Interestingly, THC treatment (0.5 and 2.0 mg/kg) had virtually no effects on skin hypothermic responses induced by all arousing stimuli (see Fig. 6 and Fig. 7), but decreased basal skin temperature relative to the control. Although the rapid, transient decrease in skin temperature following sensory stimulation results from acute vasoconstriction, and THC appears to have no effects on these adaptive vascular responses, a tonic decrease in basal skin temperature after THC could be related to lowering body core temperature and decreases in the temperature of arterial blood supply. On the other hand, lower body temperature could cause a compensatory increase in vascular tone in order to diminish heat dissipation to the external environment. Both these effects tend to decrease skin temperature.

3.2.2. Anesthetic-like effects of THC at large doses—In contrast to small and moderate doses, THC at 8.0 mg/kg strongly decreased temperatures in all locations to true hypothermic levels absent in normal conditions (see Fig. 4 and 5). Similar to smaller doses, the effect occurred rapidly (see Fig. 4) and peaked at 60 min, but between-group differences maintained up to 180 min post-injection (Fig. 5). While NAcc and muscle temperatures generally correlated, the decrease was stronger in the brain than the muscle, significantly decreasing the NAcc-muscle differential from ~20 min post-injection (see Fig. 4). This is an important change because it indicates decreased brain metabolism and diminished intra-brain heat production—effects previously reported with THC (Athanasίου et al., 2007; Fitton and Partwee, 1982). These features are typical to general anesthetics, which induce brain and body hyperthermia, with a stronger temperature decrease in brain than body sites (Kiyatkin and Brown, 2006). Despite a similar pattern, these effects were clearly milder than those induced by common anesthetics such as pentobarbital (50 mg/kg) and were associated with weaker behavioral inhibition and different changes in skin temperature. Although spontaneous locomotion was strongly diminished following THC at 8.0 mg/kg, rats showed some degree of basal activity within the entire session and clear locomotor responses to sensory stimuli and cocaine (see Fig. 8). Surprisingly, despite a visual hypo-response, the initial period of locomotor response was similar to that induced in vehicle-treated conditions, but the effect was clearly shorter in duration. In addition to decreased baselines, rats also showed clearly weaker elevations of brain and muscle temperatures following tail-pinch, social interaction and cocaine, resulting in drastically lower absolute temperature increases (~1–1.5°C) than in control tests (see Table 3). In contrast to the diminished responses in the NAcc and muscle, acute stimulus-induced decreases in skin temperature were less affected and had a tendency to be stronger than in the control. Although this change could suggest that THC, at a high dose, potentiates vasoconstriction induced by sensory stimuli, it could be related to lower basal skin temperatures. The existing data on the vasoactivity of THC are controversial, suggesting both vasoconstrictive and vasodilator effects on different arteries and bidirectional effects on the same vessels (Adams et al., 1976; Barbosa et al., 1981; O'Sullivan et al., 2006). Therefore, prolonged decrease in skin temperature could be a consequence of a body temperature decrease, while stronger stimulus-induced decreases in skin temperature could reflect an adaptive vascular response to diminish heat dissipation to the external environment (see Romanovsky, 2004 for review of related issues of temperature regulation). This feature of THC is in sharp contrast to general anesthetics, which induce relative vasoconstriction and increase heat dissipation to the external environment (Kiyatkin and Brown, 2006). Despite a decreased brain metabolism and diminished metabolic responses to arousing stimuli, THC even at large doses is unable to inhibit adaptive vascular responses—an important central mechanism in maintaining body temperature homeostasis.

4. Materials and Methods

4.1. Subjects

Fourteen Long-Evans male rats (Taconic, Germantown, NY), weighing 420–520 g and housed under a 12 h light cycle (lights on at 0700), with *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.

4.2. Surgery

All animals were implanted with three thermocouple electrodes as previously described (Kiyatkin and Brown 2005). Animals were anesthetized intraperitoneally (ip) with 3.3 ml/kg of Equithesin (sodium pentobarbital, 32.5 mg/kg and chloral hydrate, 145 mg/kg) and mounted in a stereotaxic apparatus. Four holes were drilled through the skull: three for securing screws and one for thermocouple insertion over the NAcc shell (1.2 mm anterior to bregma, 0.9 mm lateral to bregma) using the coordinates of Paxinos and Watson (1998). The *dura mater* was retracted and the thermocouple probe was slowly lowered to the desired target depth (7.4 mm, measured from the skull surface). A second thermocouple probe was implanted subcutaneously along the nasal ridge with the tip approximately 15 mm anterior to bregma. A third thermocouple probe was implanted in the deep temporal muscle (*musculus temporalis*). The probes were secured with dental cement to the three stainless steel screws threaded into the skull. During the same surgery session, 12 animals were also 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 (sc) to the head mount and secured with dental cement. Two rats were equipped with chronic ip catheter (allowing administration of drugs under stress-free conditions); the procedure of catheter implantation was described elsewhere (Blech-Hermoni and Kiyatkin, 2004). Rats were allowed three days recovery and two more days of habituation (6 h sessions) to the testing environment before the start of testing.

4.3. 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 ~09:30AM 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 12 rats underwent 6 recording sessions. During each session, after habituation to the testing chamber (~2 hours), rats were injected ip (0.1 ml per 100 g) with either a vehicle or one of three doses of Δ_9 -tetrahydrocannabinol (THC). This drug was obtained from the National Institute on Drug Abuse (as a solution in ethyl alcohol, 50 mg/ml) and first dissolved in solvent (1:5; 10% Tween-80, 20% DMSO and 70% saline) for 8.0 mg/kg injection, and then further diluted by this solvent (1:3) for 2.0 and 0.5 mg/kg doses. The solvent was equally diluted for vehicle injections. Because of the high instability of this substance and its susceptibility to oxidation, all samples were rapidly frozen after preparation, stored (up to 8 days) at –20°C, and later thawed out for use immediately before injection. Our pilot experiments revealed that the traditional storing of THC solutions at 4°C in the dark appears to be inadequate, resulting in progressive decrease in its activity (data not shown). We also took special care for proper

ip injection, which was done to escape drug administration in fat tissue, which appears to decrease its absorption and thus physiological effects. The order of these injections was counter-balanced and each rat received three vehicle and three THC injections (Day 1: vehicle or THC; Day 2: THC or vehicle, and so on). Doses of THC were gradually increased from 0.5 mg/kg to 2.0 mg/kg, and finally to 8.0 mg/kg. Therefore, each rat received each dose once, with one drug-free session after 0.5 mg/kg dose and three drug-free sessions after 2.0 mg/kg.

60 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. At ~180 min after the first injection, the 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 6 stimuli, 72 tests were performed in 12 rats for both vehicle and THC treatment.

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 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.

As a pilot study, in two rats we tested the effects of Rimonabant (SR 141716; RTI International) and vehicle (Tween-80, 20% DMSO and 70% saline) administered via chronically implanted ip catheter under stress-free conditions. Since vehicle administration affected temperatures, and the effects of Rimonabant (2 mg/kg in 0.6 ml vehicle) and vehicle (0.6 ml) were virtually identical, these data were not included in the Results and will be briefly described in the Discussion section.

4.4. 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. Both 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 (drug vs. vehicle) differences in temperature and locomotion. Basal temperature and basal locomotion were determined by calculating mean values for the 6 minutes 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" refers to the presence of a statistically significant change in the parameter or in the differences between the compared groups or conditions (with at least $p < 0.05$) revealed by either ANOVA or Student's t-test.

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, NIDA. We wish to thank Dr. Murat Oz for valuable comments regarding the issues discussed in this manuscript.

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Fig. 1.

Changes in temperature (A, B, C) and locomotion (D) induced by natural arousing stimuli and iv cocaine in control conditions. A: absolute temperatures in each recorded location, B: relative temperature changes, C: NAcc-muscle and skin-muscle temperature differential. Filled symbols indicate values significantly different from baseline. The effect of time, evaluated for temperature and locomotor activity was significant for each stimulus used (injection: NAcc – $F_{28,898}=14.29$, muscle=11.28, skin=12.36, locomotion=11.38; tail-pinch: NAcc – $F_{29,929}=37.59$, muscle=23.29, skin=18.33, locomotion=13.67; social interaction: NAcc – $F_{29,929}=38.86$, muscle=29.54, skin=11.33, locomotion=16.69; cocaine: NAcc- $F_{23,743}=116.05$, muscle=119.43, skin=16.7, locomotion=43.38).

Fig. 2.

Relationships between basal temperatures recorded from different locations in control conditions. Each graph shows correlation and regression equation for pairs of basal temperatures recorded from the same rats at the same time. Each graph also shows regression lines (solid), lines of equality (hatched), and limits of statistical variability (mean $\pm$ 3 SD). Asterisks denote degree of statistical significance of correlation (*, $p < 0.05$ and ***, $p < 0.001$).

Fig. 3. Correlative relationships between basal NAcc temperature and changes induced by the procedure of injection, tail-pinch, social interaction and iv cocaine. Each point represents basal and peak value. Each graph shows regression lines, regression equation, and correlation coefficient. Explanations are in the text.

Fig. 4. Changes in relative temperatures following ip injection of THC (0.5, 2.0 and 8.0 mg/kg) and vehicle for the first post-injection hour. Filled symbols denote values significantly different from baseline ($p < 0.05$, Fisher post-hoc test after one-way ANOVA evaluation of the effect of time).

Fig. 5.

Tonic changes in temperature and locomotion at different time points after ip injection of THC and vehicle. Each vertical panel corresponds to a different THC dose and shows basal locomotion (mean±sem) and relative changes in temperature (mean±sem) in each location assessed (as a mean for 6 min) at 0, 60, 120 and 180 min after the injection in the drug and vehicle groups. Asterisks inside of bars indicate significant difference vs. pre-injection baselines (0 min) and asterisks between bars indicate significance of between-group differences.

Fig. 6.

Changes in temperature and locomotion induced by tail-pinch, social interaction and iv cocaine in rats after ip injection of THC (0.5 mg/kg) and corresponding vehicle. Filled symbols show values significantly different from baseline. The effect of time evaluated by one-way ANOVA was significant for each stimulus in both the drug and vehicle groups. Tail-pinch: THC – NAcc $F_{8,278}=17.46$, muscle =16.95, skin =11.75, locomotion =4.07; Vehicle - $F_{8,278}=10.01$, muscle =6.16, skin =9.08, locomotion =3.63. Social interaction: THC – NAcc $F_{8,278}=8.64$, muscle =10.40, skin =5.58, locomotion =3.88; Vehicle - $F_{8,278}=8.84$, muscle =6.83, skin =4.58, locomotion =3.81. Cocaine: THC – NAcc $F_{7,247}=39.93$, muscle =44.23, skin =11.73, locomotion =7.49; Vehicle - $F_{7,247}=92.38$, muscle =64.34, skin =21.38, locomotion =5.95.

Fig. 7.

Changes in temperature and locomotion induced by tail-pinch, social interaction and iv cocaine in rats after ip injection of THC (2.0 mg/kg) and corresponding vehicle. Filled symbols indicate values significantly different from baseline. The effect of time evaluated by one-way ANOVA was significant for each stimulus in both the drug and vehicle groups. Tail-pinch: THC – NAcc $F_{7,247}=2.15$, muscle =2.54, skin =4.47, locomotion =3.43; Vehicle - $F_{8,278}=10.12$, muscle =7.20, skin =5.91, locomotion =5.47. Social interaction: THC – NAcc $F_{7,247}=11.35$, muscle =9.94, skin =8.68, locomotion =8.61; Vehicle - $F_{7,247}=10.45$, muscle =9.77, skin =4.77, locomotion =8.43. Cocaine: THC – NAcc $F_{7,247}=25.12$, muscle =30.74, skin =15.22, locomotion =8.63; Vehicle - $F_{7,247}=134.83$, muscle =108.06, skin =19.53, locomotion =9.78.

Fig. 8.

Changes in temperature and locomotion induced by tail-pinch, social interaction and iv cocaine in rats after ip injection of THC (8.0 mg/kg) and corresponding vehicle. Filled symbols indicate values significantly different from baseline. The effect of time evaluated by one-way ANOVA was significant for each stimulus in both the drug and vehicle groups. Tail-pinch: THC – NAcc $F_{8,278}=5.64$, muscle =4.44, skin =3.57, locomotion =4.81; Vehicle - $F_{8,278}=10.48$, muscle =4.47, skin =2.79, locomotion =5.83. Social interaction: THC – NAcc $F_{8,278}=14.85$, muscle =12.28, skin =8.52, locomotion =12.53; Vehicle - $F_{9,309}=11.86$, muscle =9.22, skin =3.95, locomotion =7.20. Cocaine: THC – NAcc $F_{7,247}=7.70$, muscle =15.35, skin =3.52, locomotion =3.50; Vehicle - $F_{7,247}=15.76$, muscle =19.29, skin =6.23, locomotion =4.22.

Table 1

Influence of THC (0.5 mg/kg) on temperature and locomotor responses induced by arousing stimuli and cocaine in rats

Parameters	Vehicle Basal	Response Absolute	Relative	THC 0.5 mg/kg Basal	Response Absolute	Relative
Tail-pinch						
NAcc	36.32±0.18	37.00±0.20	0.78±0.07	36.14±0.13	37.08±0.13	1.08±0.18
Muscle	35.42±0.24	36.06±0.22	0.74±0.08	35.40±0.14	36.20±0.16	0.90±0.11
Skin	34.55±0.17	33.66±0.18	-0.98±0.21	34.57±0.18	33.57±0.25	-1.03±0.18
Activity	4.46±1.31	17.08±3.20		1.87±1.13	16.60±2.25	
Social interaction						
NAcc	36.76±0.14	37.51±0.12	0.83±0.17	36.16±0.18*	36.94±0.26*	0.86±0.16
Muscle	35.83±0.18	36.50±0.14	0.85±0.14	35.26±0.20*	36.04±0.31	0.81±0.17
Skin	34.86±0.20	34.09±0.23	-0.84±0.18	34.28±0.24	33.63±0.31	-0.64±0.12
Activity	5.47±2.32	27.61±3.93		2.91±0.99	23.95±2.97	
Cocaine						
NAcc	36.57±0.15	37.62±0.10	1.10±0.13	36.31±0.16	37.43±0.14	1.15±0.17
Muscle	35.54±0.17	36.52±0.15	1.08±0.13	34.37±0.20	36.44±0.20	1.12±0.13
Skin	34.84±0.19	34.05±0.28	-0.96±0.21	34.47±0.19	33.64±0.21	-0.87±0.16
Activity	1.41±0.66	28.42±2.63		1.32±0.55	27.23±2.98	

"Basal" represents basal temperature and locomotion preceding stimulus presentation (mean for last 6 min±sem). "Response" represents absolute and relative changes (±sem) in peak temperature.

Response for "Activity" represents mean values of locomotion (counts/min; ±sem) for an equal time interval of significantly increased locomotion. Asterisks show the degree of statistical difference

* p<0.5

** p<0.01

*** p<0.001

Table 2

Influence of THC (2.0 mg/kg) on temperature and locomotor responses induced by arousing stimuli and cocaine in rats

Parameters	Vehicle Basal	Response Absolute	Relative	THC 2.0 mg/kg Basal	Response Absolute	Relative
Tail-pinch						
NAcc	36.92±0.29	37.69±0.27	1.02±0.15	36.31±0.25	36.97±0.23*	0.93±0.15
Muscle	35.85±0.29	36.59±0.29	0.91±0.09	35.35±0.32	36.03±0.31	0.88±0.17
Skin	34.85±0.32	34.13±0.38	-0.73±0.11	34.65±0.15	33.84±0.22	-0.89±0.16
Activity	2.59±0.97	20.51±3.11		9.63±0.31	15.17±2.54	
Social interaction						
NAcc	36.75±0.18	37.58±0.11	1.00±0.10	36.30±0.22	37.07±0.19*	0.85±0.15
Muscle	35.76±0.23	36.46±0.18	0.87±0.09	35.28±0.26	36.09±0.26	0.84±0.18
Skin	34.87±0.19	34.16±0.25	-0.75±0.16	34.45±0.28	33.72±0.32	-0.94±0.16
Activity	1.99±0.88	30.87±2.59		2.69±1.06	18.41±4.23*	
Cocaine						
NAcc	36.42±0.18	37.76±0.18	1.42±0.10	36.48±0.15	37.68±0.17	1.25±0.19
Muscle	35.30±0.20	36.68±0.20	1.51±0.09	35.52±0.17	36.64±0.21	1.15±0.19
Skin	34.80±0.17	33.99±0.24	-0.78±0.19	34.70±0.14	33.91±0.22	-0.85±0.20
Activity	1.29±1.10	37.46±3.83		0.71±0.71	42.26±4.60	

“Basal” represents basal temperature and locomotion preceding stimulus presentation (mean for last 6 min±sem). “Response” represents absolute and relative changes (±sem) in peak temperature. Response for “Activity” represents mean values of locomotion (counts/min; ±sem) for an equal time interval of significantly increased locomotion. Asterisks show the degree of statistical difference

* p<0.5
 ** p<0.01
 *** p<0.001

Table 3
Influence of THC (8.0 mg/kg) on temperature and locomotor responses induced by arousing stimuli and cocaine in rats

Parameters	Vehicle Basal	Response Absolute	Relative	THC 8 mg/kg Basal	Response Absolute	Relative
Tail-pinch						
NAcc	36.52±0.21	37.29±0.21	0.93±0.11	35.11±0.34 *	35.69±0.38 **	0.63±0.11 *
Muscle	35.33±0.24	36.05±0.21	0.85±0.14	34.36±0.38 *	34.90±0.43 *	0.56±0.13
Skin	34.46±0.21	33.75±0.22	-0.72±0.17	33.06±0.43 *	32.27±0.42 *	-0.79±0.11
Activity	0.87±0.61	18.67±3.38		1.70±1.17	8.67±2.82 *	
Social interaction						
NAcc	36.55±0.24	37.49±0.27	1.06±0.14	35.27±0.38 *	35.87±0.43 **	0.64±0.12 *
Muscle	35.55±0.27	36.54±0.31	1.05±0.14	34.54±0.38 *	35.14±0.43 *	0.63±0.11 *
Skin	34.68±0.17	34.19±0.29	-0.51±0.13	33.01±0.23	32.42±0.31	-0.79±0.09
Activity	1.48±0.70	27.35±4.04		5.54±2.46	14.48±5.11 *	
Cocaine						
NAcc	36.22±0.13	37.38±0.22	1.35±0.15	35.51±0.41	36.48±0.47	0.94±0.16 *
Muscle	35.16±0.16	36.30±0.24	1.32±0.12	34.43±0.41	35.32±0.49	0.87±0.13 *
Skin	34.42±0.35	33.68±0.32	-0.74±0.22	33.56±0.23	32.64±0.33 *	-0.83±0.13
Activity	3.19±0.74	30.98±3.14		1.44±0.93	22.22±2.98 *	

“Basal” represents basal temperature and locomotion preceding stimulus presentation (mean for last 6 min±sem). “Response” represents absolute and relative changes (±sem) in peak temperature. Response for “Activity” represents mean values of locomotion (counts/min; ±sem) for an equal time interval of significantly increased locomotion. Asterisks show the degree of statistical difference

* p<0.5
** p<0.01
*** p<0.001