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ENVIRONMENTAL CONDITIONS MODULATE NEUROTOXIC EFFECTS OF PSYCHOMOTOR STIMULANT DRUGS OF ABUSE

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Abstract

Psychomotor stimulants such as methamphetamine (METH), amphetamine, and 3,4-Metylenedioxymethamphetamine (MDMA or ecstasy) are potent addictive drugs. While it is known that their abuse could result in adverse health complications, including neurotoxicity, both the environmental conditions and activity states associated with their intake could strongly enhance drug toxicity, often resulting in life-threatening health complications. In this review we analyze results of animal experiments that suggest that even moderate increases in environmental temperatures and physiological activation, the conditions typical of human raves parties, dramatically potentiate brain hyperthermic effects of METH and MDMA. We demonstrate that METH also induces breakdown of the blood-brain barrier (BBB), acute glial activation, brain edema, and structural abnormalities of various subtypes of brain cells; these effects are also strongly enhanced when the drug is used at moderately warm environmental conditions. We consider the mechanisms underlying environmental modulation of acute drug neurotoxicity and focus on the role of brain temperature, a critical homeostatic parameter that could be affected by metabolism-enhancing drugs and environmental conditions and affect neural activity and functions.

Keywords

methamphetamine; hyperthermia; brain damage; brain temperature; blood-brain barrier; neurotoxicity

I. Introduction

Psychomotor stimulants such as methamphetamine (METH), amphetamine, and 3,4-Metylenedioxymethamphetamine (MDMA or ecstasy) are potent addictive drugs with neurotoxic properties. Approximately 25 millions people use amphetamine-like drugs worldwide, making them the second most commonly used group of illicit drugs after cannabis (United Nations Office of Drugs and Crime, 2008). Along with other drugs, METH and ecstasy are often referred to as “club drugs,” which tend to be used by teenagers and young adults at bars, nightclubs, concerts, and parties. It is quite difficult to estimate such drug use quantitatively, but it appears to be wide spread based on the numbers of reported medical complications. In addition to the social harms of addiction, the use of psychomotor stimulants could adversely influence human health, causing acute behavioral and

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physiological disturbances during intoxication and long-term health complications following chronic use (Kalant, 2001). By inducing powerful and prolonged physiological activation, psychomotor stimulants could be a co-factor in enhancing different latent pathological conditions, especially cardiovascular, neurological and psychiatric. By weakening the immune system, chronic drug use also increases the probability and severity of numerous viral and bacterial infections.

Considering the issue of neurotoxicity, it is usually assumed that METH and related drugs have direct toxic effects on neural cells, with relative selectivity towards specific cell groups, brain structures, and cellular organelles. In particular, METH preferentially affects midbrain dopamine (DA) cells, damaging fine axonal terminals in the striatum (Ricaurte *et al.*, 1980; Riddle *et al.*, 2006; Woolverton *et al.*, 1989) and resulting in health complications associated with pathologically altered DA transmission. Alterations in activity and responsiveness of DA and other monoamine systems are important factors in psycho-emotional and psychiatric disorders including acute METH psychosis and severe depression following long-term METH use (Kalant, 2001).

However, METH and other psychomotor stimulant drugs also induce metabolic activation and body hyperthermia (Alberts and Sonsalla, 1995; Estler, 1975; Freedman *et al.*, 2005; Gordon *et al.*, 1991; Kalant and Kalant, 2008; Makisumi *et al.*, 1998; Sandoval *et al.*, 2000). Enhanced metabolism is tightly related to oxidative stress, which is caused by an imbalance between the production of reactive oxygen and the ability of an organism to detoxify the reactive intermediates and repair the resulting damage. Disturbances in this normal reduction:oxidation state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress as a consequence of brain hyper-metabolism is usually viewed as a primary factor of METH-induced neurotoxicity (Cadet *et al.*, 2007; De Vito and Wagner, 1989; Stephens and Yamamoto, 1994). On the other hand, brain cells are exceptionally temperature-sensitive, with the appearance of structural abnormalities at ~40°C, i.e., only three degrees above a normal baseline (Chen *et al.*, 2003; Iwagami, 1996; Oifa and Kleshchnev, 1985; Sharma and Hoopes, 2003; Yamamoto and Zhu, 1998). Due to temperature dependence of most physico-chemical processes governing neural activity (see [Kiyatkin, 2010] for review), hyperthermia also enhances the toxic effects of METH on brain cells. From animal experiments, it is well known that METH is much more toxic at high ambient temperatures, whereas toxicity is diminished by low ambient temperatures [Alberts and Sonsalla, 1995; Ali *et al.*, 1994; Bowyer *et al.*, 1993; Farfel and Seiden, 1995; Gordon *et al.*, 1991; Miller and O'Callaghan, 1994, 2003). Although it is reasonable to assume that more harmful effects of METH seen in warm, humid conditions are associated with intra-brain heat accumulation due to enhanced brain metabolism diminished heat dissipation, direct data on brain temperature fluctuations induced by METH and related drugs as well as on environmental modulation of these drug-induced temperature fluctuations are limited.

In addition to the direct effects of high temperatures on brain cells and potentiation of toxic effects of drug metabolites, brain hyperthermia appears to alter permeability of the blood-brain barrier (BBB). The BBB is an important border that maintains stability of the brain environment and protects neural cells from potentially dangerous ionic and chemical perturbations occurring in the body (Rapoport, 1976; Zlokovic, 2008). Although leakage of the BBB has been documented during environmental warming (Cervos-Navarro *et al.*, 1998; Sharma *et al.*, 1992), intense physical exercise (Watson *et al.*, 2005), various types of stress (Esposito *et al.*, 2001; Ovadia *et al.*, 2001; Sharma and Dey, 1986) and morphine withdrawal (Sharma and Ali, 2006), data on drug-induced alterations in the BBB and its relationship to

brain temperature are limited. Moreover, the basic relations between BBB permeability and temperature remain unclear.

In this work we present and discuss several sets of recent data on environmental modulation of the physiological effects of METH and MDMA and a tight link between brain temperature, acute drug toxicity, and alterations of the BBB. We demonstrate that brain hyperthermia induced by psychomotor stimulant drugs plays an important role in the triggering of several pathophysiological mechanisms underlying acute and chronic drug neurotoxicity.

II. Brain temperature responses to METH and MDMA are modulated by activity state and environmental conditions

The effects of addictive drugs are usually studied in animals under well-controlled experimental conditions. In addition to standard temperature and humidity, drugs are typically administered after animals' habituation to the testing environment and under quiet resting conditions when baselines are stable and low. In contrast, humans often use the same drugs voluntarily, in different doses, under conditions of psychophysiological activation (that usually precedes drug intake) and in specific environmental conditions that often dramatically differ from those in animal experiments. For example, METH and other psychomotor stimulant drugs (i.e., MDMA) are often taken during raves, i.e., under conditions of psychophysiological and behavioral activation and in hot and humid environment that seriously impact an organism's thermoregulatory mechanisms. To examine how the effects of psychomotor stimulants are modulated by activity states and environmental conditions, we performed a series of studies, focusing on brain temperature as a primary parameter of interest (Brown *et al.*, 2003; Brown and Kiyatkin, 2004). First, we examined how METH and MDMA, at different doses, affects brain temperature and what the relationships between these temperatures and those recorded from various body locations are. Second, we examined how temperature effects of this drug are modulated during associated physiological activation and in a moderately hot environment that model the conditions of raves. To model psychophysiological activation, we used the procedure of social interaction, in which the recorded male rat was exposed to a female rat, resulting in behavioral activation and a clear temperature response. The effects of METH and MDMA were also compared in two ambient temperatures: 22–23°C (laboratory standard), and 29°C (moderately warm conditions corresponding to temperature comfort in rats).

Our studies revealed that METH induces dose-dependent temperature increases, which were generally correlative in the brain and body core. At the lowest dose (1 mg/kg, sc), the increase had the smallest amplitude and duration (~1.0°C for ~160 min) and was progressively larger and more prolonged (~3.4°C for 360 min) at high doses (9 mg/kg). At the latter dose (see Fig. 1A), brain hyperthermia even at standard ambient temperatures (22–23°C), reached clearly pathological levels (~40°C or 3.5°C above baseline). This temperature increase correlated with locomotor hyperactivity, which was evident and strong at 1 mg/kg and greatly progressed (with the addition of strong stereotypy) at high drug dose. The hyperthermic response to METH had two important features. First, although temperatures in the NAcc and muscle generally paralleled (Fig. 1A), the increases were significantly more rapid and stronger in the brain than temporal muscle, resulting in significant increases in brain-muscle differentials. Therefore, it appears that metabolic brain activation is the primary cause of brain hyperthermia and a factor behind more delayed and weaker body hyperthermia. Although similar increases in brain-muscle differentials occurred during exposure to natural arousing stimuli (i.e., tail-pinch and social interaction), in the case of METH the increase was robust and continued for more than five hours, suggesting a pathological aspect to this brain hyperthermia. Second, temperature increase in

brain sites and muscle was consistently associated with a rapid and prolonged decrease in skin-muscle temperature differential, suggesting peripheral vasoconstriction. This mechanism decreases heat dissipation to the external environment, thus contributing to overall brain and body hyperthermia.

Hyperthermic effects of METH became stronger when the drug was injected during social interaction (Fig. 1B). After presentation of the female, the recorded male showed a strong increase in brain and muscle temperatures, which additionally increased after METH injection. While the effect was not additive, METH-induced brain hyperthermia during social interaction reached significantly higher values and was maintained for a significantly longer time than in quiet resting conditions.

Hyperthermic effects of METH were also altered when the drug was administered in a warm (29°C) environment (Fig. 1C). In this case, mean temperatures after drug administration increased rapidly in all animals, in some of them the increase reached clearly pathological values (>41 °C), and 4/6 animals died within three hours. Again, temperature increases in the brain sites were consistently more rapid and stronger than in the muscle, and the increase in NAcc-muscle differential reached pathological levels not seen in any other physiological conditions. However, at the moment of death, brain-muscle differentials rapidly inverted and the brain became cooler than the body. A high toxicity of METH at moderately warm ambient temperatures (which were only 5–6°C higher than the laboratory standard, corresponding to normothermy or temperature comfort in rats [Romanovsky *et al.*, 2002]) is in sharp contrast to the known LD50 for METH, which is 55 and 57 mg/kg with intraperitoneal administration in rats and mice, respectively (Devis *et al.*, 1987; Yamamoto, 1963).

Classic features of neurotoxicity induced by amphetamine-like substances (i.e., neuronal necrosis and apoptosis) are usually linked to some toxic products (i.e., nitric oxide, catechol-quinones, peroxynitrite) of abnormally increased metabolism of endogenous neurotransmitter substances (Cadet *et al.*, 2007; Kuhn and Geddes, 2000). While these factors may contribute to neural damage following acute and chronic use of these substances, our present data suggest the importance of brain overheating as a factor responsible for fatal decompensation of an organism's vital functions during acute drug intoxication. The rise in brain temperature above certain limits may *per se* have a direct destructive action on brain cells, which will increase exponentially with slight increases above these limits. The most temperature-sensitive cellular elements are mitochondrial and cellular membranes, in which irreversible transitions in protein structure or arrangements begin to occur at temperatures higher than 40°C (Stephans and Yamamoto, 1994; Lepock *et al.*, 2003; Willis *et al.*, 2000). Therefore, 40°C could be considered the threshold of pathological hyperthermia, which could have long-term negative consequences even if the temperature will later return to its baselines.

Since the rats that died following METH and MDMA intoxication in a moderately warm environment showed some clinical features suggesting brain edema, we hypothesized that the destruction of endothelial cells in the brain and leakage of serum proteins across the BBB induced by high temperature could be an important pathogenic factor responsible for this life-threatening condition. To verify this hypothesis, we conducted a series of histochemical and morphological studies (Kiyatkin *et al.*, 2007; Sharma and Kiyatkin, 2009).

III. Adverse Environmental Conditions Enhance Histochemical and Morphological Perturbations Induced by METH: Role of Brain Temperature

In these experiments, physiological recordings were supplemented by histochemical and morphological examination of brain tissue to determine acute changes in BBB permeability, glial activation, and brain cell morphology during acute METH intoxication in standard (23°C) and warm (29°C) ambient temperatures. When the brain temperature peaked or reached clearly pathological values (>41.5°C), the rats were rapidly anesthetized, perfused, and brains were taken for analysis. The state of BBB permeability and edema were determined by albumin immunoreactivity and measuring brain water and ion (Na⁺, K⁺, Cl⁻) content. Albumin is a relatively large plasma protein (molecular weight 59 kDa, molecular diameter 70Å) that is normally confined to the luminal side of the endothelial cells and is not present in the brain under normal conditions. Thus, the appearance of albumin immunoreactivity in brain cells or neuropil indicates a breakdown of the BBB. Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that is expressed in glial cells (astrocytes) and increased GFAP immunoreactivity (or astrocytic activation) is usually viewed as an index of gliosis or a relatively slow-developing correlate of neural damage (Finch, 2003; Hausmann, 2003). Normal brain tissue has only scattered GFAP-positive cells but rapid GFAP expression has been reported previously during environmental warming and brain trauma (Sharma *et al.*, 1992; Gordth *et al.*, 2006). To determine morphological abnormalities of brain cells, slices were analyzed with light and electron microscopy to determine the extent of structurally abnormal cells and specifics of cellular abnormalities. In a separate experiment, we also evaluated expressions of heat shock proteins (HSP 72 kDa), a sensitive marker of metabolic activation and oxidative stress (Kiyatkin and Sharma, 2011).

As shown in Fig. 2A, METH induced significant BBB leakage. Compared to saline-treated controls, albumin immunoreactivity increased strongly in both METH groups and the changes were significantly larger when the drug was administered at 29° than 23°C. These changes were evident in each of four structures examined: the cortex (the sum of cingulate, parietal, temporal and piriform cortices), hippocampus, thalamus, and hypothalamus. As shown in Fig. 3A, albumin immunoreactivity was also strongly dependent on brain temperature, with virtually no positive cells at low basal temperatures and a progressive increase at high temperatures. Similar differences were found with respect to astrocytic activation (Fig. 2B). While only a few GFAP-positive astrocytes were scattered in the normal brains, their number was significantly larger in the METH-23°C group, and almost doubled in the METH-29°C group. Similar to albumin, GFAP counts were also tightly correlated with brain temperatures (Fig. 3A), suggesting that acute glial reaction is progressively stronger depending on the extent of brain temperature elevation. METH intoxication also strongly increased brain water content, and this increase was enhanced when the drug was used at warm environmental temperatures (Fig. 2C). In each brain structure, tissue water content was directly related to brain temperatures (Fig. 3B), suggesting tight relationships between brain hyperthermia and edema. Finally, METH induced a strong increase in the number of morphologically abnormal cells in each brain structure and this effect becomes much stronger when the drug was used at 29°C (Fig. 2D).

METH-induced changes in brain morphology were associated with profound changes in all other analyzed parameters (Fig. 4). The number of abnormal cells in the cortex tightly correlated with *brain and muscle temperatures* (Fig. 4A), *albumin leakage* and the extent *GFAP immunostaining* (Fig. 4B), and tissue *water accumulation* (Fig. 4C). This relationship appears to be a reflection of generalized edema, which is evident when METH is used at 23°C and progresses when the drug is used at 29°C.

METH-induced changes in brain morphology were associated with profound changes in all other analyzed parameters (Fig. 4). While no abnormal cells were found in the cortex in control animals, their number increased in METH-treated animals, showing tight linear correlation with *brain and muscle temperatures* (Fig. 4A). Cortical neural damage during METH intoxication was tightly related to *albumin leakage* and the extent *GFAP immunostaining* (Fig. 4B). The number of damaged cortical cells during METH intoxication correlates linearly with tissue *water accumulation* (see Fig. 4C). This relationship appears to be a reflection of generalized edema, which progresses when METH is used at 29°C, resulting in more profound cellular damage.

Therefore, these data suggest that acute METH intoxication results in robust breakdown of the BBB, glial activation, and numerous morphological abnormalities of different subtypes of brain cells. These effects are strongly enhanced when the drug is used in a moderately warm environment, tightly correlating with drug-induced brain and body temperature elevation.

IV. Temperature modulation of BBB permeability

Temperature stability is the essential condition for normal functions of any living cell in multicellular organisms. In addition to the known temperature modulation of neural activity, fluctuations in brain temperature could adversely affect brain cells and brain functions. While brain cells seem to well tolerate low temperatures (Arai *et al.*, 1993; Lucas *et al.*, 1994), multiple *in vitro* studies suggest that high temperature (>40.0°C) has destructive effects on various cells (Iwagami, 1996; Willis *et al.*, 2000), especially prominent in metabolically active brain cells (Chen *et al.*, 2003; Lee *et al.*, 2000; Li *et al.*, 2004; Lin *et al.*, 1991; Oifa and Kleshchenov, 1985), including neuronal, glial, endothelial and epithelial cells (Bechtold and Brown, 2003; Sharma and Hoopes, 2003). Rapid damage to brain cells has been also documented *in vivo* during extreme environmental warming (Cervos-Navarro *et al.*, 1998; Lin *et al.*, 1997; Sharma *et al.*, 1992) and acute METH intoxication (Kiyatkin *et al.*, 2007; Sharma and Kiyatkin, 2009), which both result in robust brain hyperthermia as well as increased permeability of the blood-brain barrier (BBB) and vasogenic edema. The integrity of the BBB is also compromised during opiate withdrawal (Sharma and Ali, 2006), intense physical exercise in a warm environment (Watson *et al.*, 2005) and during restraint and forced swim stress (Esposito *et al.*, 2001; Ovadia *et al.*, 2001)—conditions associated with brain hyperthermia. Although all these data implicate brain hyperthermia as a leading factor in BBB leakage and subsequent damage to brain cells, these changes may be also affected by many other factors (i.e., metabolic brain activation, oxidative stress, alterations in cerebral blood flow, hypoxia of different extent) (Cadet *et al.*, 2001; Nybo, 2008; Sharma, 2006), which contribute to alterations in BBB permeability and subsequent structural brain damage (Haorah *et al.*, 2007; Hom *et al.*, 2001; Kaur *et al.*, 2008).

To clarify the relations between brain temperature and BBB permeability, it is essential to delineate this physical factor from other possible contributors. To reach this goal, we examined several brain parameters in pentobarbital-anesthetized rats, which bodies were passively warmed to produce different levels of brain temperature, which was monitored by chronic brain thermocouple probes (Kiyatkin and Sharma, 2009). As shown in our previous study (Kiyatkin and Brown, 2005), sodium pentobarbital administered at a typical anesthetic dose (50 mg/kg, ip) under standard laboratory conditions (23°C) induces robust brain and body hypothermia (~31–33°C) associated with relative skin warming, suggesting loss of vascular tone and increased heat loss to the environment. In addition to metabolic inhibition that is a primary cause for brain and body hypothermia, anesthetized rats became very sensitive to changes in environmental temperatures, becoming hypothermic at low ambient temperatures and hyperthermic when their bodies are warmed. Therefore, by changing the

intensity of body warming, we were able to produce wide range of brain temperatures from very low, hypothermic (with no passive warming) to very high, hyperthermic (32–42°C) (with warming of different intensity). The brains were taken in all animals at the same time point after the start of anesthesia (90 min) and the initiation of body warming (but at different levels of brain temperature) and analyzed for several brain parameters. To evaluate the integrity of BBB and acute glial activation we used immunohistochemistry for endogenous albumin and GFAP. Brains were also evaluated for water and ion content and for the presence of morphologically abnormal cells.

As shown in Fig. 5A (left column), the number of albumin-positive cells is strongly dependent on brain temperature, being minimal at normothermic values (34.2–38.0°C), slightly larger (2–4-fold) at hypothermic values (34.2–32.2°C), and dramatically larger (~26-fold) at hyperthermic values (38.0–42.5°C). The increase was evident from 38–39°C, progressed at higher temperature, and plateaued at high levels at 41–42°C. Temperature-dependence of albumin immunoreactivity was evident in each tested brain structure, having some structural differences (Fig. 5B). Similar relationships were found for brain temperature and GFAP expression (Fig. 5, middle column). In this case, GFAP expression grew between 38 and 39°C and plateaued at 40°C (3–4-fold increase). This parameter showed larger between-structure variability and the thalamus showed the largest number of GFAP-positive cells at low temperatures and the increase during hyperthermia.

It is well established that albumin entry from the peripheral circulation to the brain results in increased tissue water content associated with robust alterations in ionic brain balance (Rapoport, 1976; Zlokovic, 2008). These alterations result in vasogenic edema—a dangerous and often fatal complication of various pathological processes in the brain as well as conditions associated with brain hyperthermia (i.e., heat stress, opiate withdrawal, METH and MDMA intoxication). As shown in this study, tissue water content (evaluated in the cortex and thalamus) was also strongly dependent on brain temperature (Fig. 6). With respect to normal temperatures, cortical water content was significantly higher during hyperthermia and significantly lower during hypothermia. Within the range of recorded temperatures, cortical water differed within ~4%. In the thalamus, water content was clearly higher during hyperthermia, but values at low and normal temperatures were similar. Cortical water content during anesthesia in normothermic conditions was virtually identical to that in control awake animals (see hatched lines), but significantly higher (edema) in hyperthermia and significantly lower (dehydration) in hypothermia. A similar trend was seen in the thalamus, where water content during anesthesia was lower than in control at low and moderate brain temperatures and similar to control at high temperatures. Moreover, the numbers of albumin-positive cells and tissue water were tightly interrelated both in the thalamus and cortex ($r=0.96$ and 0.89 , respectively). This correlation was highly linear in the thalamus, but had some divergence in the cortex at values that correspond to extreme hypothermia (see circle in Fig. 7A). Despite the presence of few albumin-positive cells (see also Fig. 5B), cortical water was relatively lower, suggesting that the tight correlation between brain albumin and water, which exists within the entire range of normal and high temperatures, could be distorted at very low temperatures. Therefore, in contrast to brain edema during extreme hyperthermia, the brain appears to be dehydrated during extreme hypothermia. Although it is known that brain temperatures never drop below 34–35°C in any physiological conditions, this hypothermia-related perturbations could be relevant for several unusual situations, including general anesthesia, extreme environmental cooling, and over-dose with powerful sedative drugs.

This study confirmed multiple *in vitro* observations, suggesting that brain cells are exceptionally sensitive to thermal damage and demonstrates that the number of structurally abnormal cells directly and strongly depends on brain temperature (see Fig. 5, right column).

A few abnormal cells were found at ~38.5°C, and their numbers gradually increased as temperature rose. While the counts of albumin-positive cells plateaued at high temperatures, morphological abnormalities linearly increased and peaked at the maximal detected temperature (42.4°C). This pattern was evident in each of four tested structures (Fig. 5B), although some areas (i.e., thalamus) or cortical sub-areas (i.e. piriform cortex), which showed robust BBB leakage, also showed more profound structural cell abnormalities. Similar to other parameters, structural abnormalities occurred relatively quickly and were tightly related to BBB leakage, glial activation, and increased tissue water content (see Fig. 7B–D). Therefore, even with passive warming, morphological damage reflects not only the effect of temperature *per se*, but also BBB leakage and associated edema.

These data indicate that brain hyperthermia, independently of its cause, could be a strong factor, eliciting breakdown of the BBB, glial activation, and morphological abnormalities of brain cells. Importantly, these changes appeared relatively quickly (20–80 min) and within the range of physiological hyperthermia (38.5–39.5°C), suggesting that increased BBB permeability is not solely pathological, but also a normal physiological phenomenon occurring during various conditions associated with hyperthermia. Such hyperthermia, for example, occurs in rats during copulatory behavior (Mitchum and Kiyatkin, 2003; Kiyatkin and Mitchum, 2004) and heroin self-administration (Kiyatkin and Wise, 2002). Although temperature dependence of BBB permeability was evident in all tested brain structures, suggesting its generalized nature, there were also minor but significant differences among individual brain areas.

V. Conclusions and Perspectives

While slowly developing, selective, and irreversible damage of specific central neurons is the traditional focus of neurotoxic studies of METH and related drugs, this work demonstrates that robust morphological abnormalities of neural and non-neural brain cells (i.e., glia, vascular endothelium, epithelium) could occur rapidly (within 30–80 min) during acute METH intoxication. Moreover, these abnormalities greatly enhanced when the same drug was used in moderately warm environments, resulting in larger brain temperature elevations.

Since brain cells of various subtypes are exceptionally sensitive to high temperature (Chen *et al.*, 2003; Kiyatkin, 2005; Lee *et al.*, 2000; Li *et al.*, 2004; Lin *et al.*, 1991; Sharma and Hoopes, 2003), brain hyperthermia could be viewed as an important contributor to morphological abnormalities induced by METH. However, this does not mean that high temperature *per se* is the cause of these changes. Brain hyperthermia is not only a physical factor that could harm cells; it is also an integral physiological index of METH-induced metabolic activation (see [Kiyatkin, 2005] for review) that also manifests as an enhanced release of multiple neuroactive substances, lipid peroxidation, and the generation of free radicals—numerous changes combined as oxidative stress (Cadet *et al.*, 2007; Seiden and Sabol, 1996) as well as behavioral and autonomic activation. Although all these factors may contribute to structural brain abnormalities, it is quite difficult to separate them from one another because they are inter-dependent, representing different manifestations of METH-induced metabolic activation.

METH intoxication also results in a robust increase in BBB permeability, intra-brain water accumulation (edema), and serious alterations in brain ionic homeostasis. These changes, moreover, are tightly related to both the degree of hyperthermia and the intensity of structural brain damage. Therefore, breakdown of the BBB that allows the diffusion of endogenous albumin, water, several ions, and other neuroactive and potentially neurotoxic substances is another important contributor to brain pathology and the primary mechanism

underlying decompensation of vital functions and lethality. While different chemical factors activated by METH could be involved in increased BBB permeability and edema formation, brain hyperthermia appears to play a crucial role since both these parameters strongly correlate with brain temperature.

Although our data indicate that acute METH intoxication results in rapidly developing morphological abnormalities in neural and non-neural brain cells, it remains unclear whether these abnormalities are reversible or irreversible in nature. Dramatic changes in cellular elements (e.g., degeneration of some neuronal nucleus, endothelial cell membrane bleb-formation, vesiculation of myelin and vacuolation in the neuropil with clear signs of synaptic damage and swelling), especially evident in the METH-29°C group, appear to be inconsistent with normal cell functions, pointing at irreversible damage. However, some of these changes appear to be transient and reversible and they could disappear after basic homeostatic parameters are restored to baseline. In light of the extent of these morphological abnormalities, we can speculate that they could result in irreversible cellular damage but this issue needs to be examined further. While our studies support the idea that glial activation could occur rapidly (Sharma *et al.*, 1992; Gordth *et al.*, 2006), different mechanisms appear to mediate rapid and slow glial reactions. GFAP expression is usually thought of as a late outcome of traumatic, ischemic, or hypoxic insults or a correlate of various neurodegenerative diseases (Finch, 2003; Hausmann, 2003; Gordth *et al.*, 2006), representing astrogliosis (Norton *et al.*, 1992; O'Callaghan, 1993). In contrast, rapid GFAP expression seen in association with strong edema (environmental warming, acute trauma, METH intoxication) could reflect the interaction of antibodies with GFAP that was released or during membrane damage. Thus, binding sites to GFAP could be increased due to acute breakdown of the BBB and associated edema rather than proliferation of astrocytes or elevated levels of GFAP proteins that require more time. Since damage of astrocytes and swelling of the astrocytic end foot results in increased binding of GFAP antibodies (Bekay *et al.*, 1977; Bondarenko and Chesler, 1993; Gordth *et al.*, 2006), this reaction could reflect acute, possibly reversible, damage of glial cells. Relatively smaller numbers of damaged neural cells in the post-intoxication period compared to acute METH intoxication (Bowyer and Ali, 2006) could also be related to their rapid scavenging, making it difficult to detect them using traditional techniques. Although the issue of the extent of damage and its reversibility remains unanswered and requires additional studies, it is likely that rapid cell abnormalities may initiate cascades that could precipitate cellular and molecular dysfunctions, leading to neurodegeneration—the most dangerous outcome of chronic abuse with amphetamine-like drugs.

Although heat *per se* could selectively damage brain cells, hyperthermia also increases BBB permeability, thus allowing entry to the brain from peripheral circulation of various potentially neurotoxic substances, ions, and water. Therefore, damage of brain cells under conditions of hyperthermia reflects the effects of not only temperature but also of multiple potentially dangerous influences. Robust leakage of the BBB under conditions of hyperthermia could be an important factor in brain entry of several small viruses and neurotoxic products of viral metabolism that are retained in the periphery under normal conditions. This effect could explain the unusually high incidence of neuro-AIDS in METH users as well as high co-morbidity of neuro-AIDS with malaria, a disease characterized by episodes of robust hyperthermia. High brain temperature could also promote brain entry of antibiotics that are usually retained by the BBB under normal conditions (Kearney and Aweeka, 1999; Lutsar *et al.*, 2000). Some of these drugs are neurotoxic and its entry into brain environment could explain neurological complications seen in young children treated by these drugs for viral and bacterial infections. Therefore, temperature is an extremely important variable in both normal brain functioning and development of brain pathology.

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Fig. 1.

Changes in brain and body temperatures induced by meth-amphetamine (9 mg/kg, sc) used under quiet resting conditions at 23°C (A), during social interaction with female at 23°C (B), and in a warm (29°C) environment (C). Recordings were made from the nucleus accumbens (NAcc), a representative brain structure, and the temporal muscle, non-motor head muscle that received arterial blood supply from the same source as the brain.

Fig. 2. Changes in several brain parameters (A, albumin-positive cells; B, GFAP-positive cells; C, tissue water; D, morphologically abnormal cells) during acute METH intoxication (9 mg/kg, sc) at normal (23°C) and warm (29°C) ambient temperatures.

Fig. 3. The relationships between brain temperatures and the numbers of albumin- and GFAP-positive cells (A) and tissue water (B) during acute METH intoxication. There was a tight correlation (r is coefficient of correlation) between changes in these parameters. Data are shown for cortex.

Fig. 4.

The relationships between morphological abnormalities of cortical cells and brain and muscle temperatures (A), albumin- and GFAP-immunoreactivity (B), and tissue water (C) during acute METH intoxication (9 mg/kg, sc). Each graph shows coefficients of correlation and regression equations.

Fig. 5.

The relationships between brain temperatures and several histochemical and morphological parameters (albumin, GFAP, cellular abnormalities) in pentobarbital-anesthetized rats passively warmed to different brain temperatures. Top graphs (A) show mean changes in the brain as a whole (sum of the cortex, thalamus, hippocampus, and hypothalamus) and bottom graphs (B) show changes in individual brain structures. Each point represents the value (ordinate) determined in rats at different brain temperature (abscissa).

Fig. 6.

Dependence of tissue water content in the thalamus and cortex upon brain temperature. Data were obtained from pentobarbital-anesthetized rats passively warmed to different brain temperatures. Hatched lines show mean values of tissue water in the thalamus and cortex in awake rats maintained at quiet resting conditions.

Fig. 7.

The relationships between different brain parameters (A, Albumin – tissue water; B, Albumin – GFAP; C, Albumin (GFAP) – abnormal cells; and D, Tissue water – abnormal cells) obtained in pentobarbital-anesthetized, passively warmed rats within $\sim 10^{\circ}\text{C}$ fluctuations in brain temperature ($32\text{--}42^{\circ}\text{C}$).