MDMA, Methylone, and MDPV: Drug-Induced Brain Hyperthermia and Its Modulation by Activity State and Environment

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Abstract Psychomotor stimulants are frequently used by humans to intensify the subjective experience of different types of social interactions. Since psychomotor stimulants enhance metabolism and increase body temperatures, their use under conditions of physiological activation and in warm humid environments could result in pathological hyperthermia, a life-threatening symptom of acute drug intoxication. Here, we will describe the brain hyperthermic effects of MDMA, MDPV, and methylone, three structurally related recreational drugs commonly used by young adults during raves and other forms of social gatherings. After a short introduction on brain temperature and basic mechanisms underlying its physiological fluctuations, we will consider how MDMA, MDPV, and methylone affect brain and body temperatures in awake freely moving rats. Here, we will discuss the role of drug-induced heat production in the brain due to metabolic brain activation and diminished heat dissipation due to peripheral vasoconstriction as two primary contributors to the hyperthermic effects of these drugs. Then, we will consider how the hyperthermic effects of these drugs are modulated under conditions that model human drug use (social interaction and warm ambient temperature). Since social interaction results in brain and body heat production, coupled with skin vasoconstriction that impairs heat loss to the external environment, these physiological changes interact with drug-induced changes in heat production and loss, resulting in distinct changes in the hyperthermic effects of each tested drug. Finally, we present our recent data, in which we compared the efficacy of different pharmacological strategies for reversing MDMA-induced hyperthermia in both the brain and body. Specifically, we demonstrate increased efficacy of the centrally acting atypical neuroleptic compound clozapine over the peripherally acting vasodilator drug, carvedilol. These data could be important for understanding the

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potential dangers of MDMA in humans and the development of pharmacological tools to alleviate drug-induced hyperthermia – potentially saving the lives of highly intoxicated individuals.

Keywords Active ingredients of "bath salts" • Brain metabolism • Cerebral heat production • Drug-induced intoxication • Drugs of abuse • MDMA (Ecstasy) • Psychomotor stimulants • Rave parties • Vasoconstriction

Contents

Abbreviations

ip	Intraperitoneal
iv	Intravenous
MDMA	3,4-Methylenedioxymethamphetamine (Ecstasy)
MDPV	3,4-Methylenedioxypyrovalerone
METH	Methamphetamine
methylone	3,4-Methylenedioxymethcathinone
NAc	Nucleus accumbens
sc	Subcutaneous

1 Introduction: Psychoactive Drugs of Abuse

In contrast to therapeutic psychoactive drugs, which are taken voluntarily or given by medical professionals to alleviate specific pathological symptoms, individuals take drugs recreationally to induce pleasurable, novel, or unusual psycho-emotional effects. Each psychoactive drug induces specific behavioral, physiological, and psycho-emotional effects, which vary significantly depending upon the dosage, individual activity state, and environmental conditions in which the drug is taken. While the physiological effects of such drugs may mimic physiological responses induced by naturally arousing stimuli at lower doses, drug-induced responses at higher doses may reach pathological levels, resulting in acute intoxication and posing a significant risk to human health. It is generally believed that drug dosage is the primary parameter in predicting acute intoxication, but many other factors

play important roles in determining the severity of drug-induced responses, including individual responsiveness, previous drug experience(s), concurrent poly-drug use, preexisting pathologies, and finally, the specific circumstances or context in which the drug is taken.

In this review, we will be focused on the latter variable, discussing how activity state and the environmental conditions of drug use modulate the physiological and behavioral effects of MDMA (3,4-methylenedioxymethamphetamine or "Ecstasy," "Molly"), methylone (3,4-methylenedioxymethcathinone), and MDPV (3,4-methylenedioxypyrovalerone). While the psychoactive effects of MDMA have been known for decades, methylone and MDPV are synthetic cathinones that became popular among young adults in the last few years with the emergence of "bath salts." Although MDMA, MDPV, and methylone have distinct behavioral and physiological effects, they are structurally similar psychostimulants that all induce sympathetic activation and body hyperthermia. As such, our focus here will be on drug-induced perturbations in temperature homeostasis, with a special emphasis on brain temperature as an important parameter that not only reflects the metabolic aspects of brain activity, but also affects neural activity and neural functioning [1].

2 Brain Temperature

While it is traditionally believed that brain temperature in healthy homeothermic organisms is stable and close to 37°C, abundant data obtained in different animal models suggest that relatively large fluctuations in brain temperature occur during different types of natural motivated behavior and following exposure to various environmental challenges [2–6]. It is difficult to quantify the range of physiological fluctuations in brain temperature in humans, but, as shown by direct monitoring with chronically implanted thermocouple sensors, hypothalamic temperature recorded from freely moving rats could fall to ~35°C during deep sleep [7, 8] and phasically peak at ~39.5°C at the time of ejaculation during copulatory behavior [9].

While the direct monitoring of brain temperature in rats is a relatively simple procedure, human data are limited and often restricted to neurological patients [10, 11] or indirect measurements [12–14] that are questionable with respect to their validity and accuracy. Therefore, it has not been definitely proven that similar, relatively large brain temperature fluctuations could occur in healthy humans. However, several observations suggest that this could be the case. First, monkeys show robust physiological changes in brain temperature within a range comparable to that observed in rats [3, 15]. Second, humans show ~1.0–2.0°C diurnal fluctuations in body temperature as well as clear pathological hyperthermia (>40–41°C) during acute intoxication by METH or MDMA [16–19], similar to the temperature changes seen in rats [20]. Finally, direct measurements of venous outflow from healthy human volunteers wearing water-impermeable clothing which impaired

normal heat dissipation to the external environment revealed that brain temperatures could reach 39.5–40.0°C during a 30-min bicycle exercise [21]. Importantly, even at such high brain temperatures, the physical and mental states of these volunteers remained normal, suggesting that the brain can tolerate relatively large, but transient temperature increases.

3 Basic Mechanisms Underlying Brain Temperature Homeostasis

Brain temperature is determined by the balance of two opposing forces: (1) metabolism-related intra-brain heat production and (2) heat loss via cerebral blood outflow to the rest of the body and then to the external environment. Although the brain represents only ~2% of body weight in humans, it accounts for ~20% of an organism's total energy consumption [22, 23], suggesting intense intra-cerebral heat production. This heat is removed from brain tissue by the cerebral circulation due to arrival from the lungs of cooler arterial blood [24–26].

Although mechanistic, the cooling of an internal combustion engine is a good analogy when considering brain temperature exchange. Similar to circulating coolant that continuously removes heat from a working engine, cool, oxygenated arterial blood removes heat from the brain via heat exchange. The now warmed venous blood then returns to the heart to be cooled and re-oxygenated in the lungs. Such an arrangement determines the critical role of cerebral blood flow in brain temperature homeostasis and the essential interdependence between temperature in the brain and the rest of the body. While brain temperature tends to increase due to metabolism-related intra-brain heat production, it also rises when brain-generated heat cannot be properly dissipated to the body and then to the external environment. Similarly, a decrease in cerebral metabolism tends to lower brain temperature; however, this effect could be strongly enhanced by a peripheral vasodilation that promotes heat loss to a cooler environment [27, 28].

In our experiments, we routinely used a three-point thermorecording paradigm. In addition to a brain representative site, usually the nucleus accumbens (a critical structure in brain motivation-reinforcement circuitry [29–31]), temperatures were also recorded from two peripheral locations: the temporal muscle and skin. Since the brain and temporal muscle receive arterial blood from the same common carotid artery and are equally exposed to blood-delivered heat from the body, temperature difference between these locations (NAc-Muscle temperature differential) shows the source of heat production, providing a measure of drug-induced metabolic brain activation. As a result, increases in NAc-Muscle differential reflect increased metabolic brain activation. Skin temperature is primarily determined by the state of peripheral vessels, but it also depends on the temperature of arterial blood inflow. Therefore, Skin-Muscle temperature differential serves as an accurate measure of peripheral vascular tone, another important contributor to changes in brain

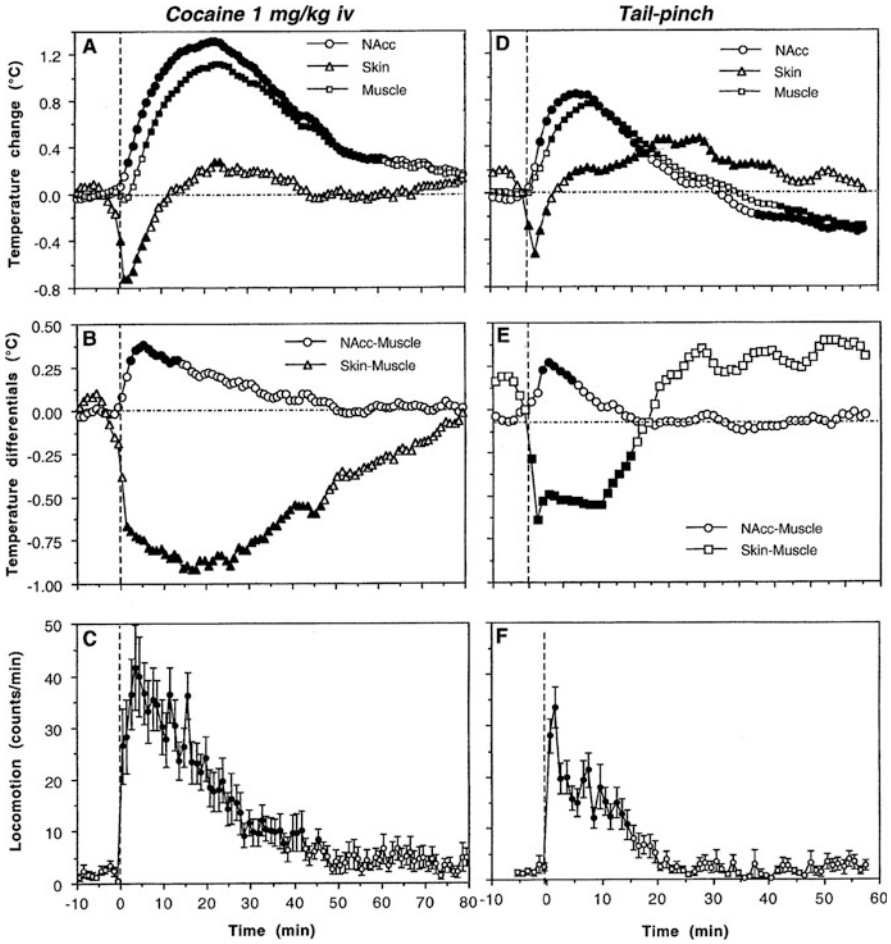

Fig. 1 Changes in brain (NAc), temporal muscle, and skin temperatures induced by iv injection of cocaine (1 mg/kg) and 3-min tail-pinch in freely moving rats under quiet resting conditions. *Top graphs* (**a**, **d**) show relative changes in temperatures, *middle graphs* show changes in NAc-Muscle and Skin-Muscle temperature differentials (**b**, **e**), and *bottom graphs* (***c–f***) show changes in locomotor activity. *Filled symbols* mark values significantly different from baseline. Original data shown in this graph were reported in Kiyatkin [1]

temperature [1]. Increases in Skin-Muscle differential reflect vasodilation, whereas decreases reflect vasoconstriction.

Figure 1 shows temperature and locomotor effects induced by two different stimuli: a three-min tail-pinch and intravenous (iv) cocaine injected to freely moving rats at a typical self-administration dose (1 mg/kg). Despite the differing natures of these stimuli, they induced similar locomotor activation and moderate increases in brain and muscle temperatures. Importantly, NAc-Muscle differentials significantly increased in both cases, suggesting metabolic brain activation as a

common factor elicited by both pharmacological and natural arousing stimuli. Similarly, both stimuli significantly decreased Skin-Muscle differentials, suggesting another common factor – prolonged skin vasoconstriction. While qualitatively similar, cocaine-induced effects on both temperature differentials were stronger and more prolonged, obviously indicating a larger elevation in temperature induced by cocaine.

This example, as well as the results of our other studies (see [1] for review), demonstrates that brain temperature increases, induced by both natural arousing stimuli and drugs, are determined by two basic mechanisms: (1) increased intrabrain heat production due to metabolic brain activation and (2) decreased heat loss due to skin vasoconstriction. Since psychostimulant drugs increase metabolism and locomotor activity [32–36] and induce peripheral vasoconstriction [34, 37], it could be assumed that drug-induced brain temperature responses will depend upon the ongoing state of the organism and the environmental conditions associated with drug use. A drug at a certain dose could induce minimal temperature effects in an environment where the adaptive mechanisms of heat loss are fully effective, but the same drug at the exact same dose could induce powerful hyperthermic effects in an environment where heat dissipation mechanisms are significantly impaired. Since peripheral vasodilation and perspiration are powerful means for heat loss in humans, drug-induced impairment of these adaptive mechanisms may be a very important determinant of drug-induced increases in brain and body temperatures.

4 Brain Hyperthermia Induced by MDMA, Methylone, and MDPV: State Dependency and Environmental Modulation

MDMA is a typical “club drug” that is often used by young adults under conditions of physical and emotional activation, often in a warm and humid environment. Similar to other psychostimulants, MDMA increases metabolism and induces hyperactivity coupled with hyperthermia [32–36]. The influence of environmental conditions and specific activity states could be especially important for MDMA because, in addition to metabolic activation, it also induces peripheral vasoconstriction [34, 37], thus diminishing heat dissipation from body surfaces and enhancing heat accumulation in the brain and body.

During recent years, there has been a rapid increase in the abuse of synthetic cathinone analogs, which are sold with innocuous names such as “bath salts” or “plant food” [38, 39]. Such products were designed to circumvent regulations controlling the sale and use of psychoactive substances. Two very popular synthetic cathinones are methylone and MDPV [40]. While low recreational doses of synthetic cathinones enhance mood and increase energy, high doses or chronic use can cause serious medical complications, including agitation, psychosis, tachycardia,

hyperthermia, and even death [41, 42]. Due to these risks, methylone and MDPV have been classified as Schedule I controlled substances in the USA.

Methylone and MDPV are structurally similar to MDMA. Like MDMA, methylone and MDPV exert their major effects by interacting with monoamine transporter proteins in the central and peripheral nervous systems [43]. Methylone is a non-selective transporter substrate that evokes the release of dopamine, norepinephrine, and serotonin, analogous to the effects of MDMA [44–46]. By contrast, MDPV is a potent transporter blocker that inhibits the uptake of dopamine and norepinephrine, with minimal effects on serotonin uptake [43, 47].

Although robust increases in body temperature have been reported in humans as the result of acute intoxication with both methylone [48] and MDPV [49–51], data on temperature effects of methylone and MDPV in laboratory animals are limited and controversial, varying according to species, dose, and experimental conditions [44, 52, 53]. MDPV is reported to cause hyperthermia in mice (3–30 mg/kg, intraperitoneal or ip administration) but only under elevated ambient temperature [53]. In rats, MDPV (1.0–5.6 mg/kg, subcutaneous or sc administration) had no evident effect on core body temperature [52].

To elucidate how MDMA, MDPV, and methylone affect brain and body temperature, and which mechanisms underlie these changes, we first examined the effects of these drugs on NAc, muscle, and skin temperatures under standard laboratory conditions (quiet rest at 22°C ambient temperatures). We also examined the effects of these drugs on locomotion. In these initial experiments, drugs were delivered using sc injections at a wide range of recreational extremes. MDMA and methylone were tested within a dosage range of 1–9 mg/kg and the more potent MDPV was delivered at a range of 0.1–1.0 mg/kg. The data for each individual drug were described in detail elsewhere [54, 55]. While these conditions are usually used in most preclinical studies in animals, humans typically use these drugs during psychophysiological activation and often in warm environments that impair normal heat dissipation from the body to the external environment. To model conditions relevant to humans, the effects of MDMA, MDPV, and methylone were examined in rats during social interaction between two animals. Under these conditions, brain and body temperatures moderately increased but skin temperature rapidly decreased, reflecting an adaptive response elicited by arousal stimulation. Finally, the effects of each of the three drugs were tested in quietly resting rats maintained in a moderately warm environment (29°C). While this temperature is ~7°C higher than standard housing conditions (22–23°C), it corresponds to the thermoneutral zone in rats, where endogenous heat production is minimal and balanced with heat loss [56].

Since our focus was on pathological hyperthermia that develops in some drug users and could result in serious health complications (including lethality), in these experiments we used relatively large drug doses (MDMA and methylone –9 mg/kg, sc; MDPV –1 mg/kg, sc), which induce relatively large effects, both behaviorally as well as on temperature. While exceeding the typical doses consumed by humans, all drugs at these doses are well tolerated by freely moving rats tested under standard environmental conditions. For example, a 9 mg/kg dose of MDMA is ~1/5 of the

LD50 (50 mg/kg; [57]), but and with sc injection this dose never results in lethality. Since the psychoactive effects of MDMA possess significant latencies after ingestion, sometimes people consume one or two additional MDMA tablets, shifting the dose close to 9 mg/kg. Similar to MDMA, methylone and MDPV are usually ingested, with typical active doses of 100–300 mg for methylone and 5–20 mg for MDPV (see www.erowid.org for human self-reports). However, these drugs can be taken at much higher doses (up to 1,000 and 200 mg, respectively) and via other administration routes.

Figure 2 shows that MDMA, MDPV, and methylone each increased brain and muscle temperatures, rapidly decreased skin temperature, and induced locomotor activation. These changes differed from a transient temperature increase induced by the saline injection, which did not result in an evident motor response (panels a–c). While brain temperature increases induced by all three drugs were approximately equal in their magnitude (1.5–1.8°C), their time-course and duration differed in each case. Methylone and MDMA induced prolonged temperature increases, but the effect of MDPV was shorter and more rapid. Injections of each drug induced rapid increases in NAc-Muscle differentials, but this effect, suggesting metabolic brain activation and enhanced intra-brain heat production, was clearly larger vs. saline injection only with MDMA (panel k). Each drug also rapidly decreased

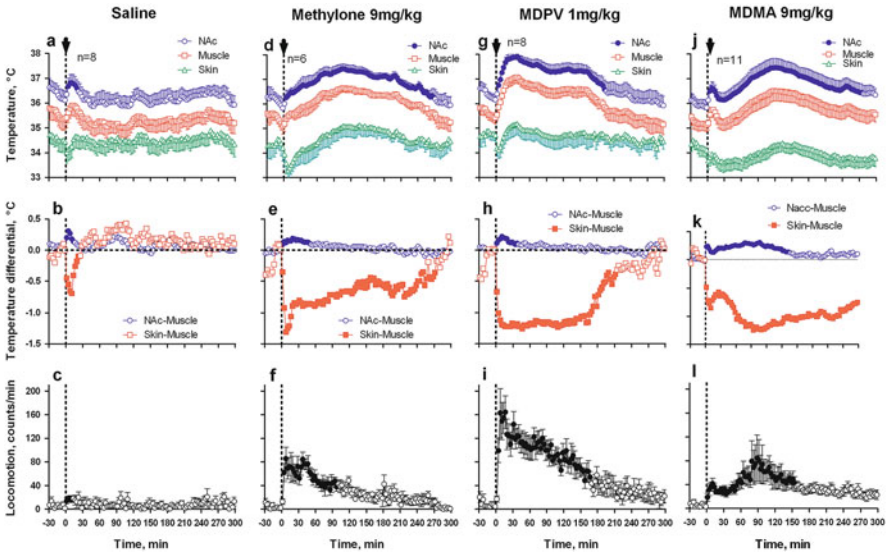

Fig. 2 Mean changes in brain (NAc), temporal muscle, and skin temperatures and locomotor responses induced by sc injections of methylone (9 mg/kg), MDPV (1 mg/kg), MDMA (9 mg/kg), and saline in freely moving rats under quiet resting conditions. *Top graphs* show mean (±SEM) values of absolute temperature changes; *middle graphs* show changes in NAc-Muscle and Skin-Muscle differentials; and *bottom graphs* show mean (±SEM) changes in locomotor activity. *Filled symbols* mark values significantly different from pre-injection baselines. *Bold arrows* mark the moment of injection. Original data shown in this graph were reported in Kiyatkin et al. [54, 55]

Skin-Muscle differential, suggesting cutaneous vasoconstriction; this effect was about the same for each drug. Finally, all three drugs induced locomotor activation; this effect was clearly the greatest for MDPV.

Consistent with our previous work [1], the introduction of a novel "guest" rat into the chamber occupied by the experimental rat undergoing recording induced locomotor activation, a rapid, strong rise in NAc and muscle temperatures (~1.5°C), and a brief decrease in skin temperature that was rapidly transformed into a more tonic, rebound-like increase (Fig. 3a–c). While the changes in NAc and muscle temperatures were generally parallel, the rise was stronger and more rapid in the brain. This resulted in a significant increase in NAc-Muscle differentials that indicated metabolic brain activation. Social interaction was also accompanied by a strong decrease in Skin-Muscle temperature differentials, indicating stimulus-induced cutaneous vasoconstriction. While NAc-Muscle differentials rapidly returned to baseline after the end of the social interaction period, Skin-Muscle differentials increased above baseline, suggesting a rebound vasodilation. These physiological parameters and locomotion showed consistent changes at the start and end of the social interaction. A saline injection during social interaction (+10 min after its onset; black arrow in Fig. 3a) had no effect on the parameters above.

When rats received methylone instead of saline, temperature differences were minimal, but the decreases in NAc and muscle temperatures and Skin-Muscle differentials after the end of social interaction were more prolonged (Fig. 3d–f). Interestingly, the combination of two hyperthermic effects (methylone + social interaction) did not result in their summation or potentiation. Mean values of all parameters did not differ statistically vs. those seen in rats that received methylone under quiet resting conditions. The mean increase in NAc temperature was calculated for the entire 5-h post-drug interval as the area under the curve was maximal with methylone used under quiet resting conditions (1.12 ± 0.23°C) and even slightly lower when methylone was used during social interaction (0.88 ± 0.18°C, no significant change).

Similar to methylone, injection of MDPV delayed brain and muscle temperature decreases after social interaction (Fig. 3g–i). However, in contrast to methylone, changes were more robust and MDPV-induced NAc temperature increases significantly potentiated during social interaction (MDPV + social interaction 1.91 ± 0.18°C vs. MDPV, quiet resting conditions 0.79 ± 0.25°C; $p < 0.01$). The "pure" effect of MDPV (difference vs. saline control for each condition) was also clearly amplified when drug was administered during social interaction (1.38°C vs. 0.74°C). When taken in an activated physiological state, MDPV also enhanced both increases in NAc-Muscle differentials and decreases in Skin-Muscle differentials, suggesting a weak potentiation of MDPV-induced metabolic and vasoconstrictive effects.

The most robust potentiation of hyperthermic effects was found with MDMA used during social interaction (Fig. 3j–l). In this case, mean NAc temperatures exceed 39°C at their peak and the overall response was the most prolonged, extending the 5-h observation window. These changes, however, were highly

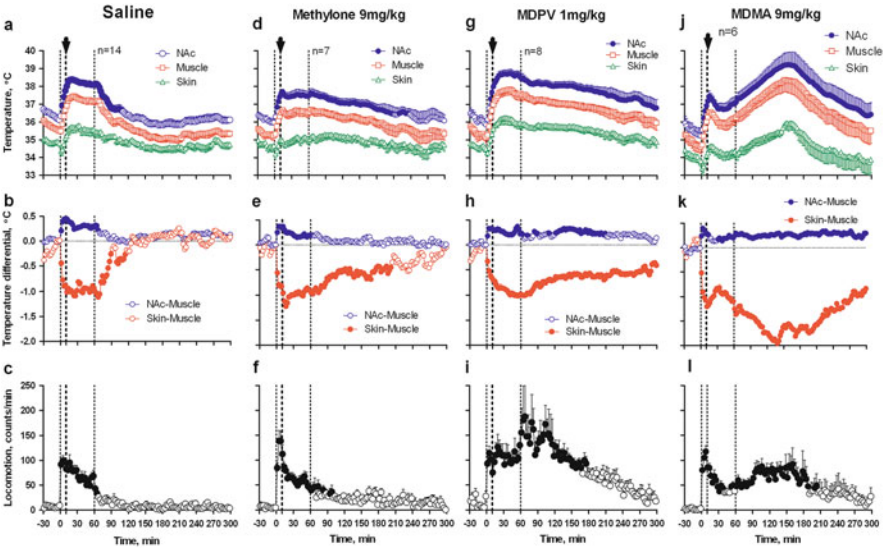

Fig. 3 Mean changes in brain (NAc), temporal muscle, and skin temperatures and locomotor responses induced by sc injections of methylone (9 mg/kg), MDPV (1 mg/kg), MDMA (9 mg/kg), and saline during social interaction. *Top graphs* show mean (±SEM) values of absolute temperature changes; *middle graphs* show changes in NAc-Muscle and Skin-Muscle differentials; and *bottom graphs* show mean (±SEM) changes in locomotor activity. *Filled symbols* mark values significantly different from the pre-injection baseline. The first and third vertical hatched lines in each graph show onset and offset of social interaction (60 min) and *black arrows* at the second hatched lines mark the moment of drug administration. Original data shown in this graph were reported in Kiyatkin et al. [54, 55]

variable in individual rats, with peak brain temperatures ranging from 37.1 to 42.1°C. The mean temperature elevations were maximal in this case (2.39 ± 0.27°C), significantly exceeding modest temperature increases seen under quiet resting conditions (0.95 ± 0.25°C; $p < 0.01$). The pure effects of MDMA on brain temperature were also significantly stronger during social interaction than in quiet resting conditions (1.86°C vs. 0.89°C), suggesting a super-additive interaction. Despite the use of a moderate, non-lethal dose of MDMA (9 mg/kg, 1/5 LD50; [57]), one of the seven rats tested with MDMA died overnight after the recording session.

The potentiation of MDMA-induced hyperthermia during social interaction could be explained by two primary contributors: a stronger rise in NAc-Muscle differentials and a strong, tonic drop in Skin-Muscle differentials. The increase in the NAc-Muscle differential was very prolonged (>5-h) although its amplitude did not differ from that seen with the drug used in quiet resting conditions. In contrast, MDMA-induced decrease in Skin-Muscle differential (-1.31 ± 0.29°C) was larger and more prolonged than with either methylone (-0.56 ± 0.13°C; $p < 0.05$) or MDPV (-0.66 ± 0.27°C; $p < 0.05$) and it did not recover at the recording end (5 h). Interestingly, robust potentiation of MDMA's hyperthermic effect profile

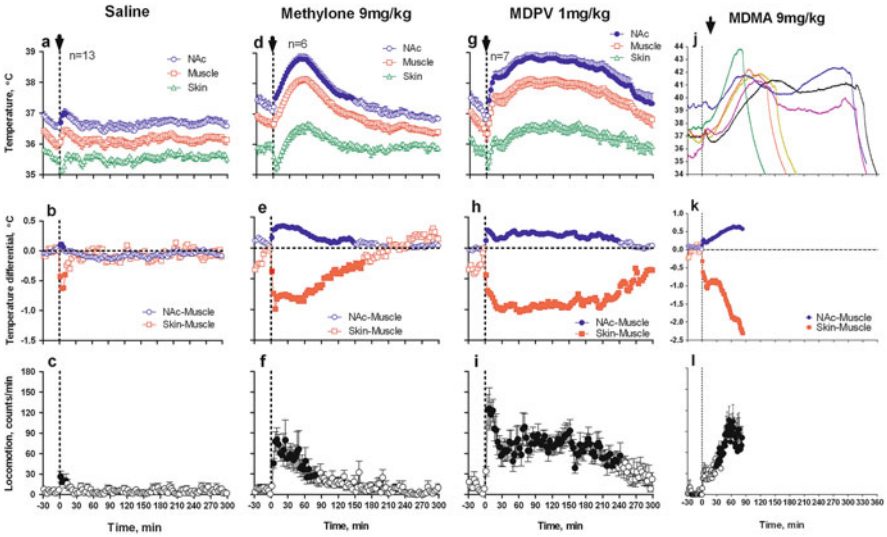

Fig. 4 Mean changes in brain (NAc), temporal muscle, and skin temperatures and locomotor responses induced by sc injections of methylone (9 mg/kg), MDPV (1 mg/kg), MDMA (9 mg/kg), and saline in freely moving rats at warm ambient temperatures (29°C). *Top graphs* show mean (±SEM) values of absolute temperature changes; *middle graphs* show changes in NAc-Muscle and Skin-Muscle differentials; and *bottom graphs* show mean (±SEM) changes in locomotor activity. *Filled symbols* mark values significantly different from the pre-injection baseline. *Black arrows* at the hatched lines mark the moment of drug administration. Since all rats exposed to MDMA died within 6 h post-injection, MDMA data are shown as individual changes (*j*) and mean values of NAc-Muscle, Skin-Muscle differentials and locomotion (*k* and *l*) for the first 80 min post-injection when all rats were still alive. Original data shown in this graph were reported in Kiyatkin et al. [54, 55]

by social interaction was not accompanied by significant changes in its locomotor effects, which remained similar in both conditions (compare Figs. 2 and 3).

The profile of temperature effects of MDMA, MDPV, and methylone was robustly modulated by warm ambient temperatures; the type of modulation was also unique to each compound (Fig. 4). Rats exposed to warm ambient temperatures (28–29°C) maintained stable but slightly higher internal temperatures (mean: 36.7 ± 0.2, 36.2 ± 0.2, and 35.2 ± 0.4°C for NAc, muscle, and skin, respectively) than rats housed under standard laboratory conditions at 22–23°C (mean: 35.9 ± 0.2, 35.2 ± 0.3, and 34.0 ± 0.26°C, respectively). The difference was minimal for the brain ($\Delta = 0.76$°C) and maximal for skin ($\Delta = 1.17$°C), suggesting weak tonic vasodilation as a way to promote dissipation of metabolic heat into the warmer environment. Saline injections under these conditions induced transient, weak temperature responses similar to that seen with saline injection at standard room temperature (Fig. 4a–c).

Although methylone (9 mg/kg) injected at 29°C increased NAc temperature (Fig. 4d–f), this change was more transient than at 23°C and its mean value assessed as the area under the curve (0.47 ± 0.15°C) was significantly lower than that seen

under quiet resting conditions ($1.12 \pm 0.23°C$, $p < 0.01$). At higher ambient temperatures, drug-induced increases in NAc-Muscle differentials were larger than those in quiet rest, but decreases in Skin-Muscle differentials were shorter in duration and significantly weaker than in control ($-0.30 \pm 0.05°C$ vs. $-0.72 \pm 0.15°C$; $p < 0.05$).

In contrast, MDPV (Fig. 4g–i), administered under warm ambient temperatures, induced stronger and more prolonged elevations in NAc temperature ($1.51 \pm 0.16°C$), which were significantly larger than that induced at 23°C ($0.79 \pm 0.25°C$; $p < 0.05$). The potentiation of its hyperthermic effect was coupled with stronger prolonged increases in NAc-Muscle differentials and stronger decreases in Skin-Muscle differentials. However, these changes were not significant vs. 23°C. Therefore, while the hyperthermic effect of methylone showed an unusual weakening, both during social interaction and at 29°C, the hyperthermic effect of MDPV is moderately enhanced by both social interaction and warm external temperatures.

In contrast to the two cathinones, MDMA administered at 29°C induced robust increases in NAc temperatures that reached fatal values (>41°C), resulting in lethality in all 6 tested rats within 6-h post-injection (Fig. 4j–l). Peak values of NAc temperatures after MDMA administration ranged from 41.4°C to 43.8°C, and their means were more than 4°C larger than in the control condition ($42.2 \pm 0.4°C$ vs. $37.7 \pm 0.4°C$; $p < 0.01$). When calculated as the pre-lethal temperature peak, the mean increase in NAc temperature was $2.29 \pm 0.22°C$, significantly larger than mean MDMA-induced NAc temperature elevation in 23°C environment ($0.95 \pm 0.25°C$; $p < 0.01$). By this parameter, the degree of potentiation for MDMA was twice that for MDPV (1.34°C vs. 0.72°C). Additionally, while significant increases in NAc-Muscle differentials were evident with MDMA at standard ambient temperatures (mean $= 0.17 \pm 0.07°C$), NAc-Muscle differentials more than doubled at 29°C ($0.36 \pm 0.04°C$; $p < 0.05$). When used at warm temperatures, MDMA also induced much stronger and more prolonged vasoconstriction as evidenced by Skin-Muscle differentials, which decreased on average more than 2°C below pre-injection baseline (see Fig. 3k).

Drug-specific differences in modulation are especially evident when we compare the hyperthermic effects of each drug under different experimental conditions (Fig. 5). The upper panel in Fig. 5a shows temperature change (as an area under the curve for the duration of significant drug effect) with respect to quiet resting baseline and the bottom panel shows "pure" or "net" effects of drugs, when changes occurring in saline control being subtracted. As can be seen, each of the three drugs induced an approximately equal brain hyperthermic effect when tested at standard laboratory conditions. However, the effects of these drugs showed distinct differences when they were administered during social interaction and at warm ambient temperatures. The effect of methylone (blue bars) administered during social interaction did not change and, surprisingly, decreased when the drug was administered at 29°C. This pattern of interaction could suggest that methylone and social interaction share common effector mechanisms (e.g., sympathetic activation) to induce brain hyperthermia. When these mechanisms are naturally activated during

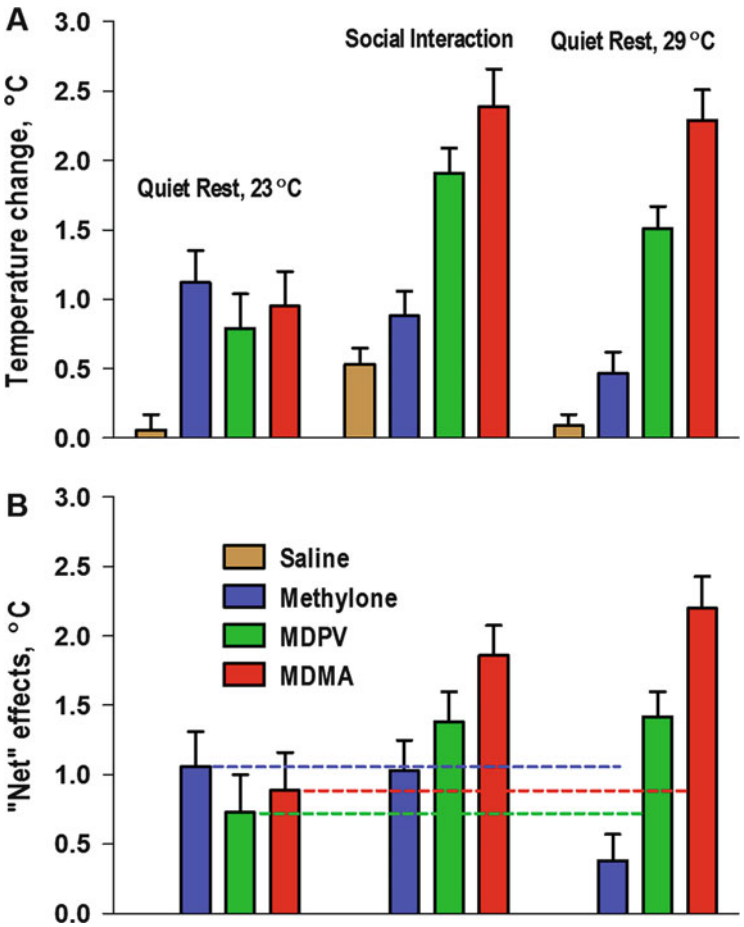

Fig. 5 Mean values of brain (NAc) temperature increases (area under curve for 5-h post-injection) induced by sc injections of methylone (9 mg/kg), MDPV (1 mg/kg), MDMA (9 mg/kg), and saline in rats under quiet resting conditions, during social interaction, and at warm ambient temperatures. *Top graphs* (**a**) show mean (±SEM) values and *bottom graphs* (**b**) show "net" or "pure" drug effects (drug–saline). *Horizontal hatched lines* in (**a**) show values in control (saline) group. *Horizontal hatched lines* in (**b**) show values induced by each drug under quiet resting conditions. Original data shown in this graph were reported in Kiyatkin et al. [54, 55]

social interaction (i.e., when brain metabolic activity is increased and/or cutaneous vessels are physiologically constricted), the effects of the drug *per se* become weaker but the overall hyperthermic response does not change.

However, a different pattern of interaction occurred with MDPV (Fig. 5, green bars). Brain temperature increases induced by this drug during social interaction and at 29°C become significantly stronger (A) and the net effect of MDPV was larger (B), suggesting an additive interaction and the involvement of different mechanisms in mediating the enhanced brain temperature response to MDPV.

While the mechanisms underlying this potentiation remain unclear, it is likely that MDPV, in addition to its central action, acts directly on peripheral vessels potentiating skin vasoconstriction and increasing intra-brain heat accumulation.

Finally, potentiation was supra-additive with MDMA (Fig. 5, red bars), which induced pathological brain temperature increases when administered during social interaction and lethality when used in moderately warm environments. This pattern of modulation was clearly evident in mean values of temperature elevation (A) and the "net" effect of drug in each condition (B).

It is difficult to speculate as to why these three structurally similar psychostimulant compounds show different types of state-dependent and environmental modulation, and why MDMA differed drastically from MDPV and methylone. Obviously multiple factors determine quantitative and qualitative differences in each compound, including the affinity and specificity of each drug to various neural substrates in the brain and the periphery, differences in drug metabolism, and drug-specific entry in brain tissue via the blood–brain barrier. Despite the importance of all these factors – many of which are still unknown and require further work – at the physiological level, MDMA, compared to cathinones, has strong, sustained effects on intra-brain heat production coupled with very strong, tonic peripheral vasoconstriction that prevents normal heat loss to the external environment. These two features facilitate the stronger and more prolonged hyperthermic effect of MDMA and the profound, often lethal, potentiation of this effect when the drug is taken in conjunction with psychophysiological activation and/or diminished heat dissipation.

The powerful augmentation of MDMA's thermal effects by environmental conditions observed in rats may help to explain the exceptionally strong, and sometimes fatal, response of some individuals induced by this drug under rave party conditions. However, some caution should be taken in extrapolating these findings to human conditions because humans have much more sophisticated mechanisms of heat loss from the body than do rats [58], thus making them more resistant to high environmental temperatures and thermogenic effects of psychomotor stimulants. In contrast to rats, humans have a well-developed ability to sweat and have a very high dynamic range of flow rates in the skin, thus allowing them to lose more metabolic heat (1 kW) than could be maximally produced in the body [59]. These differences in the effector mechanisms of heat loss could explain weaker MDMA-induced body temperature increases and their lesser dependence on ambient temperatures found in monkeys [60–62] and humans [33, 63]. Despite their high efficiency, the compensatory mechanisms of heat loss in humans could be greatly impaired under specific conditions, resulting in progressive heat accumulation in the organism. Even a simple bicycle exercise that produces ~1°C brain temperature elevation under normal conditions produced strong hyperthermia (39.0–39.5°C) when the exercise is conducted in a special water-impermeable cloth that prevents heat dissipation to the external environment [64]. Therefore, pathological brain hyperthermia induced by overdose of psychomotor stimulants under rave conditions results not only from excessive heat production due to drug-induced and associated psychophysiological activation, but also from the powerful

drug-induced peripheral vasoconstriction and impaired ability to dissipate metabolic heat due to warm, humid environment. Since partygoers are often engaged in intense social/physical interactions with other individuals (dancing, sexual activity, etc.), drug effects are additionally potentiated by psychophysiological activation typical to these conditions.

5 Pharmacological Strategies to Reverse Pathological Hyperthermia Induced by MDMA

Current emergency therapeutic options to counteract MDMA-induced pathological hyperthermia mainly focus on whole body cooling, water substitution, and sedative therapy. Indeed, body cooling should have a hypothermic effect, but the effectiveness of this treatment is limited due to strong, sustained MDMA-induced vasoconstriction and the natural vasoconstrictive effect of skin cooling. Similarly, water consumption and/or saline infusions are minimally effective in counteracting MDMA-induced hyperthermia [65].

Since our previous work established a critical role of intra-brain heat production and peripheral vasoconstriction in potentiating brain and body hyperthermic effects of MDMA, we explored two alternative pharmacological strategies for possible reversal of this effect in order to decrease brain and body temperature [55]. We assessed the effects of clozapine, an atypical neuroleptic, and carvedilol and labetalol, mixed alpha/beta adrenoceptor blockers. These medications are routinely used to treat chronic health problems in humans, and were chosen because of their preclinical success in attenuating MDMA-induced body hyperthermia [66, 67]. Clozapine acts on multiple neural receptors and glial cells in the brain, presumably inhibiting MDMA-induced metabolic neural activation, sympathetic tone, and centrally mediated vasoconstriction [68, 69]. Carvedilol and labetalol act peripherally to dilate skin vessels by blocking alpha- and beta-adrenoceptors [70, 71]. Similar to our previous studies, we used three-point thermorecording paradigm (brain site, temporal muscle, and skin) and determined changes in NAc-Muscle and Skin-Muscle differentials to determine the basic physiological mechanisms underlying brain temperature responses. This three-point recording technique allowed us to evaluate the effects of the drugs on intra-brain heat production due to metabolic neural activation and heat loss due to changes in peripheral vascular tone [1].

Our experimental protocol has three important features. First, we exposed rats to MDMA at a relatively modest, non-toxic dose [9 mg/kg or ~ 1/5 of LD50; [72]] and delivered the drug subcutaneously (in 0.3 ml of saline), providing the slowest pharmacokinetics, analogous to oral consumption in humans [87]. Second, in contrast to most studies that utilized drug administration in quietly resting laboratory animals, we administered MDMA during social interaction with another rat at the height of "psychophysiological activation," when brain and body temperatures are significantly increased [54, 73]. This protocol is more relevant for human

conditions because MDMA is recreationally used in social settings associated with high arousal (e.g., rave parties and music festivals). Finally, in contrast to most studies, where a treatment drug was administered before or at the same time as MDMA [67, 74–77], we injected each of the three test drugs – clozapine, carvedilol, and labetalol – after the MDMA injection, when brain and body temperatures were already significantly increased (>38°C). This dosing regimen closely mimics the clinical situation, in which MDMA-intoxicated patients are treated for pathological hyperthermia in hospital emergency rooms.

Although we strived for a fully factorial, within-subjects design, the variability associated with MDMA temperature response made this difficult. However, generally we exposed all rats to a 1-week-long experimental protocol where they randomly received MDMA–Saline (control), MDMA–Treatment Drug, Saline, or Treatment Drug Alone on alternating days. In rats from the first two groups, we injected MDMA 10 min after the onset of the 1-h social interaction followed by a counterbalanced injection of either a treatment drug (clozapine, carvedilol, and labetalol) or saline. In rats from two other groups, we injected either a treatment drug (clozapine, carvedilol, and labetalol) or saline under quiet resting conditions. Each rat received only two MDMA injections, either alone or with a treatment drug. We injected all "treatment" drugs ip at the same dose (5 mg/kg); this dose was chosen based on previous studies [66, 67].

As shown in Fig. 6e–f, after clozapine injection, NAc temperature rapidly decreased, resulting in a large difference vs. saline control (saline). The clozapine-induced temperature decrease was rapid and profound; the final temperature values in the clozapine treatment group were lower than the initial baseline and significantly lower than in the control group that received MDMA with saline. These post-treatment temperature values, however, remain within the physiological range; similarly, low or even lower values occur in well-habituated rats during day-time recording [1]. Muscle and skin temperatures also decreased after clozapine injection, and the difference vs. control was also significant.

In contrast to the stable increase in NAc-Muscle differentials present in the control group (Fig. 6c), this parameter decreased after clozapine injection (Fig. 6g). The decrease developed with ~10–15 min onset latency, producing a significant difference vs. control from ~30 min post-injection. However, the most rapid and strong effects of clozapine were found for the Skin-Muscle differentials, which reflect the vasomotor tone of skin vessels. While this parameter further decreased after saline injection (Fig. 6c), suggesting MDMA-induced sustained skin vasoconstriction, the Skin-Muscle differential began to rapidly increase immediately after clozapine injection (Fig. 6g), reflecting a full blockade of drug-induced vasoconstriction. The difference between clozapine and saline appeared from the second data point (i.e., within 3–6 min) and this difference continued to increase throughout the entire post-treatment interval. Finally, clozapine inhibited MDMA-induced hyperlocomotion (Fig. 6h) while maintaining a normal level of locomotion similar to that seen before MDMA injections.

Carvedilol also had a strong attenuating effect on MDMA-induced increases in NAc, temporal muscle, and skin temperatures, returning these parameters to near-

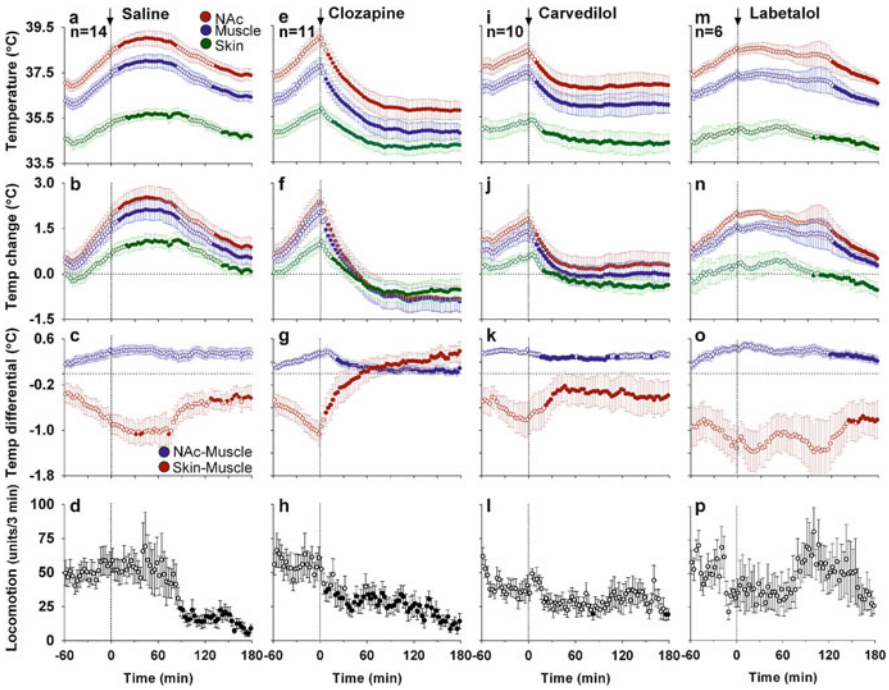

Fig. 6 The effects of clozapine, carvedilol, and labetalol on MDMA-induced temperature and locomotor responses. (**a**–**d**) MDMA–saline: absolute temperatures, relative temperatures, NAc-Muscle and Skin-Muscle differentials, and locomotion. (**e**–**h**) MDMA–clozapine, (**i**–**l**) MDMA–carvedilol, and (**m**–**p**) MDMA–labetalol. The graphs show changes in different parameters before and after administration of each testing drug and saline (0 min). In each group, the rats received a single injection of MDMA and the testing drug/saline was injected at the time of gradual NAc temperature increase in the range of 38.5°C (mean 83 min, range 57–140 min). *Filled symbols* show values significantly different from the last pre-treatment value; the absence of *filled symbols* indicates the absence of a significant effect on a specified parameter evaluated with one-way ANOVA, *n*: the number of rats (original data of this study were published in [78])

baseline levels by the end of the session (Fig. 6i–l). Carvedilol also attenuated the vasoconstrictive effects of MDMA as evidenced by a moderate increase in Skin-Muscle differentials, but this effect was short-lived and lasted only ~100 min post-treatment. Carvedilol minimally affected NAc-Muscle differential (Fig. 6k) and the effect of treatment vs. control was not significant. Lastly, carvedilol inhibited MDMA-induced locomotor activation, although locomotor activity was maintained at normal levels throughout the recording session (Fig. 6l). Labetalol had no evident effects on any of the temperature responses caused by MDMA plus social interaction (Fig. 6m–p).

To further compare the efficacy of each drug in attenuating MDMA-induced hyperthermia, we calculated the mean differences between the effects of MDMA + Treatment drug and MDMA + Vehicle (Fig. 7, left panels). Additionally, we approximated temperature change, as analyzed by the integral of the difference

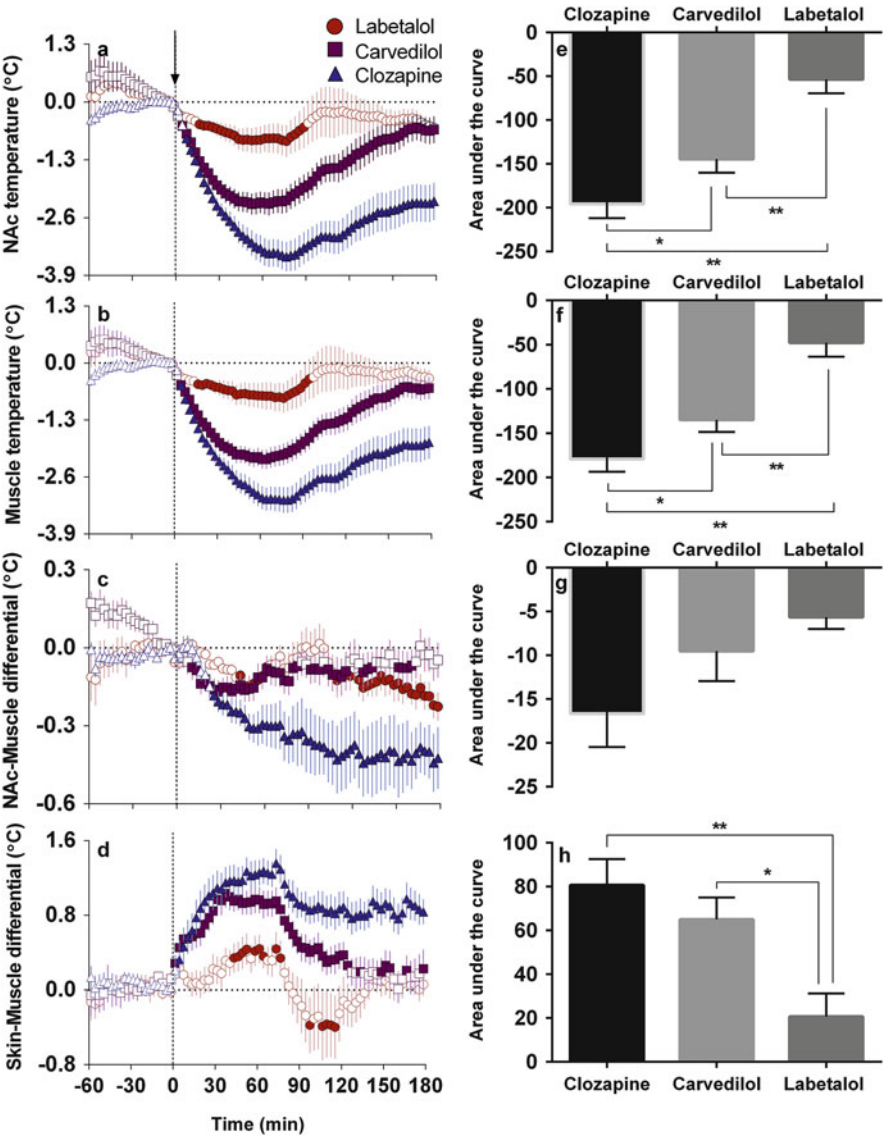

Fig. 7 Comparative effectiveness of different drugs in reversing MDMA-induced temperature responses. *Left panel* shows the time-course of the effects of each drug (difference vs. saline) on (**a**) brain and (**b**) muscle temperatures as well as (**c**) NAc-Muscle and (**d**) Skin-Muscle temperature differentials. *Right panel* shows the mean effects (area under curve for 80 min post-injection) for each testing drug (**e–h**). Time-course data were analyzed using one-way ANOVA with repeated measures; *filled symbols* show the values significantly different from the last pre-treatment value (Fisher PLSD test). *Bar graph* data were analyzed using one-way ANOVA; *asterisks* show significant between-drug differences (*$p < 0.05$; **$p < 0.01$). Original data shown in this graph were reported in Kiyatkin et al. [78]

between saline and drug treatment groups (i.e., area under the curve) for the time of maximal effect (80 min; Fig. 7, right panels).

As can be seen in Fig. 7, the attenuating effect of clozapine on NAc and muscle temperatures appeared with the shortest latencies (~6 min), and displayed the greatest magnitude and longest duration. Accordingly, the effect of clozapine on NAc and Muscle temperatures was significantly stronger ($p < 0.05$) than the effects of carvedilol and labetalol.

Clozapine also has the greatest attenuating effects on MDMA-induced intra-brain heat production and skin vasoconstriction, showing the largest decrease in NAc-Muscle differential and a strong, sustained increase in Skin-Muscle differential, respectively. In contrast, carvedilol had a much weaker effect on NAc-Muscle differentials and a less pronounced, short-lived effect on Skin-Muscle differentials. Finally, labetalol had minimal effects on MDMA-induced changes in all temperature measures.

6 Targets and Pathways to Alleviate MDMA-Induced Hyperthermia

The downstream pharmacological and biochemical mechanisms underlying MDMA-induced hyperthermia are complex and involve multiple factors, including excessive sympathetic activation, dopamine-mediated hyperactivity, decoupling of mitochondrial ATP, and heat production in both the brain and periphery (see [79] for review). As such, rather than focusing on the specific downstream neurotransmitter, receptor, or signaling mechanisms, we chose to focus on the basic physiological mechanisms underlying MDMA-induced hyperthermia and its reversal by the treatment drugs.

Clozapine (1–5 mg/kg) has been previously shown to decrease high-dose MDMA-induced body hyperthermia via vasodilation, as assessed by an ear pinna artery Doppler signal in rabbits, and blood flow Doppler signal in the tails of rats [66]. Consistent with these data, we found that a low ip dose of clozapine (5 mg/kg or ~2% of LD50; [80]) completely reversed MDMA-induced brain and body hyperthermia. The temperature decrease was exceptionally rapid, appearing within ~6 min, and both brain and muscle temperatures returned to the initial, quiet resting baseline within ~30 min post-injection. Consistent with its central, antipsychotic action, clozapine strongly reduced MDMA-induced increases in NAc-Muscle temperature differentials, suggesting a gradual decrease in intra-brain heat production due to blockade of drug-induced metabolic brain activation. Within the first 3 min post-treatment, clozapine also reversed the decreases in Skin-Muscle temperature differentials, suggesting a rapid blockade of skin vasoconstriction. In addition, clozapine decreased MDMA-induced locomotor activation while maintaining normal activity levels and showing no evident signs of sedation. Interestingly, when used in intact rats, clozapine at the same dose (5 mg/kg, ip) slightly decreased NAc

and muscle temperatures, with no effects on locomotion and NAc-Muscle temperature differentials [55]. This pattern of action suggests the absence of a true sedative effect.

Carvedilol and labetalol are mixed alpha–beta adrenoceptor blockers that directly dilate blood vessels [70, 71], thus increasing heat dissipation from skin surfaces [81, 82]. At a relatively low dose (5 mg/kg or 0.6% of LD50; [83]) carvedilol also reversed MDMA-induced brain and body hyperthermia but its effects were slower, weaker, and more transient than those of clozapine. In contrast to the strong effects of clozapine on MDMA-induced increases in NAc-Muscle differentials, peripherally acting carvedilol had virtually no effects on this metabolism-related parameter. Surprisingly, the effects of carvedilol on MDMA-induced changes in Skin-Muscle differentials, an index of cutaneous vascular tone, were weaker and incomplete compared to the effects of clozapine. Carvedilol also had minimal effects on MDMA-induced hyperlocomotion, and the rats' activity remained at normal levels, suggesting lack of sedative effects. When used in intact rats, carvedilol slightly decreased brain and muscle temperatures, and this effect was observed in conjunction with the rise of Skin-Muscle temperature differentials [55], suggesting peripheral vasodilation. Consistent with its peripheral mechanism of action, carvedilol had no effects on either NAc-Muscle differentials or animal locomotion, suggesting a slight drop in brain and body temperature, resulting from transient dilation of skin vessels and increased heat dissipation.

Although human reports suggest that labetalol taken before MDMA exposure decreases MDMA-induced hyperthermia [84], in our hands this drug was ineffective and had minimal, if any, effects on all temperature parameters. The reasons for the differences between carvedilol and labetalol are unclear and may be due to their different affinities for the various subgroups of alpha- and beta-adrenoceptors [85]. As a vasodilator, labetalol also appears to be less potent than carvedilol [86]; therefore, the different effects of the drugs in our study may be due to the choice of a uniform drug dose. However, our exploratory tests with a higher dose of labetalol (20 mg/kg) did not reveal clear effects in attenuating MDMA-induced hyperthermia and also led to adverse side-effects.

In conclusion, the data indicate that carvedilol, by acting directly on blood vessels, is modestly effective in attenuating MDMA-induced brain and body hyperthermia. In contrast, clozapine induces much more rapid and powerful hypothermic effects by both decreasing MDMA-induced brain activation and diminishing the sympathetic outflow to peripheral vessels. A therapeutic agent such as clozapine that not only mitigates, but reverses, MDMA-induced hyperthermia could be indispensable for emergency situations and could save the lives of highly intoxicated individuals.

7 Conclusions

While usually underappreciated, brain temperature is an important physiological parameter that reflects metabolic neural activity and affects all types of neural function. Despite widely held beliefs that brain temperature is a stable homeostatic parameter, the data presented in this review clearly demonstrate that brain temperature can vary within relatively large limits during normal physiological activities and after exposure to different psychoactive drugs. Our focus in this review was on psychomotor stimulants, which in some drug users are known to induce extreme hyperthermia – the most dangerous health complication produced by these drugs. While a large dose of injected or ingested drug is usually viewed as the most important parameter for inducing pathological hyperthermia, this review demonstrates that the temperature effects of drugs are strongly modulated when they are used during physiological activation and in moderately warm environments. Social interaction, a procedure used in rats to model human psychophysiological activation moderately enhances intra-brain heat production and limits heat dissipation due to peripheral vasoconstriction; its combination with the similar but more sustained effects induced by psychomotor stimulants results in a strong potentiation of drug-induced hyperthermic responses. Similar potentiation could occur when the drug is used in a warm environment where proper heat dissipation is important to maintain brain and body temperature homeostasis. Therefore, a certain drug used under adverse environmental conditions could induce unexpectedly strong effects and even lethality at doses that are usually viewed as "safe."

Among the drugs considered in this review, MDMA showed the most powerful potentiation both during social interaction and in a warm environment. While the exact molecular and receptor mechanisms underlying this potentiation remain unclear, at the physiological level large doses of MDMA induce prolonged increase in metabolic neural activity coupled with strong and sustained peripheral vasoconstriction. Although slightly less but significant vasoconstriction was also induced by MDPV and methylone, these two drugs have minimal effects on intra-brain heat production possibly reducing the effects of the cathinone drugs on state-dependent and environmental potentiation of their hyperthermic effects. Finally, we tried to demonstrate how the knowledge of physiological mechanisms underlying hyperthermia could help in finding pharmacological tools for its reversal. Using MDMA, we showed that centrally acting clozapine, an atypical neuroleptic, is more efficient in reversing pathological hyperthermia than peripherally acting vasodilators. Since there are no effective drugs that could be used during MDMA-induced overdose in emergency-room environment, these data could have translational importance, helping to save the lives of highly intoxicated individuals.

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Disclosure of Potential Conflicts of Interest No potential conflicts of interest are disclosed.

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