Pharmacology, Biochemistry and Behavior 96 (2010) 32–39

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Lack of effect of dopaminergic denervation on caudate-putamen hyperthermia or hypothermia induced by drugs and mild stressors

Caterina Marcangione [1], Annie Constantin, Paul B.S. Clarke *

Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec, Canada H3G 1Y6

ARTICLE INFO

Article history:
Received 26 January 2010
Received in revised form 30 March 2010
Accepted 7 April 2010
Available online 14 April 2010

Keywords:
Brain temperature
Caudate-putamen
Dopamine
Amphetamine
Apomorphine
Stressor

ABSTRACT

A number of drugs and psychological stressors induce brain hyperthermia and increase extracellular dopamine in the caudate-putamen. The present study tested whether caudate-putamen hyperthermia produced by such stimuli is dependent on dopaminergic transmission. Rats were infused with 6-hydroxydopamine unilaterally into the medial forebrain bundle, and after a two-week recovery period, removable thermocouples were used to monitor temperature in the depleted and intact caudate-putamen in freely-moving animals. The indirect dopamine agonist d-amphetamine (1 and 2 mg/kg s.c.) increased caudate-putamen temperature, whereas a low dose of the direct agonist apomorphine (0.1 mg/kg s.c.) reduced it. Gamma-butyrolactone, which strongly inhibits dopamine release at the dose administered (700 mg/kg i.p.), initially reduced and then increased caudate-putamen temperature. Brief (5-10 min) presentation of mild stressors, including tail pinch, produced a rapid and transient caudate-putamen hyperthermia. Quantitative ^{125}I-RTI-55 autoradiography in post-mortem tissue revealed a 97-100% loss of binding to dopamine transporters in the lesioned caudate-putamen. Despite this near-total dopamine denervation, neither basal caudate-putamen temperature, nor any of the observed temperature responses to drugs or mild stressors, was altered. We conclude that in the caudate-putamen, endogenous dopamine is unlikely to modulate temperature significantly at a local level.

1. Introduction

Brain temperature is affected by numerous behavioural (Blech-Hermoni and Kiyatkin, 2004; Blumberg et al., 1987; Brown et al., 2003; Grossman and Rechtshaffen, 1967; Kiyatkin et al., 2002; Kiyatkin and Mitchum, 2003; Kiyatkin and Wise, 2001; Moser et al., 1993; Sundgren-Andersson et al., 1998) and pharmacological (Blech-Hermoni and Kiyatkin, 2004; Blumberg and Moltz, 1987; Brown et al., 2003; Clausing et al., 1996; Colbourne et al., 1996; Parada et al., 1995; Yanase et al., 1998) manipulations. Although brain temperature often matches core temperature quite closely, it is to some extent independently regulated (Blech-Hermoni and Kiyatkin, 2004; Blumberg et al., 1987; Brown et al., 2003; Busto et al., 1987; Duncan et al., 1995; Kiyatkin et al., 2002; Kiyatkin, 2005; Kiyatkin and Mitchum, 2003; Kiyatkin and Wise, 2001; Miyazawa and Hossmann, 1992; Smirnov and Kiyatkin, 2009; Tachibana, 1969; Yanase et al., 1998; Zhu et al., 2009). Uncoupling of brain and core temperature tends to occur when heat, produced mainly by oxidative metabolism, is not matched by the cooling influence of the circulation (Kiyatkin et al., 2002; Kiyatkin, 2005; Yablonskiy et al., 2000; Zhu et al., 2009). Temperature gradients have been reported not only between brain and blood, but also within the brain itself (reviewed by Kiyatkin, 2005). Some of these gradients are relatively static and reflect factors such as proximity to the external environment. However, other gradients fluctuate in response to drug and other stimuli (Brown et al., 2003; Brugge et al., 1995; Kiyatkin et al., 2002; Kiyatkin and Mitchum, 2003; McElligott and Melzack, 1967; Parmeggiani et al., 1998; Tachibana, 1969). Hence, brain temperature is likely to be controlled by *local* factors as well as peripheral thermoregulatory mechanisms.

Drugs and psychological stressors that enhance dopaminergic transmission tend to have widespread effects on temperature mediated by sympathetic nervous system arousal. Nevertheless, several observations indicate that dopaminergic tone may also modulate brain temperature at a local tissue level. First, systemic injection of cocaine and methamphetamine, at doses which increase interstitial DA levels, typically produce temperature rises in DA-rich areas (i.e. caudate-putamen (CP) and nucleus accumbens) exceeding those occurring in other brain areas or in the deep temporal muscle (Kiyatkin et al., 2002; Kiyatkin and Mitchum, 2003) (Blech-Hermoni and Kiyatkin, 2004; Brown et al., 2003; Kiyatkin and Brown, 2004). Second, several psychological stimuli that can increase dopaminergic transmission (Horvitz, 2000), also increased temperature more rapidly in the brain than in the deep temporal muscle (Kiyatkin et al., 2002). Third, the accumbens-recorded hyperthermic effects of

* Corresponding author. Tel.: +1 514 398 3616; fax: +1 514 398 6690.
E-mail address: paul.clarke@mcgill.ca (P.B.S. Clarke).
[1] Present address: Centre de Recherche Fernand-Seguin, Département de Psychiatrie, Université de Montréal, 7331 rue Hochelaga, Montréal, Québec, Canada H1N 3V2.

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.04.003

intravenous cocaine or psychological stimuli were reduced or blocked by systemic administration of DA receptor antagonists (Kiyatkin, 2008; Kiyatkin and Brown, 2005).

Additional evidence for *local* dopaminergic control of tissue temperature is that amphetamine increases striatal glucose metabolism (Porrino et al., 1984), an effect which appears DA receptor-dependent (Trugman and James, 1993). Furthermore, dopaminergic fibers appear to innervate intraparenchymal blood vessels in rat and monkey brain (Jones, 1982; Krimer et al., 1998), and pharmacological evidence suggests possible vasodilatory and vasoconstrictive actions mediated by D1/D5 and D3 receptors, respectively (Choi et al., 2006). Such actions, if physiological, could potentially modulate blood flow locally within the striatum, thus tending to alter temperature locally.

Taken together, these findings suggest that DA may produce local brain warming, possibly by altering cerebral metabolism or blood flow, or both. Such an action could have significant implications for physiological functioning, given that cellular processes (e.g. metabolism, electrical activity, transmitter release and uptake kinetics) tend to be highly temperature-sensitive (Kiyatkin, 2005; Kiyatkin and Wise, 2001). In addition, DA-mediated tissue warming, were it to occur, would potentially confound the measurement of transmitter dynamics via intracerebral microdialysis or electrochemistry (Kiyatkin, 2005).

The present study therefore sought to determine whether endogenous DA modulates CP temperature in freely-moving rats. For this purpose, subjects first received an intracerebral infusion of 6-hydroxydopamine (6-OHDA) that resulted in a severe unilateral depletion of forebrain DA. Subsequently, temperature was monitored in the depleted and intact CP in response to drugs or mild stressors. Amphetamine and gamma-butyrolactone (GBL) were chosen as pharmacological challenges since they greatly increase and decrease DA outflow in the CP, respectively (Brown et al., 1991; Kuczenski and Segal, 1989; Zetterstrom et al., 1986). The direct DA agonist apomorphine was also tested, in a dose chosen to differentiate denervated vs. intact CP (Schwarting and Huston, 1996).

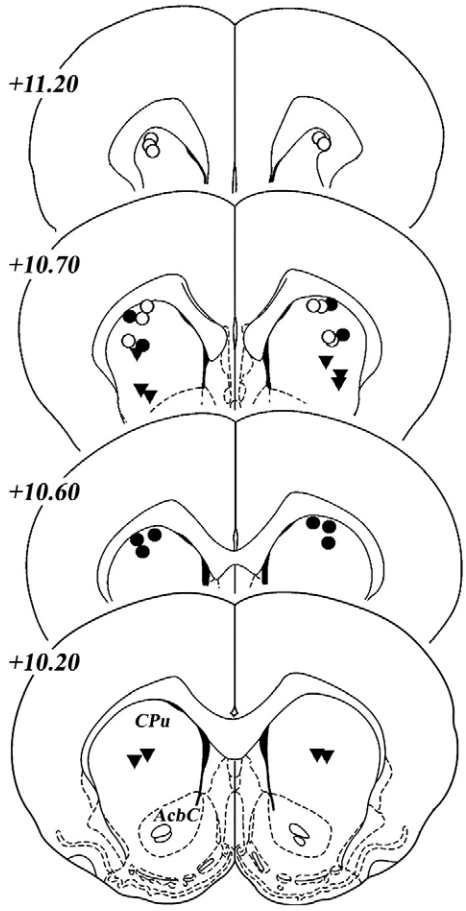

Fig. 1. Location of thermocouple tips in coronal brain sections. Drawings are modified from the atlas of Paxinos and Watson (1998). Anteroposterior levels (mm anterior to Bregma) are shown on the left. CPu, caudate-putamen; AcbC, nucleus accumbens core. Filled inverted triangles represent rats tested with amphetamine and apomorphine; filled and open circles represent GBL- and saline-tested rats, respectively.

2. Results

2.1. Histology

In the amphetamine/apomorphine experiment, five out of the eight rats had bilateral thermocouple locations within the CP, between anterior-posterior levels corresponding to interaural 10.2 and 10.9 (Fig. 1). Three out of the eight rats were excluded from further analysis because in each case only one thermocouple site could be identified. In the GBL and mild stressor experiments, 16 rats received bilateral thermocouple implants. Three of these subjects were excluded due to mislocated thermocouples, one died prematurely, and one was a statistical outlier. Hence, Fig. 1 shows the 11 remaining subjects; these all had bilateral thermocouple locations within the dorsal CP, between anterior-posterior levels corresponding to interaural 10.2 and 10.9 (Fig. 1).

2.2. Effects of amphetamine and apomorphine on caudate-putamen temperature

Temperature in the CP declined during the baseline period (i.e. the 25 min immediately before injection), with a small transient rise associated with the injection (Fig. 2). The baseline temperature did not differ significantly between the lesioned and intact side (mean difference 0.1 ± 0.1 °C, $t = 1.20$, df 4, $P > 0.2$). Each rat received the two saline sessions; data were statistically indistinguishable and were pooled. Within one hour of saline injection, CP temperature declined by approximately 1 °C and then remained stable (Fig. 2).

Both doses of amphetamine increased CP temperature (compared to the saline condition). The lower dose produced a higher initial

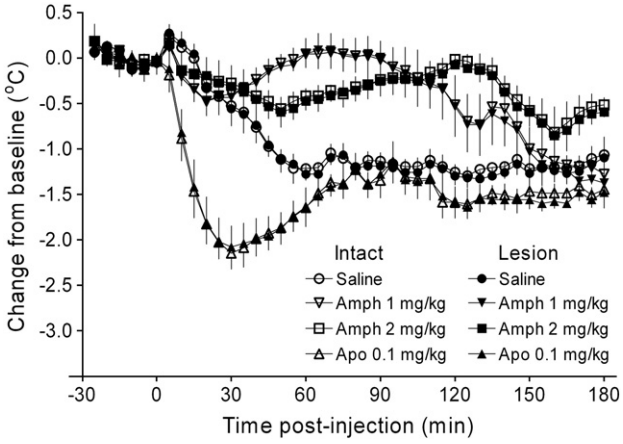

Fig. 2. Effects of d-amphetamine and R(-)-apomorphine on temperature in intact and 6-OHDA lesioned caudate-putamen (CP). Rats received a unilateral infusion of 6-OHDA in the medial forebrain bundle and starting 16 days later, each animal was tested once with amphetamine (1 and 2 mg/kg s.c.), and apomorphine (0.1 mg/kg s.c.), and twice with saline (s.c.). Tests sessions were given in random order and were initiated 30-40 min before injection. During the baseline period (i.e. the 25 min immediately pre-injection), the CP temperature was 38.4 ± 0.2 °C (mean ± SEM, n = 5 rats). The y axis shows baseline-subtracted temperature (mean ± SEM). Data from the two saline test scores were pooled. Amphetamine and apomorphine produced CP hyperthermia and hypothermia, respectively. No drug effect differed significantly between the intact and 6-OHDA lesioned CP.

response, whereas the higher dose exerted a longer effect (Fig. 2). In both cases, the peak effect was approximately 1.2 °C vs. saline. Amphetamine-induced CP hyperthermia occurred mostly between 50 and 150 min after injection, and during this period the drug effect was significant (AMPH main effect: $F=8.65$, df 2,8, $P<0.025$), and independent of lesion side (AMPH×LESION: $F=0.6$, df 2,8, $P>0.5$). Both doses produced a 0.9 °C time-averaged increase compared to saline. From 10-50 min after injection, neither the main effect of AMPH nor the AMPH×LESION interaction were significant ($F<0.7$, df 2,8, $P>0.5$ for each).

Apomorphine produced a rapid CP hypothermia (Fig. 2). The peak effect (1.6 °C vs. saline control) occurred at 30 min post-injection. Hypothermia lasted from 10-50 minutes post-injection, and during this time, the effect was significant ($F=44.73$, df 1,4, $P<0.005$), but independent of lesion side (APO×LESION: $F=0.04$, df 1,4, $P>0.8$).

In the subsequent behavioural test, apomorphine induced robust contraversive turning (i.e. away from the lesion side) in all five rats with no ipsiversive turning (mean ± SEM 4.6 ± 1.1 turns/min at 20-30 min post-injection). The 6-OHDA lesion virtually eliminated ^{125}I-RTI-55 labeling in the ipsilateral CP, reducing it by at least 99% compared to the contralateral side in all 5 rats.

2.3. Effects of GBL on caudate-putamen temperature

After saline administration, CP temperature declined by 1.0 - 1.5 °C with no detectable difference between lesion and intact sides (Fig. 3). GBL initially reduced temperature and then increased it in all subjects except one. The latter was considered to be an outlier and excluded from analysis. Data were subjected to ANOVA with one between-subject factor (GBL) and one within-subject factor (LESION). Two time periods (5-20 min and 60-180 min) were then analyzed, corresponding to the initial hypothermic and subsequent hyperthermic effect. These two time-averaged effects were statistically significant and corresponded to an initial temperature drop of 0.7 °C ($F=13.6$, df 1, 9, $P<0.01$) followed by a gain of 1.3 °C ($F=9.8$, df 1, 9, $P<0.02$) compared to the saline-treated group. Neither effect differed between lesioned and intact CP (GBL×LESION: $F<0.7$, df 1, 9, $P>0.2$ in each case). Rats treated with GBL became sedated within a few minutes of injection and remained immobile for the remainder of the 3-hour session.

Dorsal CP ^{125}I-RTI-55 labeling was reduced by 98.4 ± 0.3% in the saline-tested group and by 98.4 ± 0.5% in the GBL-tested group (mean ± SEM, n = 6 and 5 respectively) compared to the contralateral side. The range of depletion was 97-99% across animals.

2.4. Effects of mild stressors on caudate-putamen temperature

Temperature in the CP increased for several minutes after the thermocouples were connected and the six animals were placed in the test apparatus (Fig. 4). The two tail pinch stimuli elicited a rapid and transient temperature rise in the six animals tested (Fig. 4, upper panel). For analysis, data were divided into six 5 min periods starting 10 min before each tail pinch. Temperature in the CP changed significantly over these time blocks (TIME: $F=11.7$, df 5, 25, $P<0.0001$), and the effect did not differ significantly between the two tail pinch trials (TIME×TRIAL: $F=1.66$, df 5, 25, $P>0.2$), nor between lesioned and intact CP (LESION×TIME: $F=0.50$, df 5, 25, $P>0.5$). Compared to the pre-stimulus 10 min baseline, temperature was significantly elevated during both the first and second 5 min of each tail pinch, by 0.4 ± 0.1 and 0.6 ± 0.1 °C, respectively (Bonferroni $t=4.90$ and 5.72, df 5, $P<0.01$ and $P<0.005$). The maximal rise in temperature was 0.9 ± 0.1 °C (mean ± SEM, n = 6 rats). Animals tended to orient towards the clip and try to remove it; three rats also showed teeth chattering behaviour.

Grooming behaviour was reliably induced in all six rats by the two applications of water (Fig. 4, lower panel). However, the latency to groom was somewhat variable across subjects, and some animals required more than one or two drops of water before they began to

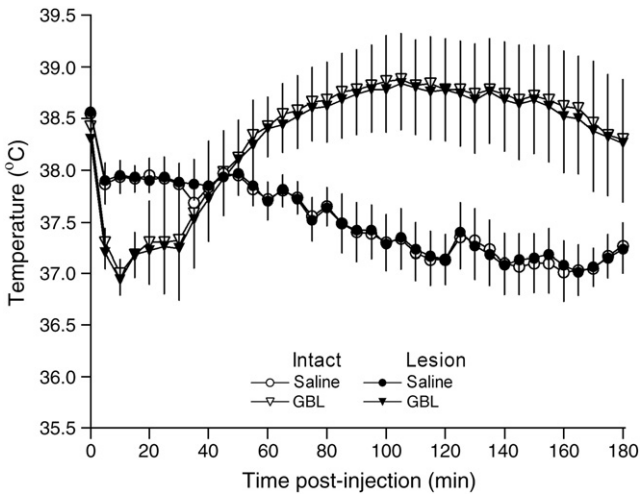

Fig. 3. Effects of GBL on temperature in intact and 6-OHDA lesioned CP. Rats received a unilateral infusion of 6-OHDA in the medial forebrain bundle and 15 days later, each rat was tested once, either with GBL (700 mg/kg i.p.) or with saline. Thermocouples were connected and temperature recordings were initiated immediately after injection; for this reason, it was not possible to perform subtraction of pre-injection baseline temperature values (as performed in Fig. 2). Values are group mean ± SEM (n = 5-6 rats per group). GBL initially reduced CP temperature relative to saline-treated animals, but later increased it. Neither the initial hypothermia nor the subsequent hyperthermia was affected by the 6-OHDA lesion.

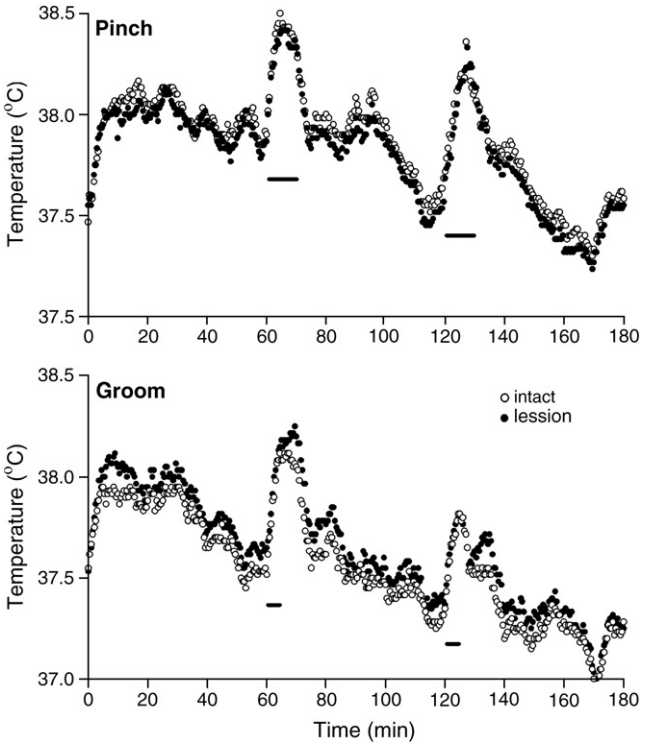

Fig. 4. Effects of two mild stressors on temperature in intact and 6-OHDA lesioned CP. Unilaterally lesioned rats were tested on separate occasions for their responses to tail pinch (upper panel) and to drops of water that induced grooming (lower panel). The order of testing was counterbalanced across rats. The stimuli were applied twice, starting at 60 and 120 min, respectively. The tail pinch stimuli were each applied for 10 min, whereas the grooming stimuli were each applied for 5 min. Values are group mean (n = 6 rats); for clarity, the SEM bars are omitted. Both types of stressors induced a rapid and transient hyperthermia which was not significantly altered by the 6-OHDA lesion.

groom. Bouts of grooming typically began with the face and extended to the whole body, and tended to last 2-3 min. New bouts of grooming were induced with one or two more drops of water, to maintain virtually continuous grooming for about 5 min. Grooming data were analyzed in 5 min blocks, as for tail pinch. ANOVA revealed no difference between lesioned and intact sides (LESION×TIME: $F=2.41$, df 5,25, $P>0.1$) nor between the two grooming tests (TRIAL×TIME: $F=0.95$, df 5,25, $P>0.4$). Compared to the pre-stimulus baseline, temperature was significantly increased during the 5 min grooming period (by 0.3 ± 0.1 °C; Bonferroni $t=5.73$, df 5, $P<0.005$), but not in the period immediately after it (Bonferroni $t=2.90$, df 5, $P>0.05$). The maximal rise in temperature was 0.7 ± 0.1 °C (mean ± SEM, $n=6$ rats). Dorsal CP ^{125}I-RTI-55 labeling was reduced by 97-99% on the lesioned side in these animals, which comprise the saline-tested group reported in Section 2.3.

3. Discussion

3.1. Summary of novel findings

The present study provides several novel findings. First, we describe a new method allowing repeated brain temperature measurements in freely-moving rats. Second, effects of d-amphetamine, apomorphine, and GBL on CP temperature are reported. Third, temperature fluctuations resulting from drug administration or mild stressors were found to be unaffected by unilateral forebrain DA depletion.

3.2. Effects of drugs and mild stressors on caudate-putamen temperature

The effects of dopaminergic drugs on peripheral (i.e. core or deep temporal muscle) body temperature have been widely characterized in rats (Blech-Hermoni and Kiyatkin, 2004; Brown et al., 2003; Chipkin, 1988; Hjorth et al., 1985; Kiyatkin, 2005; Kiyatkin and Brown, 2005; Scheel-Kruger and Hasselager, 1974; Ulus et al., 1975; Yehuda and Wurtman, 1972). In contrast, much less is known about the effects of these drugs on *brain* temperature.

Amphetamine, in sub-toxic doses, produces core hyperthermia in rats tested at room temperature (Clark and Lipton, 1985; Yehuda and Wurtman, 1972) and increases hypothalamic temperature, according to a preliminary report (Parada et al., 1995). The chemical analog methamphetamine induced a dose-dependent hyperthermia in nucleus accumbens and hippocampus after systemic injection (Brown et al., 2003). In the present study, amphetamine produced a greater initial CP hyperthermia at the lower of the two doses tested; this result was unexpected, especially given the counterbalanced, repeated-measures design.

Apomorphine, in low doses, produces a reliable core hypothermia after s.c. administration in rats (Clark and Lipton, 1985; Faunt and Crocker, 1987; Hjorth et al., 1985). This effect is dependent on D2 receptors (Chipkin, 1988; Faunt and Crocker, 1987), and appears to originate, at least in part, in the preoptic area of the anterior hypothalamus leading to increased heat loss through the tail (Cox et al., 1978). The *brain* hypothermia produced by apomorphine was quite substantial, rapid in onset, and occurred despite concurrent drug-induced rotational activity. The mechanisms underlying this hypothermic effect are discussed elsewhere (Brown et al., 2007).

In the periphery, apomorphine hypothermia is monotonically dose-dependent (Clark and Lipton, 1985; Faunt and Crocker, 1987; Hjorth et al., 1985), raising the possibility that higher doses of apomorphine, common in behavioural studies, cause profound brain hypothermia which in turn could potentially affect behavioural output. Although there is little evidence bearing directly on this question, both behavioural despair (Taltavull et al., 2003) and 22 kHz ultrasonic vocalizations (Blumberg and Moltz, 1987) have been identified as being highly sensitive to brain temperature.

To our knowledge, the effects of GBL on brain temperature have not been reported previously. The biphasic effect of GBL seen in the present study (i.e. hypothermia followed by prolonged hyperthermia) resembles the changes in rectal temperature observed after systemic administration of a similar high dose (Borbely and Huston, 1972; Snead, 1990). GBL produced hyperthermia despite heavy sedation and immobility, suggesting that the hyperthermia may have been central in origin.

The induction of prompt and mild striatal hyperthermia by tail pinch and saline injections has been previously reported (Blech-Hermoni and Kiyatkin, 2004; Brown et al., 2003; Kiyatkin et al., 2002; Kiyatkin, 2008; Kiyatkin and Wise, 2001). The present observations confirm these effects and show that experimenter-induced grooming has a similar effect.

3.3. Dopaminergic regulation of caudate-putamen temperature

In the present study, d-amphetamine, apomorphine and tail pinch produced temperature responses that grossly matched their expected effects on extracellular DA in the intact CP. Thus, administration of d-amphetamine and apomorphine at the doses used here results in a prolonged rise and fall in dialysate DA, respectively (Imperato et al., 1988; Kuczenski and Segal, 1989; Zetterstrom et al., 1986), whereas tail pinch tends to produce a smaller, transient response (Mendlin et al., 1999; Wheeler et al., 1995). In contrast, GBL affected CP temperature in a biphasic manner, yet produces only a profound and sustained reduction in dialysate DA (Brown et al., 1991; Zhang et al., 1988).

Dopaminergic denervation resulting from 6-OHDA administration was quantified by ^{125}I-RTI-55 binding to the plasmalemmal DA transporter. It has been reported that in rats lesioned with 6-OHDA, percentage losses of DAT binding and [^{3}H]DA uptake were comparable to the loss of tissue DA (Altar et al., 1987; Joyce, 1991). In the present study, all animals lost 97-100% of DAT binding in the lesioned CP. Dialysis studies indicate that such a high degree of denervation would result in lower basal extracellular concentrations of DA, and would either markedly attenuate or abolish amphetamine-induced DA outflow (Bjelke et al., 1994; Robinson and Whishaw, 1988; Zhang et al., 1988). Such lesions would also reduce the DA-suppressant effects of GBL, according to in vivo electrochemical findings (Blaha, 1996). It seems likely that severe DA depletion would also attenuate stress-induced DA release, although this question appears unaddressed in the literature.

In the present study, profound unilateral DA depletion did not detectably alter CP temperature. This was the case not only for baseline temperature, but also for hyperthermia or hypothermia associated with d-amphetamine, apomorphine, GBL, and the two mild stressors. Statistical power analysis (Section 4.7) revealed an ability to detect thermal side differences as small as 0.08, 0.12, and 0.18 °C for amphetamine, mild stressors, and apomorphine, respectively. A lesion effect was also not apparent in the early post-injection period when brain hyperthermia tends to be more marked than peripheral hyperthermia (Blech-Hermoni and Kiyatkin, 2004; Brown et al., 2003). The negative result with apomorphine is perhaps particularly noteworthy. Here, we used a low apomorphine dose designed to stimulate "supersensitive" postsynaptic D2 receptors in lesioned CP, as confirmed by the occurrence of contraversive rotation (Schwarting and Huston, 1996). In contrast, in the intact CP, this dose of apomorphine would act preferentially on D2 autoreceptors, reducing DA transmission (Imperato et al., 1988).

It is possible that thermal equilibration between the intact and lesioned CP could have masked a lesion effect. However, this explanation seems improbable for the following reasons. First, baseline temperature differences can be maintained even between adjacent rat brain structures (Brown et al., 2003; Kiyatkin et al., 2002; Kiyatkin and Mitchum, 2003; Kiyatkin and Wise, 2001; McElligott and

Melzack, 1967; Tachibana, 1969). Second, stimulus-evoked temperature changes have been localized to distinct subregions within the feline lateral geniculate nucleus (McElligott and Melzack, 1967). In the latter study, thermal responses recorded at one sensor could not be detected at a second sensor located 3 mm away; our bilateral thermocouples were more distant (approx. 5 mm apart). Thirdly, as noted above, side differences as small as 0.08, 0.12, and 0.18 °C would have been detectable for amphetamine, mild stressors, and apomorphine, respectively. These detection thresholds represent a small fraction of the bilateral temperature changes evoked by our stimuli. For example, amphetamine and apomorphine produced changes of +1.2 °C and -1.3 °C, respectively, sustained for many minutes, whereas the non-pharmacological stressors produced smaller temperature increments (peak ~0.7-0.9 °C).

Since both 6-OHDA and ^{125}I-RTI-55 target the plasmalemmal DA transporter, our approach also cannot exclude a role for dopaminergic terminals that lack this protein. However, this possibility also seems unlikely, for two reasons. First, DAT and tyrosine hydroxylase are highly colocalized in nigrostriatal DA terminals (Hersch et al., 1997), and second, we have previously obtained 99% depletion of CP DA using the same 6-OHDA lesion parameters (Clarke et al., 1990).

Lastly, compensatory mechanisms almost certainly occurred following the unilateral 6-OHDA lesion. However, compensatory changes are unlikely to explain the present negative results, for the following reason. All the lesioned rats rotated in response to the direct DA agonist apomorphine, and hence would have rotated in response to systemic amphetamine, since the latter does not require nearly as much DA depletion (Schwarting and Huston, 1996). Therefore, the lesioned rats, when challenged with amphetamine, would have experienced an asymmetry of striatal dopamine transmission. It is unclear how compensatory mechanisms could have masked such an asymmetry.

3.4. Technical aspects

Removable sensors have seldom been used for measuring brain temperature in rodents (Colbourne et al., 1993); virtually all published work appears to have employed chronic indwelling probes. The main advantage of removable probes is economic. Once assembled, they require little if any maintenance. Unlike indwelling sensors, they can be reused many times and they are not lost at the end of the experiment. Two types of thermocouple probes were tested. Both were sensitive to rapid temperature fluctuations (time constant ~0.1 s). The wider Physitemp model proved the more durable, since the Omega probes were sometimes bent during insertion, especially if the animals had not been extensively habituated. The Physitemp thermoprobes (330 µm diameter) are comparable in size to implanted thermocouples used by others (Kiyatkin and Wise, 2001) and to commercially available microdialysis probes, but much larger than carbon fiber microelectrodes used for in vivo electrochemistry. The main limitation of these removable thermoprobes is that repeated insertion causes a modest degree of tissue damage; to our knowledge, comparative data for chronically implanted probes have not been published.

3.5. Functional implications

Although numerous observations provide correlative evidence consistent with a local brain-warming effect of DA (see Introduction), the present findings suggest that any such effect must be very small, accounting for fluctuations of less than 0.1-0.2 °C. This estimate was obtained using pharmacological effects that likely exceed the physiological range of DA function. Although certain aspects of neuronal activity are highly temperature-sensitive (Kiyatkin, 2005; Kiyatkin and Wise, 2001), it seems unlikely that such small temperature changes would have important biological consequences (Wirtshafter et al., 1978). One specific implication of our findings is in the field of in vivo electrochemistry. Small temperature fluctuations affect electrode kinetics and can measurably affect the size of electrochemical signals (Marcangione et al., 1999), yet brain temperature has rarely been recognized as a potential confound in the in vivo literature. In this context, several validation studies have assessed DA selectivity in part by systemically administering drugs that increase or decrease DA release to animals bearing unilateral 6-OHDA lesions (Blaha, 1996; Marsden et al., 1988). The present findings suggest that this approach may be valid, at least for the drug conditions used here.

The wider functional significance of brain hypothermia or hyperthermia is largely unknown, but could be profound (reviewed by Kiyatkin, 2005). For example, certain cellular processes are highly temperature sensitive and hyperthermia poses a therapeutic challenge in brain-damaged individuals (Kiyatkin, 2005; Kiyatkin and Wise, 2001). In addition, brain hyperthermia has been proposed as an index of neuronal activation (Kiyatkin and Wise, 2001), arousal (Kiyatkin and Mitchum, 2003), and as a factor in the organization of adaptive responses to unexpected environmental stimuli (Kiyatkin and Wise, 2001). Ascending dopaminergic neurons play an important role in some of these functions, but not apparently in the local control of caudate-putamen temperature.

4. Materials and Methods

4.1. Animals

Male Long-Evans rats (Charles River, St. Constant, QC, Canada) weighing between 285 and 400 g at time of surgery were housed individually in a temperature- and humidity- controlled animal colony lit from 7 am to 7 pm. Food and water were available ad libitum, except during test sessions, which occurred between 8:30 am and 5:30 pm. All experimental protocols were approved by the McGill Medical Faculty Animal Care Committee, in accordance with Canadian Council on Animal Care guidelines, in order to minimize pain and discomfort.

4.2. Surgery

Each rat was pretreated with the monoamine oxidase inhibitor pargyline (50 mg/kg i.p. 30 min prior to surgery), and anesthetized with either ketamine HCl (80 mg/kg i.p.) and xylazine HCl (16 mg/kg i.p.) in the first experiment, or with Na pentobarbital (55 mg/kg i.p.) in subsequent experiments. Atropine methyl nitrate (0.04 mg/kg s.c.) was administered to minimize bronchial secretions when Na pentobarbital was used. Each rat was placed in a stereotaxic instrument (Kopf) with the tooth bar positioned 4.2 mm below the ear bars. Solutions of 6-OHDA HBr were thawed just prior to use and kept on ice in the dark. 6-OHDA was infused unilaterally into the medial forebrain bundle; the injection side was counterbalanced across animals. Coordinates (in mm) were set as follows: 5.9 anterior, 2.3 lateral, and 2.2 dorsal to interaural zero (Paxinos and Watson, 1998). Infusions (4 µg base in 2 µl) were made over 10 min via a 30 gauge stainless steel cannula connected by PE 10 tubing to a 5 µl Hamilton syringe driven by a syringe pump (MF-9090, BioAnalytical Systems, West Lafayette, IN, USA). The cannula was removed after a 5 min delay to allow for diffusion. Following the medial forebrain bundle infusion, each rat was bilaterally implanted with 22 gauge guide cannulae (model C313G, Plastics One Inc., Roanoke, VA, USA) to permit subsequent insertion of thermocouples into the dorsal CP (bregma +1.2, lateral ±3.0, skull surface –4.0). Guide cannulae were secured with the aid of four stainless steel skull screws and dental cement. A stainless steel stylet (model C313DC, Plastics One) was inserted into each guide cannula so as to protrude 1.5 to 2.0 mm past the tip. The wound was sutured and rats received an analgesic

(dipyrone 100 mg/kg s.c. in Experiment 1, buprenorphine 0.05 mg/kg s.c. in subsequent experiments).

Rats were handled in the days before and after surgery. One week following surgery, animals were tested for spontaneous ipsiversive turning (Schwarting and Huston, 1996) in an open field and only those displaying robust ipsiversion were tested further.

4.3. Measurement of caudate-putamen temperature

Thermocouple probes (model KMTSS-010U-6, K-type, 250 µm diameter, Omega, Laval, QC, Canada or model MT-29/3, T-type, 330 µm diameter, Physitemp, Clifton, NJ, USA) were inserted and secured inside a cannula connector assembly (C313C, Plastics One). This was done such that the probe, once inserted into the intracranial guide cannula, would protrude 1.5 to 2 mm. The thermocouples were connected via a slip-ring commutator (CAY-675-12, Airflyte Electronics Co., Bayonne, NJ, USA) to a thermocouple thermometer unit (DuaLogR® Thermocouple Thermometer model P-91100-50, Labcor, Anjou, QC, Canada) from which data were subsequently uploaded to computer via an RS-232 interface adapter (Cole-Palmer Instrument Co.). Thermocouples were calibrated against a mercury thermometer marked in 0.1 °C divisions and traceable to NIST standards (Fisher Scientific, Montreal, QC, Canada). They were cleaned with 70% ethanol prior to insertion. Temperature was recorded in the CP at 5 min intervals for drug tests and at more frequent (30 s) intervals for tests with mild stressors.

4.4. Quantitative [^{125}I]RTI-55 autoradiography

The extent of the 6-OHDA lesion was quantified by autoradiographic labeling of the plasmalemmal DA transporter by [^{125}I]RTI-55, using a method adapted from published protocols (Boja et al., 1992; Coulter et al., 1995). The rats were anesthetized with Na pentobarbital (65 mg/kg i.p.) and decapitated. The brains were rapidly removed and frozen in 2-methylbutane at -50 °C for 30 sec and stored at -40 °C. Cryostat-cut coronal sections (20 µm thick) were collected at 0.5 mm intervals throughout the extent of the CP. At each anteroposterior level, six adjacent sections were thaw-mounted on gelatin-subbed slides, air-dried at RT for 20 to 30 min, and stored with desiccant at -40 °C. Additional sections were air-dried and Nissl-stained with cresyl violet in order to localize the thermocouple.

For radiolabelling, sections were thawed at RT and incubated for 120 min at RT in 10 mM sodium phosphate buffer containing 10 pM [^{125}I]RTI-55, 0.1 M sucrose, and 50 nM citalopram HBr to protect 5-HT transporters (Coulter et al., 1995). Non-specific binding was defined by the addition of the DA uptake inhibitor GBR 12909 (10 µM). Sections were rinsed in ice-cold radioligand-free buffer (1×1 min plus 2×20 min) and distilled water (5 sec), then blow-dried. Sections were exposed to X-ray film (Kodak X-OMAT AR) together with [^{125}I] autoradiographic standards (Amersham) for 48 hours in light-proof cassettes. Film autoradiographs were quantified using an M4 MCID microcomputer-based system (Imaging Research, St. Catherines, ON, Canada). One circular sample of 2 mm diameter was measured in each dorsal CP from two sections at a given rostrocaudal level.

4.5. Drugs

Drugs and suppliers were as follows: [^{125}I]RTI-55 (2200 Ci/mmol, NEN Life Sciences Products); 6-hydroxydopamine HBr (6-OHDA), atropine methyl nitrate and pargyline HCl (Sigma); gamma-butyrolactone (i.e. GBL, Research Biochemicals Inc.); Na pentobarbital (MTC Pharmaceuticals, Cambridge, ON, Canada); buprenorphine HCl (Reckitt & Colman Pharmaceuticals); dipyrone (Vetoquinol, Lavaltrie, QC, Canada); R(-)-apomorphine HCl (ICN Biomedicals, Aurora, OH, USA); ketamine HCl (Vetalar™, Vetrepharm™, London, ON, Canada); xylazine HCl (Anased™, Novopharm, Toronto, ON, Canada); and d-amphetamine sulphate (Bureau of Drug Research, Ottawa, ON, Canada). Amphetamine and pargyline were dissolved in 0.9% saline. GBL was dissolved in distilled water to make an injection volume of 1 ml/kg body weight. 6-OHDA HBr was dissolved in 0.9% saline made with preboiled distilled water and with 0.1 mg/ml ascorbic acid added. The apomorphine vehicle was 0.3 mg/ml Na metabisulphite in distilled water, brought to pH 7.2 with 0.5 M NaOH prior to addition of drug. The solutions of 6-OHDA and apomorphine were shielded from light. Doses are expressed either as salt (d-amphetamine, pargyline) or as free base (apomorphine, 6-OHDA). Systemic administration was in a volume of 1 ml/kg, given by i.p. injection except for apomorphine, d-amphetamine, and atropine methyl nitrate (s.c.).

4.6. Plan of experiments

4.6.1. Effects of d-amphetamine and apomorphine on caudate-putamen temperature

Temperature tests were performed 16 to 27 days after surgery. Each animal received five tests: once with each dose of d-amphetamine (1 and 2 mg/kg s.c.) and apomorphine (0.1 mg/kg s.c.), and twice with saline (s.c.). The five tests were given in random order and were spaced two days apart. On test days, thermocouples were first inserted and each rat was habituated for 30-40 min to its test chamber, injected, and then left undisturbed and unobserved in the test cage for the 3-hour session. Three weeks after the final temperature test, all rats were tested once more with apomorphine (0.1 mg/kg s.c.), and contraversive and ipsiversive rotations were counted 20-30 min post-injection. Animals were sacrificed the next day and their brains were collected for quantitative [^{125}I]RTI-55 autoradiography.

4.6.2. Effects of GBL on caudate-putamen temperature

Rats were tested 15 days after surgery. Animals were randomly allocated to two groups and tested with either GBL (700 mg/kg i.p.) or saline (i.p.). GBL-treated rats were given a low dose of atropine methyl nitrate (0.04 mg/kg s.c.) to minimize bronchial secretions. Atropine methyl nitrate does not readily cross the blood brain barrier, and even in high doses (e.g. 1 mg/kg) is reported not to affect core temperature (Overstreet and Russell, 1982; Simonic et al., 1988). In pilot studies, we performed i.p. injections in rats which had thermocouples already inserted and were hence tethered to an electrical commutator. However, this procedure appeared somewhat stressful, and to minimize this aspect the following test procedure was used instead. Each rat was habituated to the test cage for 20 min, briefly removed for injection and insertion of thermocouples, and left in the test cage for the 3-hour session. One week after in vivo testing, the GBL-tested group was sacrificed and their brains were collected for quantitative [^{125}I]RTI-55 autoradiography. The saline-tested animals were then used in the next experiment.

4.6.3. Effects of mild stressors on caudate-putamen temperature

One week after their saline test, each rat was tested with two types of mild stressor (tail pinch and grooming stimulus), in separate 3-hour sessions occurring two days apart in counterbalanced order. Each tail pinch of 10 min duration was applied at the base of the tail with a modified metal hair clip, padded with heat-shrink tubing such that the pressure of the clip would not illicit vocalization. Each grooming stimulus comprised one or two drops of water delivered from a plastic transfer pipette onto the rat's snout, and repeated as necessary in order to induce continuous grooming for 5-10 min (Lowry et al., 1997). At the start of each session, rats were connected to the thermocouples and habituated for 1-hour to the test chamber, then subjected to two mild stressor trials spaced 50 min apart. Rats were sacrificed one week after the final in vivo test and their brains were collected for quantitative [^{125}I]RTI-55 autoradiography.

4.7. Statistical analysis

Statistical analysis was performed using commercial software (SYSTAT version 10, SPSS Inc., Chicago, IL, USA). Data were subjected to repeated measures ANOVA. Each rat was used as its own control with regard to lesion effect (i.e. ipsilateral vs. contralateral side), and also with respect to drug effect (except for GBL, where a between-subject design was used). Probability values are 2-tailed. For repeated measures ANOVA, the Huyhn-Feldt P value is reported. Probability values derived from multiple t-tests were subjected to the Bonferroni correction. In order to determine the minimum statistically detectable temperature difference in a given experiment, power analysis was performed; this was based on paired t-tests (i.e. lesion vs. intact side), using the mean and error variance values obtained (Zar, 1984).

Acknowledgement

This research was supported by the Canadian Institutes for Health Research (MOP-10516) and the Natural Sciences and Engineering Research Council of Canada (155055), and by a career award from the FRSQ to P.B.S.C.

References

Altar CA, Marien MR, Marshall JF. Time course of adaptations in dopamine biosynthesis, metabolism, and release following nigrostriatal lesions: implications for behavioral recovery from brain injury. J Neurochem 1987;48:390–9.

Bjelke B, Stromberg I, O'Connor WT, Andbjer B, Agnati LF, Fuxe K. Evidence for volume transmission in the dopamine denervated neostriatum of the rat after a unilateral nigral 6-OHDA microinjection. Studies with systemic D-amphetamine treatment. Brain Res 1994;662:11–24.

Blaha CD. Evaluation of stearate-graphite paste electrodes for chronic measurement of extracellular dopamine concentrations in the mammalian brain. Pharmacol Biochem Behav 1996;55:351–64.

Blech-Hermoni Y, Kiyatkin EA. State-dependent action of cocaine on brain temperature and movement activity: implications for movement sensitization. Pharmacol Biochem Behav 2004;77:823–37.

Blumberg MS, Moltz H. Hypothalamic temperature and the 22 kHz vocalization of the male rat. Physiol Behav 1987;40:637–40.

Blumberg MS, Mennella JA, Moltz H. Hypothalamic temperature and deep body temperature during copulation in the male rat. Physiol Behav 1987;39:367–70.

Boja JW, Mitchell WM, Patel A, Kopajtic TA, Carroll FI, Lewin AH, et al. High-affinity binding of [^{125}I]RTI-55 to dopamine and serotonin transporters in rat brain. Synapse 1992;12:27–36.

Borbely AA, Huston JP. Gamma-butyrolactone: an anesthetic with hyperthermic action in the rat. Experientia 1972;28:1455.

Brown EE, Damsma G, Cumming P, Fibiger HC. Interstitial 3-methoxytyramine reflects striatal dopamine release: an in vivo microdialysis study. J Neurochem 1991;57:701–7.

Brown PL, Wise RA, Kiyatkin EA. Brain hyperthermia is induced by methamphetamine and exacerbated by social interaction. J Neurosci 2003;23:3924–9.

Brown PL, Bae D, Kiyatkin EA. Relationships between locomotor activation and alterations in brain temperature during selective blockade and stimulation of dopamine transmission. Neuroscience 2007;145:335–43.

Brugge JF, Poon PW, So AT, Wu BM, Chan FH, Lam FK. Thermal images of somatic sensory cortex obtained through the skull of rat and gerbil. Exp Brain Res 1995;106:7-18.

Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab 1987;7:729–38.

Chipkin RE. Effects of D1 and D2 antagonists on basal and apomorphine decreased body temperature in mice and rats. Pharmacol Biochem Behav 1988;30:683–6.

Choi JK, Chen YI, Hamel E, Jenkins BG. Brain hemodynamic changes mediated by dopamine receptors: Role of the cerebral microvasculature in dopamine-mediated neurovascular coupling. Neuroimage 2006;30:700–12.

Clark WG, Lipton JM. Changes in body temperature after administration of amino acids, peptides, dopamine, neuroleptics and related agents: II. Neurosci Biobehav Rev 1985;9:299–371.

Clarke PBS, Wyder KJ, Jakubovic A, Fibiger HC. Effects of B-HT 920 on nigrostriatal and mesolimbic dopamine systems in normosensitive and supersensitive rats. Br J Pharmacol 1990;99:509–15.

Clausing P, Bloom D, Newport GD, Holson RR, Slikker Jr W, Bowyer JF. Individual differences in dopamine release but not rotational behavior correlate with extracellular amphetamine levels in caudate putamen in unlesioned rats. Psychopharmacology 1996;127:187–94.

Colbourne F, Nurse SM, Corbett D. Spontaneous postischemic hyperthermia is not required for severe CA1 ischemic damage in gerbils. Brain Res 1993;623:1–5.

Colbourne F, Sutherland GR, Auer RN. An automated system for regulating brain temperature in awake and freely moving rodents. J Neurosci Methods 1996;67:185–90.

Coulter CL, Happe HK, Bergman DA, Murrin LC. Localization and quantification of the dopamine transporter: Comparison of [3H]WIN 35, 428 and [125I]RTI-55. Brain Res 1995;690:217–24.

Cox B, Kerwin R, Lee TF. Dopamine receptors in the central thermoregulatory pathways of the rat. J Physiol 1978;282:471–83.

Duncan WC, Johnson KA, Wehr TA. Antidepressant drug-induced hypothalamic cooling in Syrian hamsters. Neuropsychopharmacology 1995;12:17–37.

Faunt JE, Crocker AD. The effects of selective dopamine receptor agonists and antagonists on body temperature in rats. Eur J Pharmacol 1987;133:243–7.

Grossman SP, Rechtshaffen A. Variations in brain temperature in relation to food intake. Physiol Behav 1967;2:379–83.

Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI. Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. J Comp Neurol 1997;388:211–27.

Hjorth S, Carlsson A, Clark D, Svensson K, Sanchez D. Dopamine receptor-mediated hypothermia induced in rats by (+)-, but not by (-)-3-PPP. Eur J Pharmacol 1985;107:299–304.

Horvitz JC. Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience 2000;96:651–6.

Imperato A, Tanda G, Frau R, Di Chiara G. Pharmacological profile of dopamine receptor agonists as studied by brain dialysis in behaving rats. J Pharmacol Exp Ther 1988;245:257–64.

Jones BE. Relationship between catecholamine neurons and cerebral blood vessels studied by their simultaneous fluorescent revelation in the rat brainstem. Brain Res Bull 1982;9:33–44.

Joyce JN. Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. I. Effects of intranigral or intracerebroventricular 6-hydroxydopamine lesions of the mesostriatal dopamine system. Exp Neurol 1991;113:261–76.

Kiyatkin EA. Brain hyperthermia as physiological and pathological phenomena. Brain Res Brain Res Rev 2005;50:27–56.

Kiyatkin EA. Brain temperature responses to salient stimuli persist during dopamine receptor blockade despite a blockade of locomotor responses. Pharmacol Biochem Behav 2008;91:233–42.

Kiyatkin EA, Brown PL. Modulation of physiological brain hyperthermia by environmental temperature and impaired blood outflow in rats. Physiol Behav 2004;83:467–74.

Kiyatkin EA, Brown PL. Dopamine-dependent and dopamine-independent actions of cocaine as revealed by brain thermorecording in freely moving rats. Eur J Neurosci 2005;22:930–8.

Kiyatkin EA, Mitchum RD. Fluctuations in brain temperature during sexual interaction in male rats: an approach for evaluating neural activity underlying motivated behavior. Neuroscience 2003;119:1169–83.

Kiyatkin EA, Wise RA. Striatal hyperthermia associated with arousal: intracranial thermorecordings in behaving rats. Brain Res 2001;918:141–52.

Kiyatkin EA, Brown PL, Wise RA. Brain temperature fluctuation: a reflection of functional neural activation. Eur J Neurosci 2002;16:164–8.

Krimer LS, Muly III EC, Williams GV, Goldman-Rakic PS. Dopaminergic regulation of cerebral cortical microcirculation. Nat Neurosci 1998;1:286–9.

Kuczenski R, Segal D. Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. J Neurosci 1989;9:2051–65.

Lowry JP, Boutelle MG, Fillenz M. Measurement of brain tissue oxygen at a carbon paste electrode can serve as an index of increases in regional cerebral blood flow. J Neurosci Methods 1997;71:177–82.

Marcangione C, Lennox RB, Clarke PBS. In vitro characterization of temperature dependence of chronoamperometric signals. Soc Neurosci Abs 1999;25:384.12 Ref Type: Abstract.

Marsden CA, Joseph MH, Kruk JL, Maidment NT, O'Neill RD, Schenk JO, et al. In vivo voltammetry - present electrodes and methods. Neuroscience 1988;25:389–400.

McElligott JG, Melzack R. Localized thermal changes evoked in the brain by visual and auditory stimulation. Exp Neurol 1967;17:293–312.

Mendlin A, Martin FJ, Jacobs BL. Dopaminergic input is required for increases in serotonin output produced by behavioral activation: an in vivo microdialysis study in rat forebrain. Neuroscience 1999;93:897–905.

Miyazawa T, Hossmann KA. Methodological requirements for accurate measurements of brain and body temperature during global forebrain ischemia of rat. J Cereb Blood Flow Metab 1992;12:817–22.

Moser E, Mathiesen I, Andersen P. Association between brain temperature and dentate field potentials in exploring and swimming rats. Science 1993;259:1324–6.

Overstreet DH, Russell RW. Selective breeding for diisopropyl fluorophosphate-sensitivity: behavioural effects of cholinergic agonists and antagonists. Psychopharmacology 1982;78:150–5.

Parada MA, Puig de Parada M, Hernandez L, Hoebel BG. Triple electrical channels on a triple fluid swivel and its use to monitor intracranial temperature with a thermocouple. J Neurosci Methods 1995;60:133–9.

Parmeggiani PL, Azzaroni A, Calasso M. A pontine-hypothalamic temperature difference correlated with cutaneous and respiratory heat loss. Respir Physiol 1998;114:49–56.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. San Diego, CA: Academic Press; 1998.

Porrino LJ, Lucignani G, Dow-Edwards D, Sokoloff L. Correlation of dose-dependent effects of acute amphetamine administration on behavior and local cerebral metabolism in rats. Brain Res 1984;307:311–20.

Robinson TE, Whishaw IQ. Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. Brain Res 1988;450:209–24.

Scheel-Kruger J, Hasselager E. Studies of various amphetamines, apomorphine and clonidine on body temperature and brain 5-hydroxytryptamine metabolism in rats. Psychopharmacologia 1974;36:189–202.
Schwarting RKW, Huston JP. The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 1996;50:275–331.
Simonic A, Roganovic-Dordevic J, Golubovic V. Influence of various cholinergic and anticholinergic drugs on rectal temperature in rats. Arch Int Pharmacodyn Ther 1988;294:273–82.
Smirnov MS, Kiyatkin EA. Phasic and tonic fluctuations in brain, muscle and skin temperatures during motivated drinking behavior in rats: physiological correlates of motivation and reward. Brain Res 2009;1310:87-102.
Snead III OC. gamma-Hydroxybutyric acid-induced seizures bear no relation to core temperature. Epilepsia 1990;31:253–8.
Sundgren-Andersson AK, Ostlund P, Bartfai T. Simultaneous measurement of brain and core temperature in the rat during fever, hyperthermia, hypothermia and sleep. Neuroimmunomodulation 1998;5:241–7.
Tachibana S. Relation between hypothalamic heat production and intra- and extracranial circulatory factors. Brain Res 1969;16:405–16.
Taltavull JF, Chefer VI, Shippenberg TS, Kiyatkin EA. Severe brain hypothermia as a factor underlying behavioral immobility during cold-water forced swim. Brain Res 2003;975:244–7.
Trugman JM, James CL. D1 dopamine agonist and antagonist effects on regional cerebral glucose utilization in rats with intact dopaminergic innervation. Brain Res 1993;607:270–4.
Ulus IH, Kiran BK, Ozkurt S. Involvement of central dopamine in the hyperthermia in rats produced by d-amphetamine. Pharmacology 1975;13:309–16.
Wheeler D, Boutelle MG, Fillenz M. The role of *N*-methyl-D-aspartate receptors in the regulation of physiologically released dopamine. Neuroscience 1995;65:767–74.
Wirtshafter D, Asin KE, Kent EW. Nucleus accumbens lesions reduce amphetamine hyperthermia but not hyperactivity. Eur J Pharmacol 1978;51:449–52.
Yablonskiy DA, Ackerman JJ, Raichle ME. Coupling between changes in human brain temperature and oxidative metabolism during prolonged visual stimulation. Proc Natl Acad Sci U S A 2000;97:7603–8.
Yanase T, Hara S, Mukai T, Kuriiwa F, Iwata N, Kano S, et al. Characterization of temperature rise of the brain and the rectum following intracerebroventricular administration of alpha-amino-3- hydroxy-5-methyl-4-isoxazolepropionate and kainate in rats. Brain Res 1998;798:304–10.
Yehuda S, Wurtman RJ. The effects of D-amphetamine and related drugs on colonic temperatures of rats kept at various ambient temperatures. Life Sci [I] 1972;11: 851–9.
Zar JH. Biostatistical analysis. New York: Prentice-Hall; 1984.
Zetterstrom T, Herrera-Marschitz M, Ungerstedt U. Simultaneous measurement of dopamine release and rotational behaviour in 6-hydroxydopamine denervated rats using intracerebral dialysis. Brain Res 1986;376:1–7.
Zhang WQ, Tilson HA, Nanry KP, Hudson PM, Hong JS, Stachowiak MK. Increased dopamine release from striata of rats after unilateral nigrostriatal bundle damage. Brain Res 1988;461:335–42.
Zhu M, Ackerman JJ, Yablonskiy DA. Body and brain temperature coupling: the critical role of cerebral blood flow. J Comp Physiol B 2009;179:701–10.