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Relationship between Methamphetamine-Induced Dopamine Release, Hyperthermia, Self-Injurious Behaviour and Long Term Dopamine Depletion in BALB/c and C57BL/6 Mice

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Abstract: Differential sensitivity to neurotoxic effects of methamphetamine on striatal dopaminergic neurones between C57BL/6 and BALB/c mice has been established. In the present studies, the interaction of methamphetamine-induced dopamine release, self-injurious behaviour, the neural immune response, and the long-term (3 day) dopamine depletion were examined in these strains after administration of 8 mg/kg methamphetamine. BALB/c mice showed increased hyperthermia compared to the C57BL/6 strain, as well as induction of interleukin-1β. Additionally, homovanillic acid (HVA) levels, as well as HVA/DA turnover ratios were elevated in the striatum and frontal cortex of BALB/c mice, both compared to untreated mice and to the C57BL/6 strain after a single injection of methamphetamine. Pretreatment with acetaminophen eliminated the methamphetamine-induced hyperthermia in BALB/c mice and reduced body temperature in C57BL/6 mice. However, acetaminophen pretreatment did not affect any parameters of dopaminergic toxicity in the striatum or frontal cortex of the BALB/c strain following repeated methamphetamine injections. Furthermore, acetaminophen pretreatment did not alter the incidence of self-injurious behaviour in BALB/c mice. Therefore, hyperthermia and methamphetamine-induced toxicity appear to be independent phenomena while self-injurious behaviour may provide a better predictor of toxicity, which, in turn, may be related to dopamine release.

Dopaminergic toxicity after repeated administration of methamphetamine has been demonstrated in rodents (Wagner *et al.* 1980), monkeys (Seiden *et al.* 1976; Woolverton *et al.* 1989; Melega *et al.* 1997) and man (Wilson *et al.* 1996; McCann *et al.* 1998). The mechanism through which methamphetamine causes this damage has been linked to dopamine release (Wagner *et al.* 1983; Kita *et al.* 1998 & 2000) as well as the hyperthermic response that follows its administration (Bowyer *et al.* 1993 & 1994; Ali *et al.* 1994; Albers & Sonsalla 1995; Wallace *et al.* 2001). With respect to methamphetamine-induced hyperthermia, it has been demonstrated that lowering body temperature by changing ambient room temperature during methamphetamine administration attenuates methamphetamine-induced neurotoxicity (Bowyer *et al.* 1993; Metzger *et al.* 2000; Xie *et al.* 2000). However, compounds that lower body temperature do not protect subjects against the dopaminergic toxicity induced by methamphetamine (Wagner *et al.* 1983; Bowyer *et al.* 1994; Albers & Sonsalla, 1995; Melega *et al.* 1998; Yuan *et al.* 2001). Furthermore, not all neuroprotective agents blocked methamphetamine-induced hyperthermia (Albers & Sonsalla 1995; Itzhak *et al.* 2000). Thus, it is possible to dissociate the hyperthermic and neurotoxic effects of methamphetamine.

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Recently, it was observed that there are strain differences in sensitivity to methamphetamine-induced dopaminergic toxicity with BALB/c and DBA mice being more sensitive than C57BL/6 mice (O'Callaghan & Miller 1994; Kita *et al.* 1998). In addition, BALB/c mice were found to be more sensitive to methamphetamine-induced self-injurious behaviour (Kita *et al.* 1998). These behaviours, which include self-biting of the forepaws, chest and neck areas, have been demonstrated to be sensitive to changes in dopaminergic activity following amphetamine (Kelly *et al.* 1975; Kelley *et al.* 1989; Breese *et al.* 1984). In a separate study, Kita *et al.* (2000) linked the long-term dopamine depletion and self-biting to dopamine release, but not to hyperthermia, suggesting that the increased incidence of self-injurious behaviour may be a valid marker of long-term toxicity. In a preliminary study performed in rats, we found that acetaminophen pretreatment at 200 mg/kg was able to significantly abolish the hyperthermic response produced by amphetamine at 15 mg/kg without altering amphetamine-induced changes in dopamine metabolism or turnover. Accordingly, in the present study, we sought to compare the acute effects of methamphetamine on dopamine release, hyperthermia, and self-injurious behaviour to the long-term dopamine depletion in both BALB/c and C57BL/6 mice. In addition, an attempt was made to offset the hyperthermic effect of methamphetamine by pretreating mice with acetaminophen. Finally, in an effort to determine if the neural-immune response reflects the long-lasting dop-

aminergic toxicity, the induction of interleukin-1β in the hypothalamus was measured following methamphetamine administration.

Materials and Methods

Experiment 1.

Animals and drug treatment. Twenty-four six-week old BALB/c and 22, six-week old C57BL/6 mice were housed individually in plastic cages and maintained according to the NIH Guide for the Care and Use of Laboratory Animals. Mice were treated with 8 mg/kg methamphetamine HCL (Sigma, St. Louis, MO, USA) subcutaneously and sacrificed 0 min. (n=6 BALB/c and n=5 C57BL/6), 30 min. (n=6 BALB/c and n=5 C57BL/6), 60 min. (n=6 each group), or 120 min. (n=6 each group) after injection. The drug was dissolved in saline and the animals were injected with 10 ml/kg body weight. All animals were housed in a colony room with temperature maintained at 22°, and a 12 hr on/12 hr off light cycle and were given free access to food and water. Immediately prior to sacrifice, measurement of body temperature was taken using a rectal probe coupled to a BAT-10 thermometer (Physitemp, Clinton, NJ, USA). In addition, animals were observed for incidence of self-injurious behaviour by an independent experimenter blind to treatment conditions. Self-injurious behaviour has been previously described (Kelly *et al.* 1975; Kita *et al.* 1998). After sacrifice, the brains were dissected on ice and the frontal cortex and striatum stored in liquid nitrogen for neurochemical analysis by HPLC (methods described in detail in Halladay *et al.* 1998). The hypothalamus was also dissected, immersed in RNAlater (Ambion, Austin, TX, USA) and stored in -80° for later determination of interleukin-1β and β-actin mRNA using RT-PCR.

Determination of murine interleukin-1β and β-actin. Total RNA from hypothalamic samples was extracted using the Totally RNA kit (Ambion, Austin, TX, USA). RNA was quantified using a geneQuant RNA/DNA reader (Pharmacia, Peapack, NJ, USA). Determination of interleukin-1β and β-actin transcripts was performed by RT-PCR using reagents purchased from Perkin-Elmer (Roche, Branchburg, NJ, USA) and a Perkin-Elmer DNA Thermal Cycler 480. Briefly, approximately 1 μg total RNA was reversed transcribed in a 20 μl reaction containing 5 mM $MgCl_2$, 1mM each of dNTPs, 1U/μl RNAse Inhibitor, 2.5 U/μl MuLV Reverse Transriptase, 2.5 μM Oligo $d(T)_{16}$ and 2 μl Perkin Elmer 10X PCR Buffer II. Reverse Transcription proceeded for 15 min. at 42°, and was followed by a 5 min. denaturation at 99°, and a final cooling at 5°. PCR amplification of interleukin-1β and β-actin cDNA was then conducted in separate tubes. A 50 μl PCR reaction was established containing 2 mM $MgCl_2$, 1.25 U AmpliTaq DNA Polymerase, 5 μl 10X PCR Buffer II, 1 μM total primers and 8 μl RT reaction. This involved an initial 5 min. denaturation step at 94°, followed by 5 min. annealing at 60°, after which there were 35 cycles of 1.5 min at 72°, 45 sec. at 94° and 45 sec. at 60°, and then a final extension of 10 min. at 72°. Primers were ordered from Stratagene (La Jolla, CA, USA). PCR products were run on an agarose gel and visualized by ethidium bromide staining, with product size determined using a 100 bp DNA ladder (Promega, Madison, WI, USA).

Experiment 2.

Animals and drug treatment. Thirty-six BALB/c and 32 C57BL/6 mice were housed in conditions identical to that in Experiment 1. The animals were injected with either saline or methamphetamine 8 mg/kg, subcutaneously, four times with 2 hr inter-injection intervals. Thirty min. prior to each injection, each animal was administered either acetaminophen (200 mg/kg intraperitoneally) or saline. Acetaminophen (Sigma, St. Louis, MO, USA) was dissolved in warm saline and animals were injected with a volume of 20 ml/kg body weight. Body temperature was measured rectally using a monitoring thermometer (Physitemp, NJ, USA) immediately prior to every methamphetamine or saline injection, as well as immediately prior to the first acetaminophen or saline injection (30 min. before methamphetamine). Animals were sacrificed three days after drug administration, brains dissected on ice and the frontal cortex and striatum stored in liquid nitrogen for assay of neurochemical concentration by HPLC.

Statistical analysis. Statistical analysis of neurochemistry and body temperature in striatum and frontal cortex was performed using either a one or two factor analysis of variance (ANOVA). Differences in self-injurious behaviour were examined using a chi-square and Fisher's Exact Test.

Results

Experiment 1.

Body temperature. There was a significant effect of strain ($F(1,38)=10.5$, $P=0.002$), time of sacrifice ($F(3,38)=29.5$, $P=0.0001$) and an interaction of strain and time ($F(3,38)=6.5$, $P=0.001$) on changes in body temperature following methamphetamine administration. C57BL/6 mice showed a hyperthermic effect 30 min. after methamphetamine. BALB/c mice showed a much greater hyperthermic effect, and this effect was significant at all 3 time points (fig. 1).

Self-injurious behaviour. While none of the C57BL/6 mice demonstrated self-injurious behaviour, 6/6 (100%) of the BALB/C mice exhibited taffy pulling as well as biting of chest and forepaws, $G^2=42.6$, $P<0.0001$ (table 1).

Neurochemistry 0–2 hr after methamphetamine treatment. Both strains showed a significant reduction in striatal 3,4-dihydroxyphenylacetic acid (DOPAC) levels following methamphetamine administration ($F(339)=29.3$, $P=0.0001$) (fig. 2). Homovanillic acid (HVA) levels remained unchanged in C57BL/6 mice; however, levels of this metabolite were increased in BALB/c mice 60 min. after methamphetamine

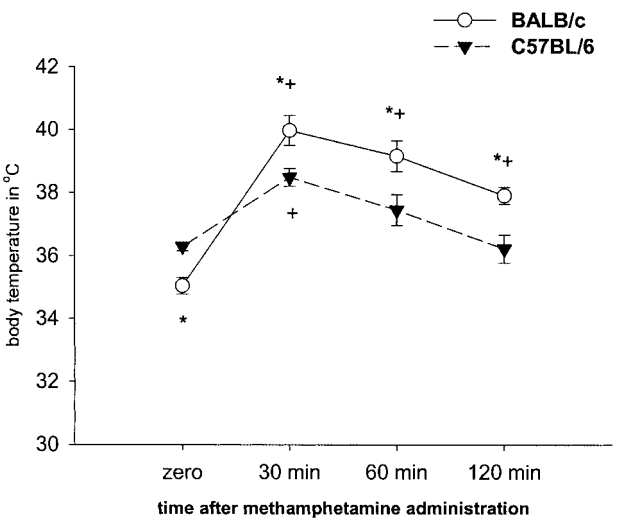


Fig. 1. Effect of a single methamphetamine injection (8 mg/kg subcutaneously) on body temperature in C57BL/6 and BALB/c mice. * indicates significantly different from C57BL/6 mice, + indicates different from time zero (untreated mice), $P<0.05$.

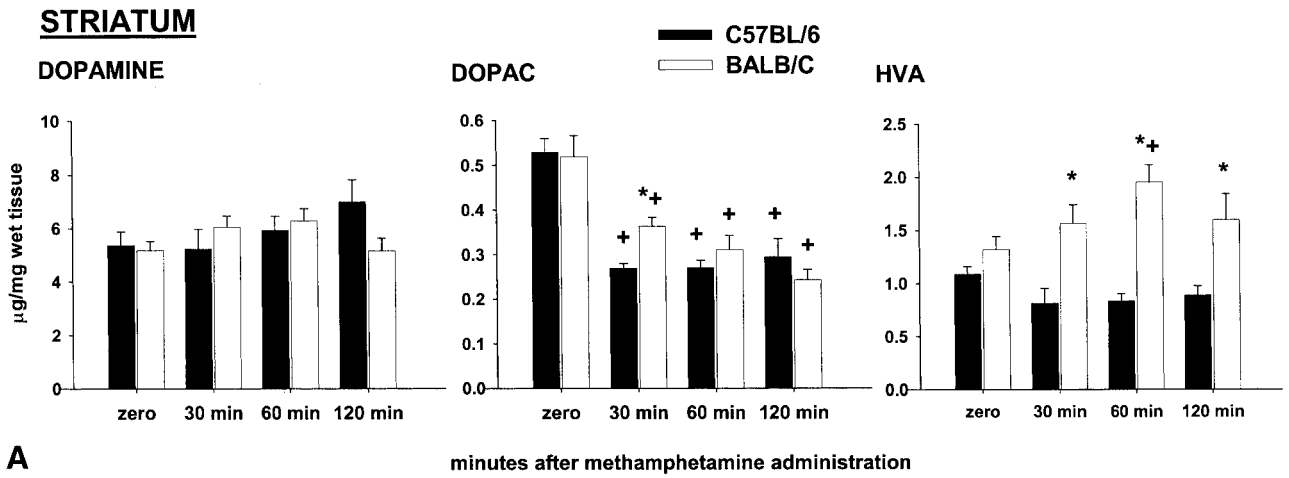
Table 1.

Experiment 1.

Strain	Treatment	SIB incidence +0 min.	SIB incidence +30 min.	SIB incidence +60 min.	SIB incidence +120 min.
BALB/C	Methamphetamine	0/5	6/6	6/6	6/6
C57BL/6	Methamphetamine	0/6	1/5	0/6	1/6

Self-injurious behaviour (SIB) at 0, 30, 60 and 120 min. after a single METH injection in BALB/c and C57BL/6 mouse strains.

administration, and remained consistently higher than C57BL/6 mice at all time points (F(3,38)=2.9, P=0.04). Finally, concentrations of 5-hydroxyindoleacetic acid (5-HIAA) were also elevated in both strains following methamphetamine (F(3,38)=3.2, P=0.03). In the frontal cortex, dopamine levels were elevated in the BALB/c strain compared to C57BL/6 mice both 60 and 120 min. after methamphetamine administration (F(1,38)=15.7, P=0.0003) (fig. 3). The concentration of the metabolite DOPAC was also significantly higher in the BALB/c strain compared to the C57BL/6's (F(1,38)=33, P=0.0001) and time zero (no treatment) (F(3,38)=36.1, P=0.0001) following methamphetamine. Homovanillic acid levels were also higher in the BALB/c strain at all time points (F(1,37)=137, P=0.0001),



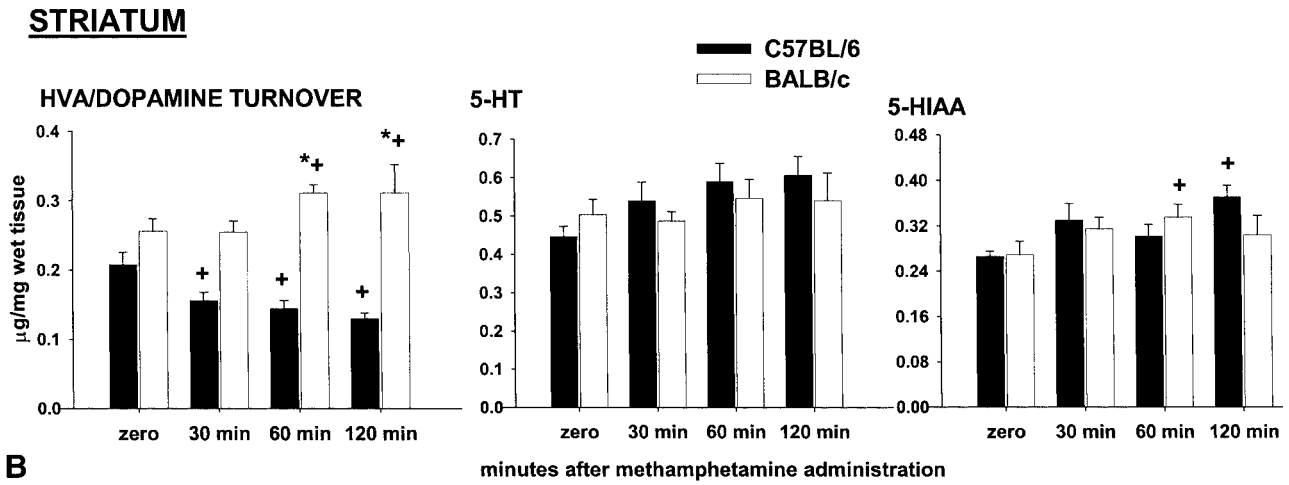


Fig. 2A. Effects of a single methamphetamine administration (8 mg/kg subcutaneously) on changes in dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) concentrations in the striatum either without injection (zero) or 30, 60 and 90 min. after drug administration in C57BL/6 and BALBc mice. * indicates significantly different from C57BL/6 mice, + indicates different from time zero (untreated mice), P<0.05.

Fig. 2B. Effects of a single methamphetamine administration (8 mg/kg subcutaneously) on changes in dopamine turnover (HVA/DA) serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the striatum either without injection (zero) or 30, 60 and 90 min. after drug administration in C57BL/6 and BALBc mice. * indicates significantly different from cC57BL/6 mice, + indicates different from time zero (untreated mice), P<0.05.

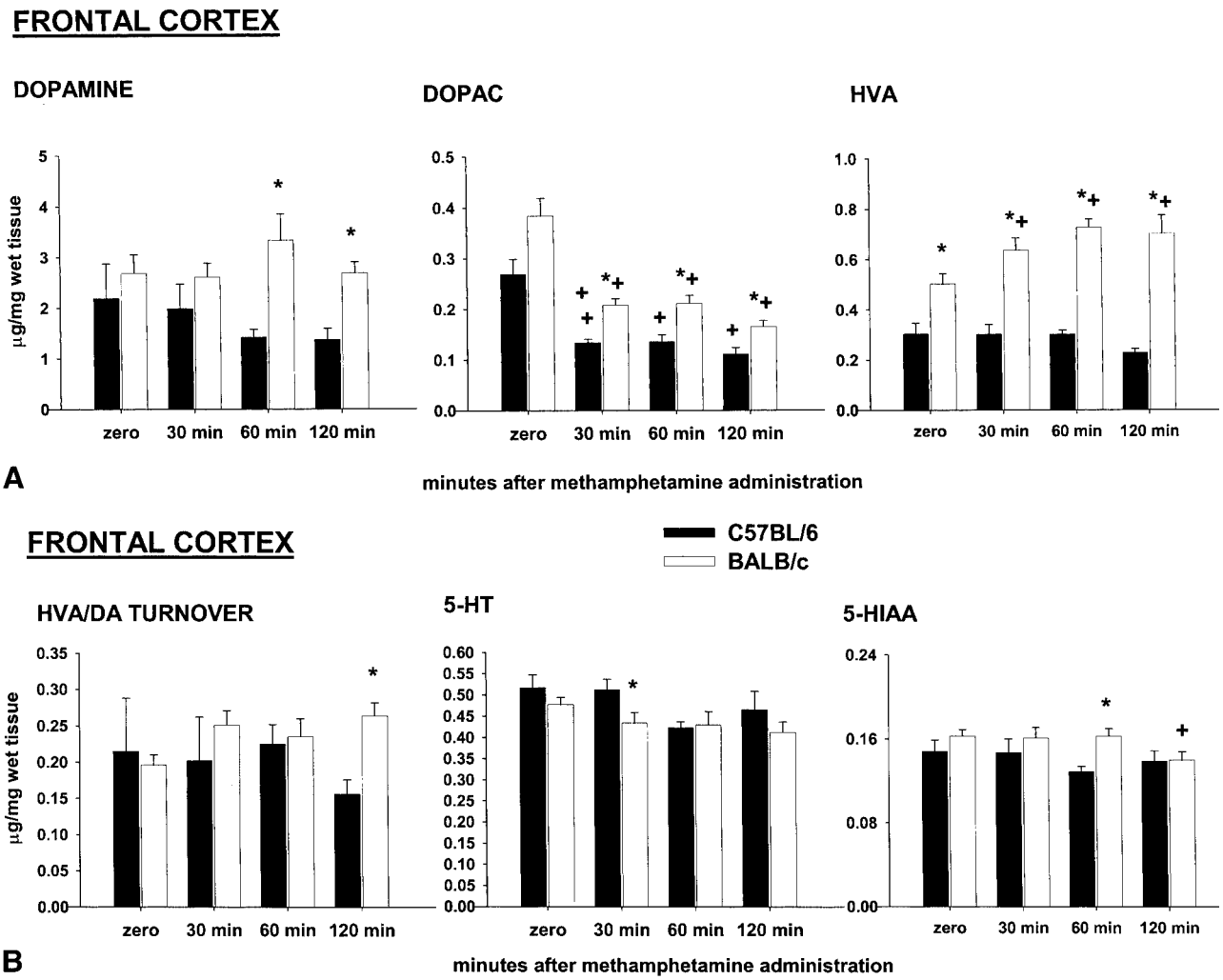


Fig. 3A. Effects of a single methamphetamine administration (8 mg/kg subcutaneously) on changes in dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) concentrations in the frontal cortex either without injection (zero) or 30, 60 and 90 min. after drug administration in C57BL/6 and BALB/c mice. * indicates significantly different from C57BL/6 mice, + indicates different from time zero (untreated mice), $P<0.05$.

Fig. 3B. Effects of a single methamphetamine administration (8 mg/kg subcutaneously) on changes in dopamine turnover (HVA/DA) serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the frontal cortex either without injection (zero) or 30, 60 and 90 min. after drug administration in C57BL/6 and BALB/c mice. * indicates significantly different from C57BL/6 mice, + indicates different from time zero (untreated mice), $P<0.05$.

as well as following methamphetamine ($F(3,37)=3.9$, $P=0.01$). There were significant effects of strain ($F(1,38)=4.5$, $P=0.04$) and time following methamphetamine ($F(3,38)=2.7$, $P=0.05$) on serotonin (5-HT) and 5-HIAA concentrations such that BALB/c mice showed lowered 5-HT levels compared to C57BL/6 mice 30 min. after methamphetamine treatment only. Furthermore, while both strains showed lower 5-HIAA concentrations concequent to methamphetamine treatment compared to untreated animals, levels of 5-HIAA were elevated in BALB/c mice at 60 min. ($F(1,38)=6.2$, $P=0.01$).

Determination of murine interleukin-1β and β-actin. Fig. 4 shows results of a RT-PCR conducted on RNA from pooled hypothalami (n=3 per group) of methamphetamine-treated C57BL/6 and BALB/c mice sacrificed 2 hr after injection. To confirm initial integrity of the RNA and successful cDNA synthesis of the RT step, PCR was conducted using primers for β-actin. There was ample hypothalamic β-actin mRNA for both mouse strains following methamphetamine treatment. In contrast, the degree of amplification of cDNA derived from interleukin-1 mRNA was different between the two strains. In the C57BL/6 group, no interleukin-1 transcript could be visualized (see arrowhead), whereas a distinct band was evident at the expected size (447 bp) for the BALB/C strain. It should be noted that for both strains, saline-treated animals did not show an interleukin-1 transcript (not shown). Since the samples were pooled so that 1 µg RNA could be obtained, the gel presented represents a

n=1 per group. This part of the study has been replicated (Kusnecov, unpublished observations); however, the gel in fig. 4 reveals a clear difference between strains.

Experiment 2.

Body temperature. A 3-factor ANOVA revealed a significant effect of strain ($F(1,56)=10.1$, $P=0.002$), methamphetamine ($F(1,56)=15.6$, $P=0.0001$) and acetaminophen ($F(1,56)=88.2$, $P=0.0001$). The C57BL/6 strain did not show a hyperthermic effect following methamphetamine, while the BALB/c mice did (fig. 5). Body temperature in the BALB/c strain was reduced with acetaminophen, and furthermore, the pretreatment served to attenuate methamphetamine-induced increases in body temperature. While the pretreatment only served to return the methamphetamine-induced hyperthermia to saline-treatment levels in the BALB/c group, acetaminophen lowered core body temperature in the C57BL/6 strain to below that of saline-treated animals (fig. 5).

Self-injurious behaviour. As in Experiment 1, C57BL/6 mice did not show any self-injurious behaviour after methamphetamine treatment. There was also no difference in self-injurious behaviour in the BALB/c strain receiving methamphetamine and acetaminophen plus methamphetamine (both at 80%), and a significant decrease in self-injurious behavior incidence at the 4th injection compared to the 2nd injection (Fisher's Exact Test, $P<0.05$, table 2). Statistical analysis revealed no predictive value of acetaminophen pretreatment on the time of onset ($\lambda=0.111$, $P=0.76$).

Neurochemistry 3 days after treatment. An analysis of the two control groups (sal-sal and acetaminophen-sal) revealed no significant differences between these two treatment conditions, therefore, the groups were combined into one control condition. ANOVA of striatal dopamine levels revealed a significant effect of strain ($F(1,55)=38.7$, $P<0.0001$), and treatment ($F(2,55)=24.2$, $P<0.0001$) and an interaction of strain and treatment condition ($F(2,44)=11.5$, $P<0.0001$). While both strains showed a long-term depletion of striatal dopamine, the lesion was significantly greater in the BALB/c mice (fig. 6). Furthermore, acetaminophen pretreatment reversed the dopamine lesion in C57BL/6 mice while having no effects in the BALB/c strain. After methamphetamine, DOPAC levels remained unchanged in the C57BL/6 strain, but were significantly depleted in the BALB/c group ($F(2,54)=19.4$, $P<0.0001$), both with and without acet-

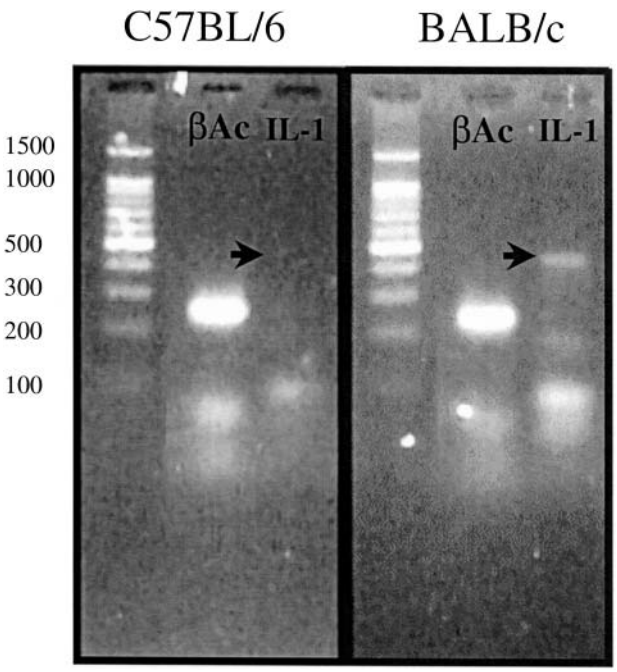


Fig. 4. Result of a RT-PCR conducted on RNA from pooled hypothalami of methamphetamine treated C57BL/6 and BALB/c mice sacrificed 2 hr after injection. Both strains show ample hypothalamic β-actin mRNA. The degree of amplification of cDNA derived from interleukin-1 mRNA was noticeably different between the two strains. In methamphetamine-treated BALB/c animals only, a distinct band was evident at 447 bp, marked by arrows (above).

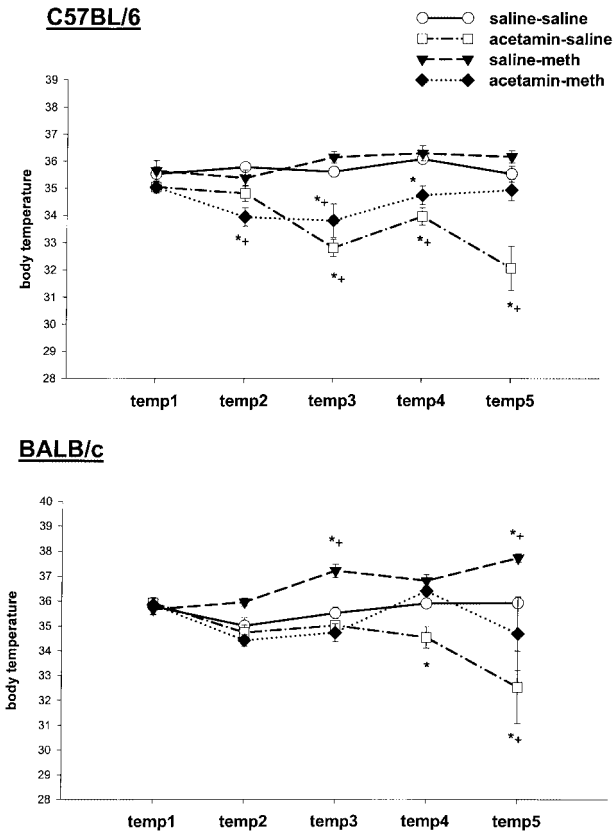


Fig. 5. Changes in body temperature in C57BL/6/black mice (top graph) and BALB/c mice (bottom graph) in animals treated with saline, acetaminophen (200 mg/kg intraperitoneally), methamphetamine (8 mg/kg subcutaneously), or acetaminophen pretreatment to methamphetamine. Methamphetamine was administered 4 times, 2 hr apart. Acetaminophen was administered 30 min. prior to each methamphetamine injection in animals treated with the two drugs. Body temperature was taken prior to each methamphetamine or acetaminophen injection. * indicates significantly different from saline treated animals, + indicates different from temperature 1 (baseline temp), $P<0.05$.

Table 2.

Experiment 2.

Strain	Treatment	SIB incidence +1 meth	SIB incidence +2 meth	SIB incidence +3 meth	SIB incidence +4 meth
BALB/C	Methamphetamine	7/10	7/10	6/10	1/10
	Acetaminophen+ methamphetamine	6/9	7/9	5/9	1/9
C57BL/6	Methamphetamine	0/8	0/8	0/8	0/8
	Acetaminophen+methamphetamine	0/8	0/8	0/8	0/8

Effects of acetaminophen pretreatment on METH-induced SIB following single or multiple injections. Animals were administered acetaminophen 200 mg/kg intraperitoneally 30 min. prior to each METH injection, which were spaced 2 hr apart. Observations were made 1 hr after each METH injection.

aminophen pretreatment ($F(2,54)=16.2$, $P<0.0001$) (fig. 6). HVA levels were reduced in both strains following methamphetamine treatment ($F(2,51)=13.9$, $P<0.0001$). The methamphetamine-induced reduction in HVA levels was reversed by acetaminophen pretreatment in the C57BL/6 but not BALB/c mice ($F(2,51)=5.2$, $P=0.008$).

Analysis of turnover ratios revealed a significant effects of both strain ($F(1,54)=3.9$, $P=0.05$) and treatment condition ($F(2,54)=7.3$, $P=0.002$) such that methamphetamine resulted in increased DOPAC/DA turnover ratios in the striatum in both strains. This effect was alleviated in the C57BL/6 but not the BALB/c strain when methamphetamine was preceded with an acetaminophen injection (fig. 7). Serotonin turnover was also affected by strain ($F(1,47)=15.8$, $P<0.0001$) and lesion condition ($F(2,47)=5.2$, $P=0.009$). 5-HIAA/5-HT ratios were elevated in C57BL/6 mice as compared to BALB/c mice, with both strains showing reduced serotonergic turnover following methamphetamine. When pretreated with acetaminophen, this decrease in turnover ratio was reversed in the C57BL/6 strain and unchanged in BALB/c mice.

In the frontal cortex, dopamine levels were reduced in BALB/C following methamphetamine treatment ($F(1,45)=13.4$, $P=0.0007$). BALB/c mice showed higher baseline dopamine and HVA levels in the frontal cortex, effects that were also seen in Experiment 1 ($F(1,45)=3.9$, $P=0.05$) and ($F(1,45)=5.6$, $P=0.02$). DOPAC and HVA levels were also reduced after methamphetamine administration in the BALB/c strain, with no effect in C57BL/6 mice ($F(1,45)=16.5$, $P=0.0002$) and ($F(1,45)=9.2$, $P=0.003$), respectively. HVA/DA turnover was also elevated in BALB/C mice receiving methamphetamine treatment ($F(1,44)=4.9$, $P=0.03$). This effect was unchanged with acetaminophen pretreatment. Levels of serotonin were elevated in C57BL/6 mice, regardless of methamphetamine treatment ($F(1,45)=14.2$, $P=0.0001$).

Discussion

The present studies revealed a substantial difference in sensitivity to methamphetamine-induced toxicity between strains with BALB/c mice exhibiting enhanced release of dopamine, increased hyperthermia, increased incidence of self-biting, induction of interleukin-1β, and increased depletion of dopamine as compared to C57BL/6 mice. Furthermore, it was demonstrated that acetaminophen blocked the hyperthermic effect induced by methamphetamine but not the long-lasting depletion of dopamine. These data are consistent with previous observations that methamphetamine-induced hyperthermia is not necessarily linked to methamphetamine-induced toxicity. In the present study, BALB/c mice showed a hyperthermic response to methamphetamine and a long-lasting dopamine depletion. Acetaminophen pretreatment protected these mice against the former but not the latter. In C57BL/6 mice, the mild dopamine depletion after methamphetamine was not accompanied by hyperthermia. However, the acetaminophen pretreatment in C57BL/6 mice actually lowered body temperature after methamphetamine and did protect against the long-lasting depletion. This replicates previous work which shows strain differences in both the hyperthermic and neurotoxic effects of methamphetamine in mice (Kita *et al.* 1998). In a previous study, we demonstrated that acetaminophen reversed the amphetamine-induced hyperthermia but did not alter the dopamine release following amphetamine administration (unpublished results). These results demonstrate that hyperthermia is not a prerequisite condition for methamphetamine-induced dopamine depletion. Rather, a more predictive factor appears to be increased dopamine release, measured by turnover ratio, and incidence of self-injurious behaviour.

Another factor that may participate in the strain-dependent differences presented here is the induction of interleukin-1β seen in the hypothalamus of the BALB/c mouse. Similar methamphetamine-induced inductions of this cytokine have been previously reported in other species (Yamaguchi *et al.* 1991); and interleukin-1β administration has been shown to exacerbate cortical induced excitotoxic lesions (Grundy *et al.* 1999). While an interleukin-1β antagonist did not affect the methamphetamine-induced dopamine-depletion in rats (Bowyer *et al.* 1994), the effects of this dosing regimen on short-term neurochemistry was not ascertained. It has been suggested that interleukin-1 and dopamine activity regulate one-another, as previous studies have reported increased dopamine turnover in the cortex and median eminence after interleukin-1β and interleukin-1 (Lacosta *et al.* 1998; MohanKumar *et al.* 1998; Brebner *et al.* 2000). In addition, Tringali *et al.* (1997) demonstrated

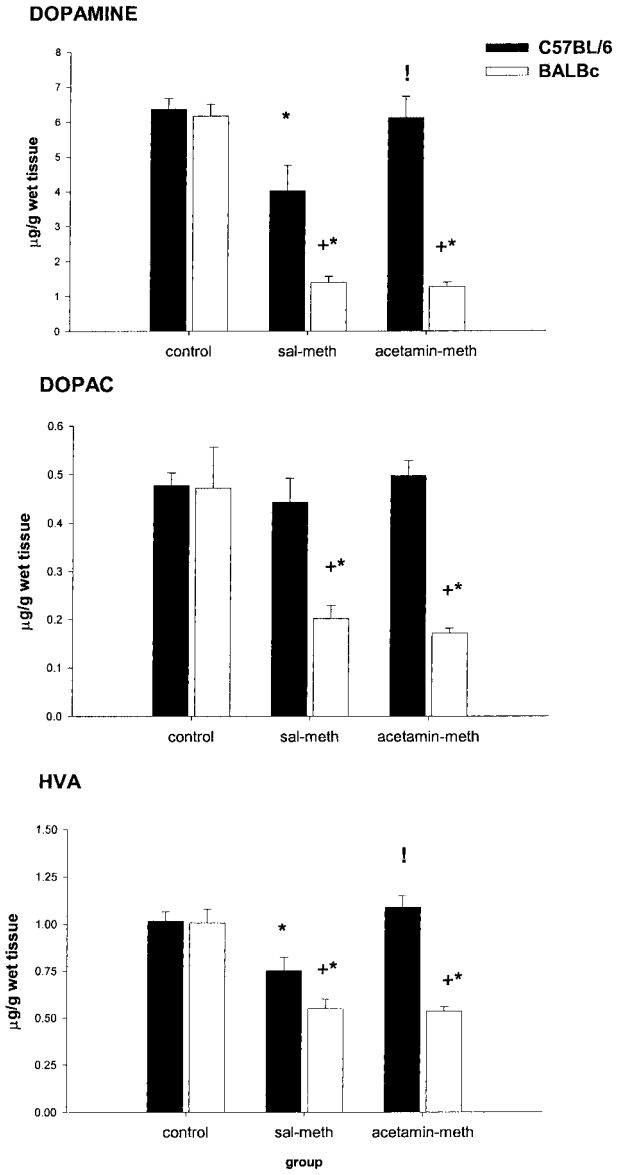


Fig. 6. Effects of acetaminophen (200 mg/kg intraperitoneally), methamphetamine (8 mg/kg subcutaneously), or acetaminophen pretreatment to methamphetamine on changes in striatal dopamine (top), DOPAC (middle) and HVA (bottom) concentrations 3 days after drug administration. Methamphetamine was administered 4 times, 2 hr apart. Acetaminophen was administered 30 min. prior to each methamphetamine injection in animals treated with the two drugs. * indicates significantly different from controls, + indicates significantly different from C57BL/6 mice, ! indicates significantly different from saline-methamphetamine treated animals, P<0.05.

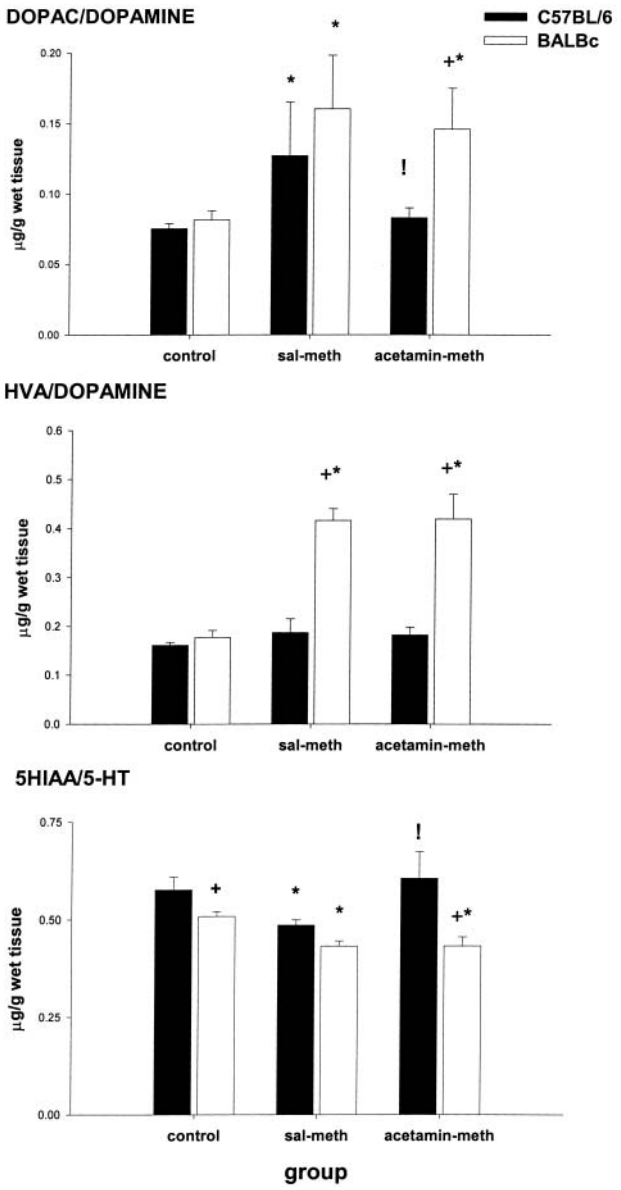


Fig. 7. Effects of acetaminophen (200 mg/kg intraperitoneally), methamphetamine (8 mg/kg subcutaneously), or acetaminophen pretreatment to methamphetamine on changes in striatal DOPAC/DA turnover (top), HVA/DA turnover (middle) and 5-HIAA/5-HT turnover (bottom) 3 days after drug administration. Methamphetamine was administered 4 times, 2 hr apart. Acetaminophen was administered 30 min. prior to each methamphetamine injection in animals treated with the two drugs. * indicates significantly different from controls, + indicates significantly different from C57BL/6 mice, ! indicates significantly different from saline-methamphetamine treated animals, P<0.05.

that hypothalmic neurones in culture show interleukin-1β in the presence of dopamine. Taken together, these combined results suggest that the magnitude of dopamine release is reflected in interleukin-1β response. This dopamine release and corresponding induction of interleukin-1β, in turn, may contribute to the magnitude of the dopamine depletion induced by methamphetamine. Furthermore, Ladenheim *et al.* (2000) showed that the sensitivity to methamphetamine-induced neurotoxicity was reduced in transgenic mice lacking interleukin-6. In the present study, the strain difference in sensitivity to the long-lasting depletion of dopamine induced by methamphetamine were reflected by the hypothalamic interleukin-1β induction measured two hours after the methamphetamine. The BALB/c mice showed a strong induction of interleukin-1β at 2 hr and this effect was accompanied by hyperthermia and an increase in dopamine turnover. C57BL/6 mice did not show the interleukin-1β ex-

pression and the hyperthermic response was both mild and short-lived. Finally, C57BL/6 mice showed a slight decrease in dopamine turnover rather than an increase as observed in the BALB/c strain. Since both strains showed a hyperthermic response to methamphetamine, the induction of interleukin-1β was most likely a reflection of the enhanced methamphetamine-induced dopaminergic activity in the BALB/c mice.

The strain differences between methamphetamine-induced self-injurious behaviour are also in accordance with previous work. BALB/c mice showed both an increase in incidence and intensity of self-biting. This effect was completely unrelated to methamphetamine-induced hyperthermia, as acetaminophen pretreatment did not alter the incidence of self-injurious behaviour in the BALB/c strain, while offsetting methamphetamine-induced increases in core body temperature. The self-injurious behaviour does appear to be related to dopamine release, as C57BL/6 mice, showing moderate dopamine activity after methamphetamine, also show no self-injurious behaviour. BALB/c mice, on the other hand, exhibit a greater dopaminergic response to methamphetamine and also high rates of self-biting. Interestingly, after the fourth injection of methamphetamine in Experiment 2, the incidence of self-biting in this strain significantly dropped compared to the other three injection points. This effect was also reported by Kita *et al.* (2000) who previously suggested that the magnitude of dopamine release may be used as a predictor of self-injurious behaviour. They reported a sharp decrease in striatal dopamine and DOPAC after the third injection of methamphetamine, as well as a drop in dopamine metabolite levels which had been elevated after the first two injections. The results of the current study replicate the Kita *et al.* (2000) observations of an inverted U-shaped time-course of self-injurious behaviour in mice after multiple injections of methamphetamine, where rates increase after the first and second injections and then drop off after the latter two treatment doses. In addition, it provides further evidence that dopamine release is a necessary prerequisite for self-injury.

In conclusion, the current set of experiments demonstrated both a strain-dependent difference between C57BL/6 and BALB/c mice in the acute effects of methamphetamine which may partially explain the differences in the long-term neurochemical consequences of high-dose methamphetamine exposure. Furthermore, these differences demonstrate that hyperthermia and dopamine depletion are not causally linked. Other factors, such as magnitude of dopamine release and induction of cytokines, may play a more important role in the development of methamphetamine-induced neurotoxicity.

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