

Methamphetamine-Induced Neurotoxicity Is Attenuated in Transgenic Mice with a Null Mutation for Interleukin-6

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Received March 13, 2000; accepted August 30, 2000

This paper is available online at <http://www.molpharm.org>

ABSTRACT

Increasing evidence implicates apoptosis as a major mechanism of cell death in methamphetamine (METH) neurotoxicity. The involvement of a neuroimmune component in apoptotic cell death after injury or chemical damage suggests that cytokines may play a role in METH effects. In the present study, we examined if the absence of IL-6 in knockout (IL-6^{-/-}) mice could provide protection against METH-induced neurotoxicity. Administration of METH resulted in a significant reduction of [¹²⁵I]RTI-121-labeled dopamine transporters in the caudate-putamen (CPu) and cortex as well as depletion of dopamine in the CPu and frontal cortex of wild-type mice. However, these METH-induced effects were significantly attenuated in IL-6^{-/-} animals. METH also caused a decrease in serotonin levels in the CPu and hippocampus of wild-type mice, but no reduction was observed in IL-6^{-/-} animals. Moreover, METH induced decreases in [¹²⁵I]RTI-55-labeled serotonin transporters in the

hippocampal CA3 region and in the substantia nigra-reticulata but increases in serotonin transporters in the CPu and cingulate cortex in wild-type animals, all of which were attenuated in IL-6^{-/-} mice. Additionally, METH caused increased gliosis in the CPu and cortices of wild-type mice as measured by [³H]PK-11195 binding; this gliotic response was almost completely inhibited in IL-6^{-/-} animals. There was also significant protection against METH-induced DNA fragmentation, measured by the number of terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeled (TUNEL) cells in the cortices. The protective effects against METH toxicity observed in the IL-6^{-/-} mice were not caused by differences in temperature elevation or in METH accumulation in wild-type and mutant animals. Therefore, these observations support the proposition that IL-6 may play an important role in the neurotoxicity of METH.

Methamphetamine (METH) is a drug of abuse that has steadily gained in popularity. Its continuous use is accompanied by long-term consequences. Repeated use of METH can cause neurological deficits that may lead to serious psychiatric and neurologic signs and symptoms in users (Lan et al., 1998). In addition, neuroimaging (McCann et al., 1998) and post-mortem studies (Wilson et al., 1996) have documented neurochemical deficits in the brains of METH abusers. METH neurotoxicity has also been demonstrated in rodents (O'Callaghan and Miller, 1994) and nonhuman primates (Villemagne et al., 1998). Previous studies from this laboratory have shown that repeated doses of METH cause loss of striatal dopamine transporters (DAT) (Hirata et al., 1996;

Hirata and Cadet, 1997) and significant depletion of dopamine (DA) in mice striata (Sheng et al., 1996b). Although the cellular and molecular events associated with METH neurotoxicity remain to be fully dissected, reactive oxygen species (Cadet et al., 1994; Jayanthi et al., 1998), nitric oxide (NO) (Sheng et al., 1996a; Itzhak et al., 1998; Deng and Cadet, 1999), and the activation of genes related to cell death (Cadet et al., 1997; Hirata and Cadet, 1997) seem to be involved. These different pathways might interact or work in parallel to cause the long-term negative consequences of the drug.

In addition to these pathways, other molecules, such as cytokines, which are known to cause neurotoxic damage, might also to participate in the toxic effects of METH. A cytokine that might contribute to METH-induced effects is interleukin-6 (IL-6). Accumulating evidence supports an essential role for IL-6 in the development, differentiation, regeneration, and degeneration of central nervous system

This work was partially supported by National Institutes of Health Grant HD 24605 (T.H.M.).

B.L. and I.N.K. have contributed equally to this work.

ABBREVIATIONS: METH, methamphetamine; DAT, dopamine transporters; DA, dopamine; NO, nitric oxide; IL-6, interleukin-6; CNS, central nervous system; IL-6^{+/+}, wild-type mice; IL-6^{-/-}, interleukin-6 knockout mice; CPu, caudate-putamen; NE, norepinephrine; DOPAC, 3,4-dihydroxyphenyl acetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; SERT, serotonin transporters; MBBS, mitochondrial benzodiazepine binding sites; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeled/labeling; GC, gas chromatography; MS, mass spectrometry; AMPH, amphetamine; IL-1 β , interleukin-1 β .

(CNS) (for review, see Gruol and Nelson, 1997; Benveniste, 1998). Areas expressing high levels of IL-6 and its receptors' mRNA in the brain are the hippocampus, hypothalamus, cortex, and cerebellum (Schobitz et al., 1992; Gadiant and Otten, 1995). IL-6 levels in CNS are low under baseline physiological conditions, but injury, inflammation, and brain diseases trigger IL-6 gene expression and protein synthesis (Gruol and Nelson, 1997). For example, elevated levels of IL-6 are detected in the cerebrospinal fluid of patients with several CNS abnormalities, including Alzheimer's disease, multiple sclerosis, meningitis and stroke (for review, see Zhao and Schwartz, 1998). Consistent with a role of IL-6 in neuropathological processes, elevated levels of IL-6 mRNA and protein have also been found in the CNS of animal models of neurodegenerative diseases (Gijbels et al., 1990; Grau et al., 1990). The prominent neurodegeneration observed in transgenic mice that chronically overexpress IL-6

provide very cogent support for the idea of IL-6 as a pro-toxin (Campbell et al., 1993; Chiang et al., 1994).

The aim of the present study was, thus, to investigate to what extent IL-6 might also play a role in the neurodegenerative effects of METH by using mice with a null mutation of the cytokine. Herein, we report that the IL-6 null genotype confers significant protection against the monoaminergic pathology and apoptosis caused by this illicit substance.

Materials and Methods

Animals and Treatment. C57BL/6J wild-type (IL-6^{+/+}) inbred mice and IL-6 knockout (IL-6^{-/-}) mice on the C57BL/6J background obtained from The Jackson Laboratory (Bar Harbor, ME) were used in the experiments. The generation and derivation of these mice have been described previously (Kopf et al., 1994). Mice were housed in groups of three and kept in a temperature- and light-controlled room

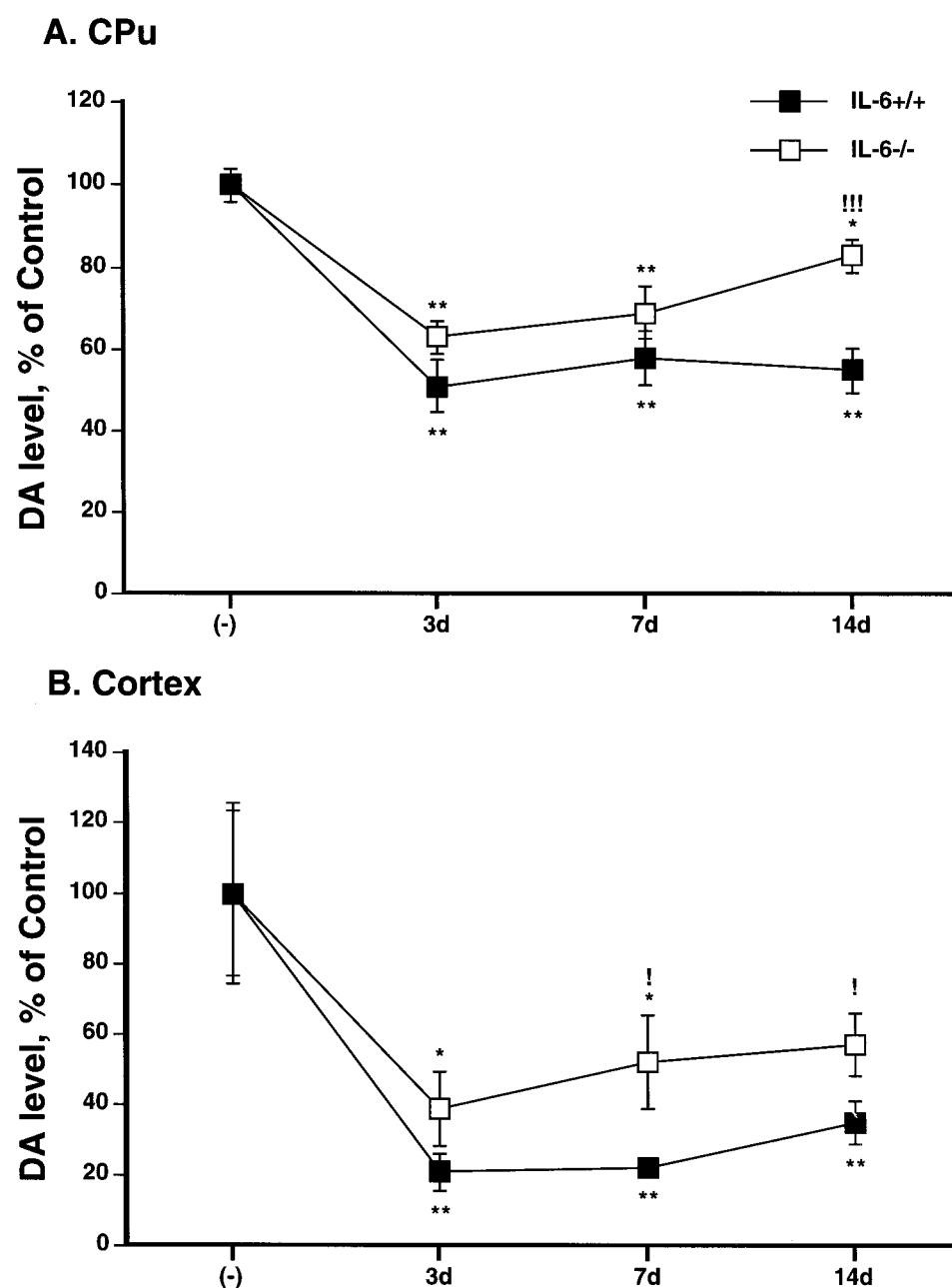


Fig. 1. Effects of repeated METH administration (5 mg/kg \times 4) on the levels of DA in the CPu (A) and frontal cortex (B). Values represent means \pm S.E.M. (percentage of respective controls) of 7 to 10 mice per group. There were no significant differences in DA levels between the saline-injected groups. The absolute DA values (nanograms per milligram of tissue weight) for saline and METH-treated at 3, 7, and 14 days are as follows: CPu, 13.95 \pm 0.24 (saline), 7.23 \pm 0.90 (3 days), 8.21 \pm 0.94 (7 days), and 7.68 \pm 0.76 (14 days) (IL-6^{+/+}); 14.26 \pm 0.55 (saline), 9.12 \pm 0.55 (3 days), 9.88 \pm 0.90 (7 days) and 11.91 \pm 0.58 (14 days) (IL-6^{-/-}). Frontal cortex, 0.740 \pm 0.191 (saline), 0.171 \pm 0.041 (3 days), 0.165 \pm 0.016 (7 days) and 0.260 \pm 0.045 (14 days) (IL-6^{+/+}); 0.737 \pm 0.172 (saline), 0.255 \pm 0.068 (3 days), 0.385 \pm 0.097 (7 days) and 0.422 \pm 0.065 (14 days) (IL-6^{-/-}). * P < .05; ** P < .01 versus respective controls; ! P < .05; !!! P < .001 versus IL-6^{+/+} mice treated with METH.

with a 12-h/12-h light/dark cycle and were fed ad libitum. All animal use procedures were according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee.

IL-6^{-/-} mice and their IL-6^{+/+} control counterparts received four intraperitoneal injections of 5 or 10 mg/kg METH or 0.9% saline at 2-h intervals. Core body temperature was recorded in IL-6^{+/+} and IL-6^{-/-} animals at 1 h intervals by the use of mouse rectal probe (YSI, Yellow Springs, OH). The mice were killed at several time points after drug treatment. Brain tissues were processed for the various assays as described below.

HPLC Analysis. At 3, 7, and 14 days after METH injections (5 mg/kg \times 4) mice were killed by cervical dislocation, the brains were removed and placed on a ice-cooled plate. Frontal cortex, caudate-putamen (CPu), and hippocampus were dissected and immediately frozen on dry ice and stored at -70°C until extraction. Brain regions obtained from each animal were weighed, ultrasonicated in 10%

perchloric acid containing 10 ng/mg of the internal standard dihydroxybenzylamine, and centrifuged at 20,000g for 10 min. Concentrations of norepinephrine (NE), DA, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in brain tissue extracts were measured by HPLC with electrochemical detection. The analytical column was Bondapak C-18 (5 μm , 4.6 \times 250.0 mm; Waters, Milford, MA), and the mobile phase contained 0.08 M sodium acetate, 1 mM sodium EDTA, 5 mM heptane sulfonic acid, 4.0% acetonitrile, pH 4.0, flow rate 1.5 ml/min, 25°C. The installation consisted of Waters 717 Plus automated injection system, Waters piston pump, Waters manometric module, and Bio-analytical Systems LC-4C amperometric detector. The glassy carbon electrode was used at a potential of 0.75 V. Peak areas and sample concentrations were calculated with a Hewlett Packard integrator. Contents of NE, DA, DOPAC, 5-HT, and 5-HIAA were calculated as nanograms per milligram of tissue weight.

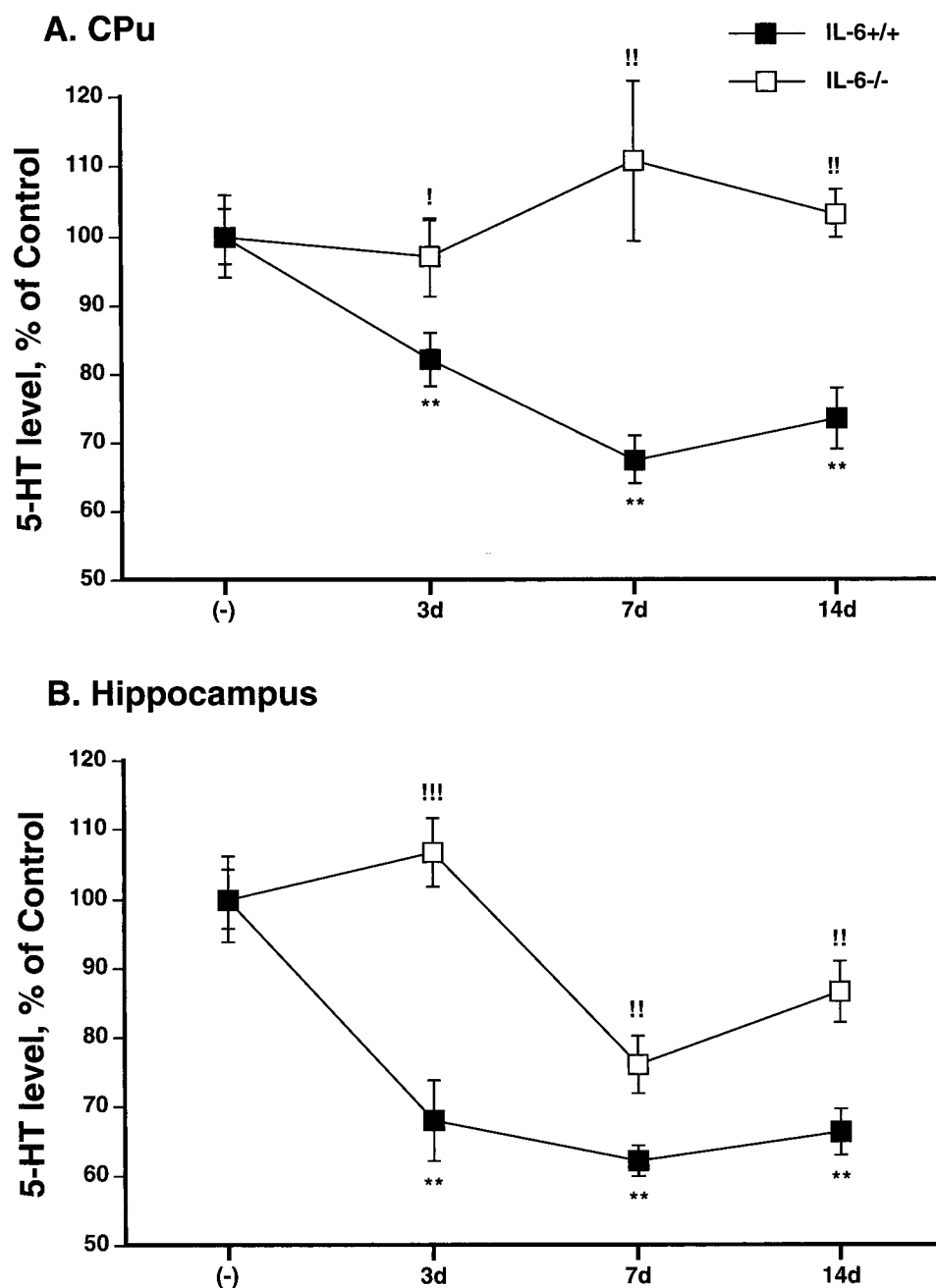


Fig. 2. Effects of repeated METH administration (5 mg/kg \times 4) on the levels of 5-HT in the CPu (A) and hippocampus (B). Values represent means \pm S.E.M. (percentage of respective controls) of 7 to 10 mice per group. There were no significant differences in 5-HT levels between the saline injected groups. The absolute 5-HT values (nanograms per milligram of protein) for saline and METH-treated at 3, 7, and 14 days are as follows: CPu, 1.68 \pm 0.10 (saline), 1.38 \pm 0.06 (3 days), 1.20 \pm 0.24 (7 days) and 1.24 \pm 0.07 (14 days) (IL-6^{+/+}); 1.44 \pm 0.15 (saline), 1.40 \pm 0.08 (3 days), 1.59 \pm 0.16 (7 days), and 1.48 \pm 0.34 (14 days) (IL-6^{-/-}). Hippocampus, 2.33 \pm 0.14 (saline), 1.58 \pm 0.13 (3 days), 1.45 \pm 0.05 (7 days), and 1.55 \pm 0.08 (14 days) (IL-6^{+/+}); 2.15 \pm 0.09 (saline), 2.29 \pm 0.11 (3 days), 1.64 \pm 0.09 (7 days), and 1.86 \pm 0.10 (14 days) (IL-6^{-/-}). ** P < .01 versus respective controls; ! P < .05; !! P < .01; !!! P < .001 versus IL-6^{+/+} mice treated with METH.

Autoradiographic Assays. Binding assays for DAT were performed as described previously by this laboratory with [125 I]RTI-121 (specific activity, 2200 Ci/mmol) and 10 μ M GBR-12909 to determine nonspecific binding which was 5% of total binding (Hirata et al., 1996). Autoradiographic determination of 5-HT transporters (SERT) was performed as described previously by this laboratory with [125 I]RTI-55 (specific activity, 2200 Ci/mmol) (Andrews et al., 1996). LR1111 was used to inhibit binding of [125 I]RTI-55 to DAT. Nonspecific binding was determined in the presence of 10 μ M paroxetine and represented <10% of the total binding. [125 I]RTI-121 and [125 I]RTI-55 binding was done in animals sacrificed at 14 days.

[3 H]PK-11195 is a specific high-affinity ligand for mitochondrial benzodiazepine binding sites (MBBS), which is reported to bind exclusively to glial cells (Myers et al., 1991; Banati et al., 1997). For investigation of MBBS mice were sacrificed 3 and 14 days after METH (5 mg/kg \times 4) treatment. Previous reports documented that the increase in gliosis peaked at 3 days and was significant till 21 days (Kuhlman and Guilarte, 1999). The brains were rapidly removed and frozen in isopentane and stored at -70°C until processing. Brains sections were cut at 20 μ m on a cryostat (-20°C) and thaw mounted on gelatin-coated slides. Slides were desiccated and stored at -70°C before assay. Adjacent sections were incubated for the MBBS on microglia and astrocytes, for 60 min with 3.0 nM [3 H]PK-11195 (86 Ci/mmol) (DuPont-NEN, Boston, MA) in a binding buffer consisting of 50 mM Tris-HCl, pH 7.4, and 120 mM NaCl at room temperature. Sections were washed in a buffer at 4°C twice for 5 min each and dipped three times in distilled ice-cold water and dried under a stream of cool air. Nonspecific binding was determined in the presence of 10 μ M unlabeled PK-11195 (RBI, Natick, MA). Dried sections were apposed to radiosensitive films (Hyperfilm; Amersham Pharmacia, Piscataway, NJ) with ^3H microscopes for 1 week. [3 H]PK-11195, [125 I]RTI-121, and [125 I]RTI-55 bindings were quantified using a Macintosh computer-based analysis system (NIH Image; National Institutes of Health, Bethesda, MD) with standard curves generated from ^3H or ^{125}I microscopes, respectively.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick-End Labeling (TUNEL) Histochemistry. TUNEL histochemistry was carried out as described previously by us (Deng et al., 1999). Briefly, IL-6+/+ and IL-6-/- mice were given 4 injections of 10 mg/kg METH or saline at 2-h intervals. For TUNEL assay, at 1 week after METH administration, animals were perfused transcardially, under deep pentobarbital anesthesia, first with saline followed by 40 ml of 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C . After perfusion, brains were removed, postfixed overnight in 4% paraformaldehyde, and then allowed to equilibrate in 30% sucrose for 24 h. Coronal sections (30 μ m) were then cut in a cryostat (Bright Instrument Company, Huntingdon, UK). The sections contained parietal cortices (bregma levels, 1.0 to -1.3) were collected. A TUNEL procedure for frozen tissue sections was performed using an in situ cell-death detection kit according to the manufacturer's manual (Boehringer Mannheim, Mannheim, Germany). Briefly, slide-mounted sections were rinsed in 0.3% hydrogen peroxide-methanol to block endogenous peroxidase. They were then rinsed in 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice to increase permeability of the cells. To label damaged nuclei, 50 μ l of the TUNEL reaction mixture was added onto each sample in a humidified chamber followed by a 60-min incubation at 37°C . The peroxidase reaction was visualized with DAB-substrate solution. Procedures for negative controls were carried out as described in the manufacturer's manual and consisted of not adding the label solution (terminal deoxynucleotidyl transferase) to the TUNEL reaction mixture. No TUNEL-positive cells were observed in the negative controls. TUNEL-positive cells were counted in the parietal cortex using a Zeiss microscope (600 \times 900 μ m).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of METH and Amphetamine (AMPH). IL-6-/- mice and their IL-6+/+ control counterparts received intraperitoneal injections of METH (5 mg/kg) or 0.9% saline at 2-h intervals. Animals were sacrificed 30 min after the first, third, and fourth injections. Their CPus, frontal cortices, and hippocampi were dissected, immediately frozen on dry ice,

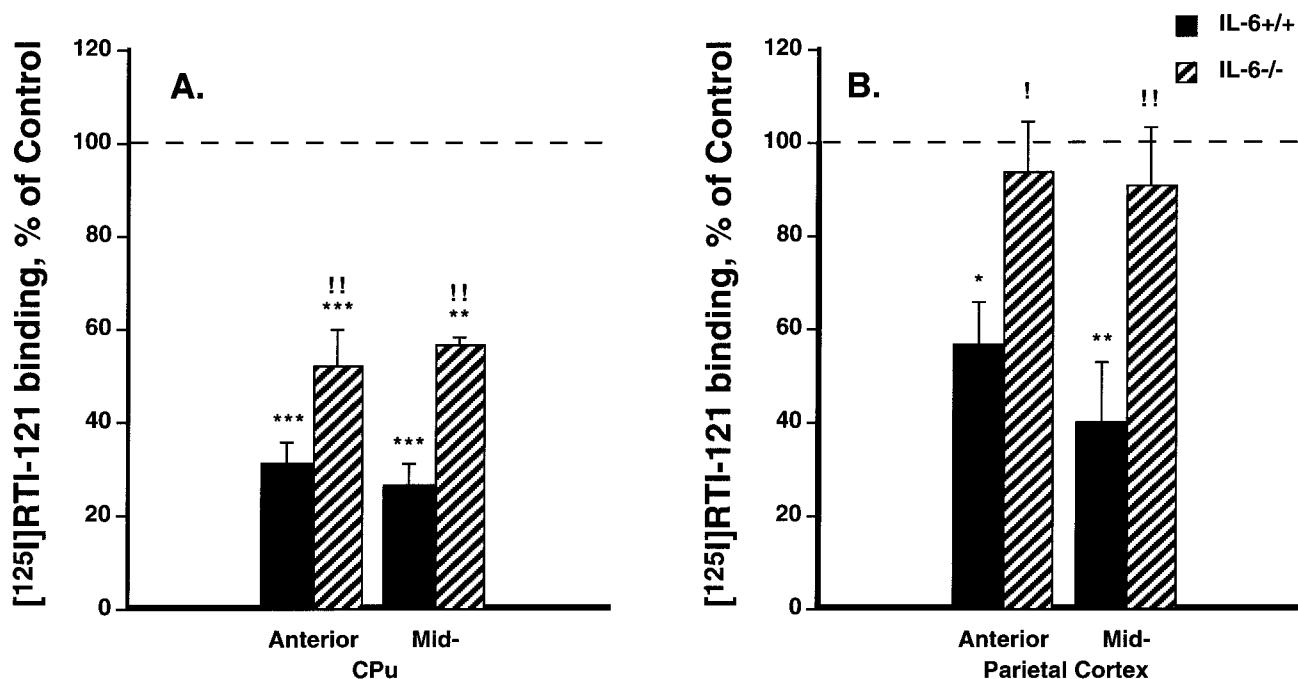


Fig. 3. Effect of METH treatment (5 mg/kg \times 4) on the [125 I]RTI-121-labeled DAT in the CPu (A) and parietal cortex (B). Values represent mean percentages of each saline injected group \pm S.E.M. $N = 5$ to 6 animals per group. There were no significant differences between wild-type and IL-6-/- saline-injected mice. The absolute values (nanoCuries per milligram of tissue) for saline and METH-treated groups are: Ant CPu, 14.43 ± 3.98 and 4.50 ± 0.68 (IL-6+/+); 15.61 ± 3.20 and 8.15 ± 1.22 (IL-6-/-); Mid-CPu, 15.69 ± 2.04 and 4.24 ± 0.69 (IL-6+/+); 13.37 ± 0.85 and 7.65 ± 0.21 (IL-6-/-). Ant parietal cortex, 1.05 ± 0.13 and 0.60 ± 0.09 (IL-6+/+); 0.99 ± 0.13 and 0.94 ± 0.11 (IL-6-/-); mid-parietal cortex, 1.03 ± 0.08 and 0.41 ± 0.14 (IL-6+/+); 0.95 ± 0.06 and 0.87 ± 0.12 (IL-6-/-). * $P < .05$; ** $P < .01$; *** $P < .001$ compared with respective saline-treated group; ! $P < .05$, !! $P < .01$ compared with METH-treated IL-6+/+ mice.

and stored at -70°C until extraction. Samples were weighed and homogenized in sodium acetate buffer (2.0 M, pH 4.0). Internal standards and 2 ml of the buffer were added to an aliquot of homogenate. Samples were kept for 10 min at room temperature, followed by addition of acetonitrile while vortexing. After centrifugation, the supernatants were decanted onto conditioned Clean Screen DAU solid phase extraction columns (United Chemical Technologies, Bristol, PA) and samples were eluted with methylene chloride/2-propanol/ammonium hydroxide (80:20:2), a 20- μl aliquot of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide + 1% *tert*-butyldimethylchlorosilane (Pierce Chemical, Rockford, IL) was then added to the eluate, followed by evaporation under nitrogen at 40°C until dry. The residues were reconstituted in acetonitrile, followed by another centrifugation step. Samples were transferred to autosampler vials, 20 μl of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide + 1% *tert*-butyldimethylchlorosilane was added. The mixture was heated at 80°C for 15–20 min, a 20 μl of *N*,*O*-bis(trimethyl)trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemical) were added, and samples were heated again at 80°C for 45 min. A 1- μl aliquot of each sample was injected on GC-MS to measure quantitatively the *tert*-butyldimethylsilyl derivative of METH and the trimethylsilyl derivative of AMPH.

Analyses were performed on a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard 5973 mass selective detector. A split-splitless capillary inlet system operated in the splitless mode and an HP-1 fused-silica capillary column (12 m \times 0.2 mm, inner diameter, 0.33- μm film thickness) were used for the analyses. Quantitative analysis was performed by positive chemical ionization GC-MS analysis in selected-ion monitoring mode. The internal standards used in the selected-ion mode were [$^2\text{H}_{11}$]METH and [$^2\text{H}_5$]AMPH (Radian International, Austin, TX). The target *m/z* ions for the *tert*-butyldimethylsilyl derivative of METH and the trimethylsilyl derivative of AMPH were 130 and 158, respectively. Detection limits for both METH and AMPH were 33 pg/mg, equivalent to the lowest calibration concentration. The dynamic range for the assay

was 33 pg to 33 ng/mg for both analytes. Correlation coefficients were ≥ 0.99 .

Statistical Analysis. All data are presented as means \pm S.E.M. The data were analyzed by analysis of variance followed by Fisher's protected least significant difference test using the statistical program Statview 4.02 or Statistical procedures for social sciences test. Criteria for significance were set at the 0.05 level.

Results

HPLC. The effects of METH (5 mg/kg \times 4) on the levels of DA in the CPu of IL-6+/+ and IL-6-/- mice are presented in Fig. 1A. METH caused marked reduction in DA levels in the striata of mice killed at 3 (–48%), 7 (–41%), and 14 (–45%) days after drug treatment. These changes were significantly attenuated in IL-6-/- METH-treated animals, and corresponded to 36%, 31%, and 16% reductions, respectively. In the cortex, administration of METH caused even greater reductions in DA levels in wild-type mice. The decreases were –77%, –78%, –65%, at 3, 7, and 14 days, respectively (Fig. 1B). In the IL-6-/- mice, these decreases were –65%, –48%, and –43%, respectively. METH did not affect DOPAC levels in CPu and cortices of any of the strains of mice (data not shown).

The effects of METH on 5-HT content in CPu of wild-type and IL-6-/- mice are shown in Fig. 2A. Significant decreases were noted in the levels of 5-HT in METH-treated wild-type mice sacrificed at 3 (–18%), 7 (–29%), and at 14 (–26%) days after treatment. However, METH had no significant effects on the levels of 5-HT in IL-6-/- mice. METH caused no significant changes in 5-HIAA levels. 5-HT levels were significantly altered in the hippocampus of IL-6+/+

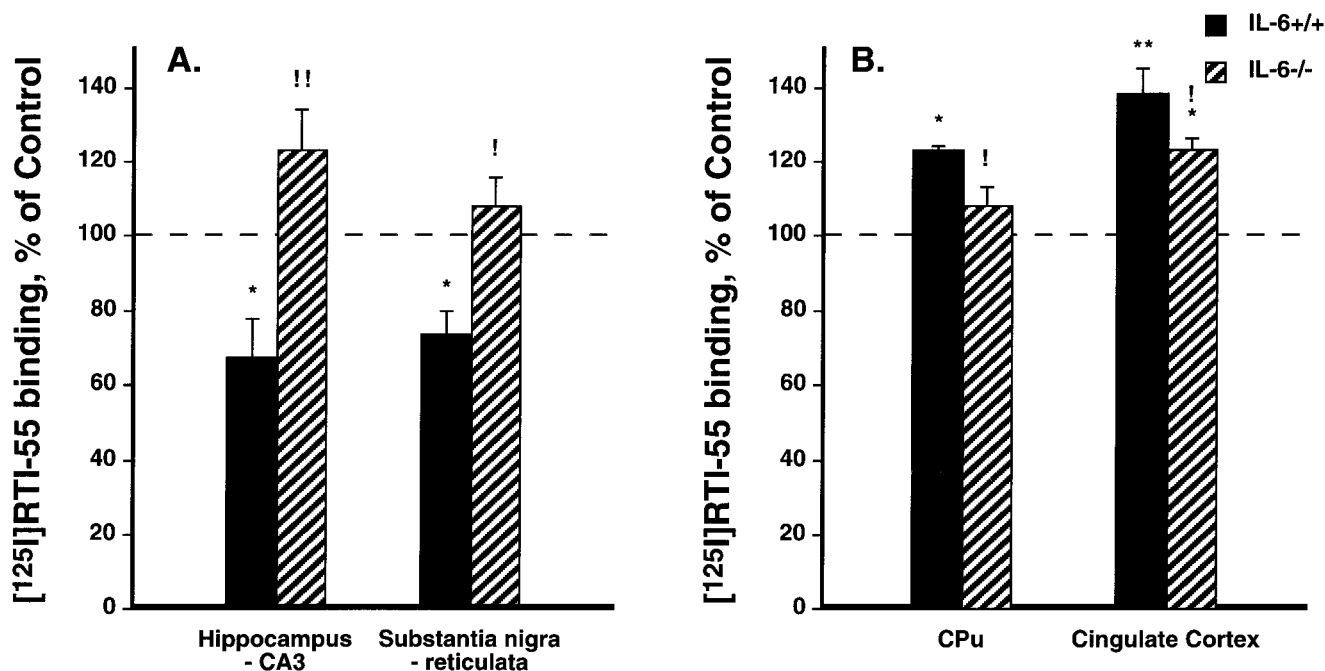


Fig. 4. Effect of METH treatment (5 mg/kg \times 4) on the [^{125}I]RTI-55-labeled SERT in the hippocampus-CA3, substantia nigra-reticulata (A) and CPu and cingulate cortex (B), on wild-type and IL-6-/- mice. Values represent mean percentages of each saline injected group \pm S.E.M. $N = 4$ to 5 animals per group. There were no significant differences between wild-type and IL-6-/- saline-injected mice. The absolute values (nanoCuries per milligram of tissue) for saline and METH-treated groups are: hippocampus-CA3, 11.55 ± 0.75 and 7.81 ± 1.19 (IL-6+/+); 9.18 ± 1.02 and 11.32 ± 1.26 (IL-6-/-); substantia nigra-reticulata, 30.75 ± 1.81 and 22.58 ± 2.02 (IL-6+/+); 26.34 ± 2.11 and 28.42 ± 2.07 (IL-6-/-); CPu, 5.85 ± 0.32 and 7.19 ± 0.06 (IL-6+/+); 6.76 ± 0.68 and 7.35 ± 0.30 (IL-6-/-); cingulate cortex, 2.86 ± 0.08 and 3.93 ± 0.20 (IL-6+/+); 2.76 ± 0.12 and 3.39 ± 0.08 (IL-6-/-). * $P < .05$; *** $P < .001$ compared with saline-treated mice of similar genotypes; ! $P < .05$; !! $P < .01$ in comparison with METH-treated wild-type mice.

mice sacrificed at 3 (–32%), 7 (–38%), or 14 (–33%) days postdrug (Fig. 2B). The depletion of 5-HT was significantly attenuated in METH-treated IL-6^{–/–} mice. METH also caused significant changes in 5-HIAA levels in the hippocampi of wild-type mice. These corresponded to decreases of 28%, 34%, and 42% at 3, 7, and 14 days, respectively. These effects were inhibited in IL-6^{–/–} animals. No reduction in 5-HIAA levels was seen at 3 days, and only 14% and 15% decreases were observed at 7 and 14 days, respectively. METH caused no changes in the levels of NE (data not shown).

Dopamine Transporter Binding. The effects of METH (5 mg/kg \times 4) on [¹²⁵I]RTI-121 labeled DAT in the anterior and mid-CPu in IL-6^{+/+} and IL-6^{–/–} mice sacrificed 2 weeks after drug treatment are presented on Fig. 3A. In wild-type animals, METH produced significant decreases in the DAT binding at the anterior CPu (–69%) and mid-CPu (–73%). METH effects were attenuated in IL-6^{–/–} drug-treated mice, with DAT binding showing reductions of 48% and 43% at the respective brain levels. METH caused significant reductions in DAT binding in the anterior (–43%) and mid-parietal cortex (–60%) of wild-type mice (Fig. 3B). Administration of METH to IL-6^{–/–} mice had no effect on DAT binding in these two regions. In contrast to its toxic effects on the cortical and striatal areas, METH injections failed to affect DAT binding in either the ventral tegmental area and substantia nigra pars compacta of either genotype (data not shown).

Serotonin Transporter Binding. The effects of METH on [¹²⁵I]RTI-55-labeled SERT in the brains of wild-type and IL-6^{–/–} mice are shown in Fig. 4. Administration of the drug to IL-6^{+/+} animals produced significant decreases in SERT binding in the hippocampal CA3 area (–33%) and in the substantia nigra pars reticulata (–27%). In contrast, IL-6^{–/–} mice showed complete protection against the deleterious effects of METH (Fig. 4A). Unexpectedly, METH caused significant increases in SERT binding in the CPu (+23%) and in the cingulate cortex (+38%) in wild-type mice. Interestingly, there were significant increases in the SERT binding in the cingulate cortex (+23%) but not in the CPu of IL-6^{–/–} mice (Fig. 4B). Nevertheless, the magnitude of this increase in the cingulate cortex of the IL-6^{–/–} was less than seen in the wild-type animals.

Mitochondrial Benzodiazepine Binding. MBBS, also referred to as the peripheral benzodiazepine receptor, has been proposed to be a general marker for gliosis in the nervous system because of its purported exclusive localization to glial cells (Myers et al., 1991; Banati et al., 1997). As shown in Fig. 5, administration of METH (5 mg/kg \times 4) to wild-type mice produced a significant increase in [³H]PK-11195 binding in the CPu, +27% and +26% at 3 and 14 days, respectively, as well as in the cingulate cortex (+15%) at 14 days. No increases were seen in the cingulate cortex at 3 days as well as in the hippocampus and substantia nigra at either 3 or 14 days after drug treatment (data not shown). METH had

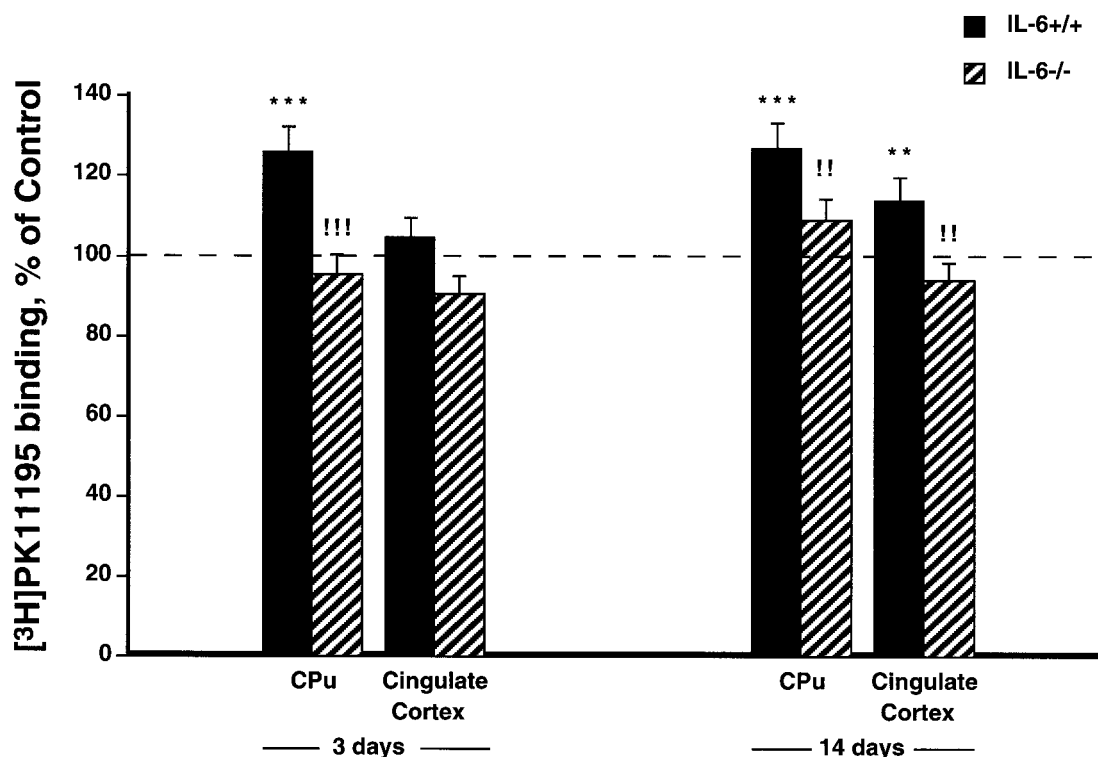


Fig. 5. Effects of METH treatment (5 mg/kg \times 4) on [³H]PK-11195-labeled mitochondrial benzodiazepine binding sites in the CPu and cingulate cortex of IL-6^{+/+} and IL-6^{–/–} mice. Values represent means \pm S.E.M. of five to seven animals per group. The absolute values (nanoCuries per milligram of tissue) for saline and METH-treated groups at 3 days are: CPu, 26.59 \pm 0.33 and 33.78 \pm 1.87 (IL-6^{+/+}); 28.06 \pm 0.92 and 26.85 \pm 1.03 (IL-6^{–/–}), cingulate cortex, 30.25 \pm 1.09 and 32.56 \pm 1.83 (IL-6^{+/+}); 31.76 \pm 1.27 and 25.86 \pm 1.43 (IL-6^{–/–}). At 14 days: CPu, 20.29 \pm 0.36 and 25.69 \pm 0.68 (IL-6^{+/+}), 21.23 \pm 0.54 and 23.13 \pm 0.49 (IL-6^{–/–}), cingulate cortex, 22.48 \pm 0.71 and 25.76 \pm 0.69 (IL-6^{+/+}), 24.04 \pm 1.11 and 22.56 \pm 0.73 (IL-6^{–/–}). ** P < .01; *** P < .001 compared with saline-treated mice of similar genotypes; !! P < .01, !!! P < .001 compared with METH-treated wild-type mice.

no significant effect on [^3H]PK-11195 binding in any of these brain regions in IL-6 $^{-/-}$ mice.

TUNEL Staining. To determine the contribution of IL-6 to METH-induced apoptotic cell death, mouse brains were examined for the occurrence of TUNEL-positive nuclei 7 days after drug (10 mg/kg \times 4) treatment. Representative photomicrographs of TUNEL-positive cells in the parietal cortex of wild-type and IL-6 $^{-/-}$ mice treated with saline and METH are presented on Fig. 6, A to D. Very few positive cells are seen in the parietal cortices of saline-treated mice of both genotypes (Figs. 6, A and C). However, administration of METH caused marked increases in TUNEL-positive staining in nondopaminergic cells at 1 week after drug treatment (Figs. 6, B and D). Quantification of these effects revealed that METH caused significant differences in the number of TUNEL-positive cells found in the brains of METH-treated IL-6 $^{+/+}$ versus METH-treated IL-6 $^{-/-}$ mice (Fig. 6E).

Temperature. Animal core-body temperature was recorded at 1-h intervals for the duration of METH injection regimen (7 h). The results showed that animals treated with METH had a hyperthermic response to the drug (Fig. 7). METH caused increases of core body temperature from 37.5 to 38.9°C both in wild-type and IL-6 $^{-/-}$ mice. Statistical

analysis revealed no significant differences between the wild-type and mutant mice.

Levels of METH and AMPH. The levels of METH and its major metabolite AMPH in the CPus, frontal cortices, and hippocampi of wild-type and IL-6 $^{-/-}$ mice are presented in Table 1. The time-points chosen for measurement of the drug levels correspond to the time of significant increase of extracellular drug levels in the CPu after a single dose (Melega et al., 1999) and after neurotoxic regimen (Clausing et al., 1995) of AMPH analogs. Levels of METH in the CPu were not significantly different between IL-6 $^{+/+}$ and IL-6 $^{-/-}$ animals at any of the time points examined. No differences were observed in the striatal AMPH levels between wild-type and mutant mice after the first and fourth injections of METH. However, after third injection, the IL-6 $^{-/-}$ mice showed a 24% higher concentration of the drug. In the frontal cortex, METH and AMPH levels were not different between IL-6 $^{+/+}$ and IL-6 $^{-/-}$ mice sacrificed at any of the time points. In the hippocampus, there were some differences in METH levels between the two genotypes, with the IL-6 $^{-/-}$ mice showing 18 to 21% higher levels than the IL-6 $^{+/+}$ animals after the third and fourth injections of METH. The levels of METH were 22% lower in the IL-6 $^{-/-}$ mice after first injection. No

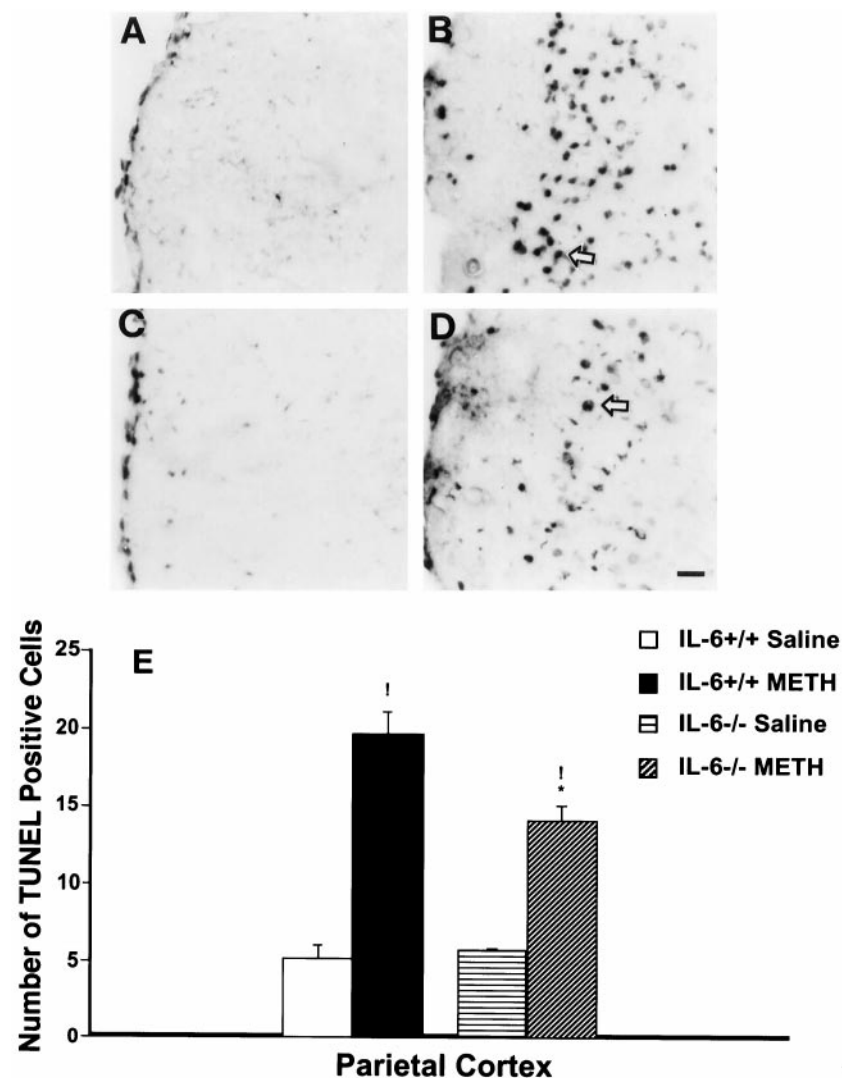


Fig. 6. A-D, representative photomicrographs of TUNEL-stained cell bodies in the cortices of mice. Very few positive cells could be seen in the cortices of saline-treated mice of both genotypes (A, C). METH (10 mg/kg \times 4) caused marked increases in TUNEL-positive cells in mice sacrificed at 7 days (B, D) after treatment. The arrows point to typical positive cells. E, statistical analysis of the quantitative data obtained from counting TUNEL-positive cells. Values represent means \pm S.E.M. of five to eight animals per group. * P < .05 compared with saline-treated mice of similar genotypes; ! P < .05 compared with METH-treated IL-6 $^{+/+}$ mice. Scale bar, 100 μm .

differences in AMPH levels were seen after the first and fourth injections; however, there was a 35% higher concentration of AMPH in the IL-6^{-/-} mice compared with control mice after the third injection of METH (Table 1).

Discussion

The main finding of the study is that METH neurotoxicity is attenuated in IL-6^{-/-} mice. This is supported by the following observations: 1) METH resulted in less depletion of DA and DAT binding in the CPu and cortices of IL-6^{-/-} mice; 2) METH-induced reduction in 5-HT levels in CPu and hippocampus and alterations in SERT binding were almost completely abolished in IL-6^{-/-} mice; 3) METH-induced reactive gliosis was markedly inhibited in the CPus and cortices of the IL-6^{-/-} mice; and 4) DNA fragmentation measured by TUNEL-positive cells was less prominent in nonmonoaminergic cortical cells of METH-treated IL-6^{-/-} mice. Although the present data identify IL-6 as another potential mediator of the long-term effects of METH, the observation that there was only partial protection against the toxic effects on monoaminergic terminals and nonmonoaminergic cell bodies in IL-6^{-/-} mice suggests that METH

may be causing its effects through both IL-6- dependent and -independent pathways. Previous studies have documented increased IL-6 gene expression in cultured astrocytes after ischemic injury (Yu and Lau, 2000) as well as in the striatum after quinolinic acid administration (Schiefer et al., 1998). These observations further enhance the idea that IL-6 overproduction is associated with neurodegenerative damage. In any case, future studies documenting the effects of METH on IL-6 cytokine are needed. These are presently ongoing in our laboratory.

Increased body temperature has been reported to play a role in METH neurotoxicity (Miller and O'Callaghan, 1994; LaVoie and Hastings, 1999). IL-6 has been demonstrated to be an essential component of the interleukin-1 β (IL-1 β)- and lipopolysaccharide-induced fever (Chai et al., 1996). Thus, it was conceivable that the protection observed in METH-treated IL-6^{-/-} mice might have been caused by a lack of temperature elevation. However, this supposition is not supported by our demonstration that METH caused increases in body temperature in both groups of mice.

Another possible explanation for the differential effects of METH in these two genotypes might be differential pharma-

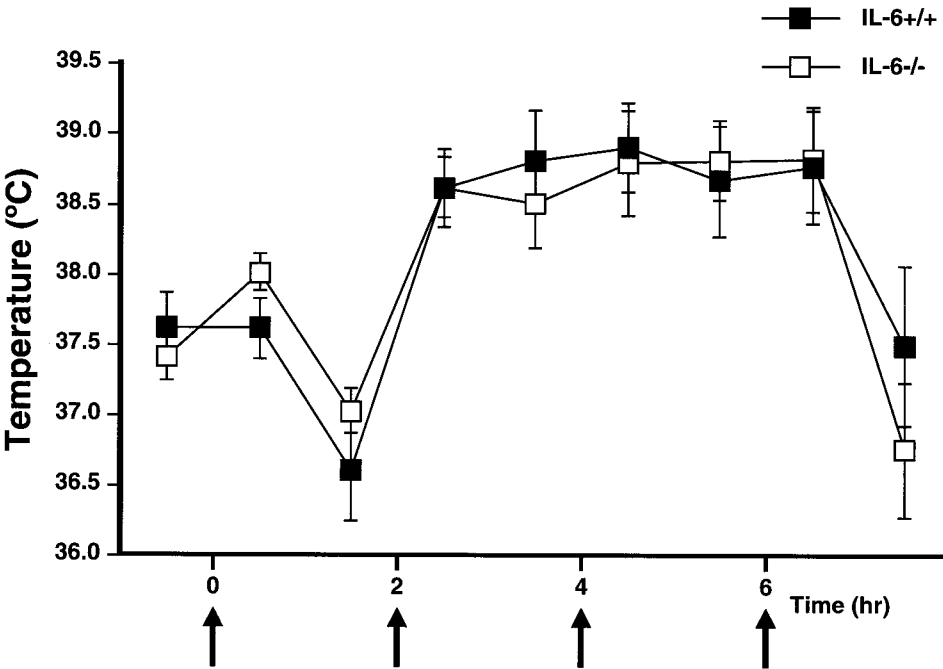


Fig. 7. Effects of METH (10 mg/kg \times 4) on core temperature in IL-6^{+/+} and IL-6^{-/-} mice. Core temperature was recorded in animals every 1 h during the administration of METH (the four injections of METH indicated by arrows) (mean \pm S.E.M.; n = 10–12). There were no statistical differences in the METH-induced temperature elevation between the two genotypes of mice. Saline-treated animals did not exhibit changes in core body temperature over time (data are not shown for the sake of clarity). Recorded ambient temperature was between 28.5 and 30.0°C.

TABLE 1
Levels of METH and AMPH (nanograms per milligram of tissue weight) in the brain structures of IL-6^{+/+} and IL-6^{-/-} mice 30 min after first, third, and fourth injections of METH (5 mg/kg)
METH and AMPH levels were measured by GC-MS as described under *Materials and Methods*. Data are presented as mean \pm S.E.M. of three to four mice per group.

	5 mg/kg \times 1		5 mg/kg \times 3		5 mg/kg \times 4	
	METH	AMPH	METH	AMPH	METH	AMPH
CPu						
IL-6 ^{+/+} mice	10.64 \pm 0.75	0.97 \pm 0.03	13.10 \pm 1.54	1.53 \pm 0.08	11.91 \pm 0.91	1.65 \pm 0.04
IL-6 ^{-/-} mice	8.53 \pm 0.52	0.76 \pm 0.05	14.60 \pm 0.70	1.89 \pm 0.09**	14.67 \pm 0.96	1.78 \pm 0.09
Frontal Cortex						
IL-6 ^{+/+} mice	3.41 \pm 0.15	0.35 \pm 0.03	4.27 \pm 0.79	0.52 \pm 0.10	4.20 \pm 0.41	0.58 \pm 0.03
IL-6 ^{-/-} mice	2.78 \pm 0.14	0.52 \pm 0.10	4.84 \pm 0.26	0.67 \pm 0.05	4.90 \pm 0.10	0.65 \pm 0.05
Hippocampus						
IL-6 ^{+/+} mice	3.32 \pm 0.15	0.36 \pm 0.02	3.75 \pm 0.13	0.49 \pm 0.02	4.02 \pm 0.20	0.60 \pm 0.01
IL-6 ^{-/-} mice	2.59 \pm 0.16*	0.31 \pm 0.01	4.43 \pm 0.11*	0.66 \pm 0.05**	4.87 \pm 0.27**	0.66 \pm 0.03

* P < .05. ** P < .01, compared with IL-6^{+/+} mice (analysis of variance followed by protected least significant difference test).

cokinetic handling of the drug by the animals. As reported above, there were no substantial differences in the levels of METH or AMPH measured in the CPus or cortices of these animals. It should be noted that, if anything, both AMPH and METH showed higher levels in the hippocampus of IL-6^{-/-} mice. Thus, if the pharmacokinetic explanation was correct, then the IL-6^{-/-} mice would have been more profoundly affected by METH toxic effects. However, as reported above, this is not the case (see Figs. 2 and 4). This argument thus suggests that other mechanistic possibilities need to be entertained. In what follows, we discuss potential scenarios via which METH could perturb the IL-6 pathway to cause its neurotoxic effects.

The accumulated evidence supports the view that formation of toxic radicals, generated from METH-induced DA release, might be the main triggers of METH toxicity to DA-ergic neurons (for review, see Cadet and Brannock, 1998). Similar oxidative mechanisms might be involved in the toxic effects of METH on other monoaminergic systems. For example, it seems that DA is required for METH-evoked 5-HT-ergic dysfunction because inhibition of DA synthesis by α -methyltyrosine attenuates (Schmidt et al., 1985), whereas L-3,4-dihydroxyphenylalanine potentiates (Schmidt et al., 1991) METH-induced 5-HT deficits. Moreover, oxygen-based radicals might also participate in METH-induced deleterious effects on the 5-HT-ergic system (Wrona et al., 1995).

In addition to its involvement in METH-induced damage to monoaminergic systems, our observations support a role for IL-6 in METH-induced apoptosis. This might occur through a scenario similar to the one described above because free radicals are known causes of apoptosis (Clement and Pervaiz, 1999).

In addition to DA-mediated generation of reactive oxygen species, METH might produce free radicals via IL-6-induced up-regulation of Fas and Fas ligand in astrocytes (Choi et al., 1999). This idea is supported by the report that stimulation of Fas cell death pathway is associated with increased production of hydrogen peroxide and superoxide radicals (Jayanthi et al., 1999) in a fashion similar to what has been reported for METH (Cadet et al., 1994; Jayanthi et al., 1998). Any superoxides produced in this manner could sequentially react with NO to form the neurotoxic peroxynitrite, with subsequent damage to cell bodies and nerve terminals. The observations that NO production occurs in microglia and astrocytes (Xiao and Link, 1998) support this contention.

Interestingly, METH caused decreases in SERT binding in the hippocampus and substantia nigra pars reticulata but increases in the CPu and cingulate cortex. Although the decreases are most probably related to depletion of 5-HT uptake sites located on 5-HT terminals, the increases might be related to changes in SERT that have been shown to be located on astrocytes (Bel et al., 1997; Hirst et al., 1998). This proposition is consistent with the observation of increased [³H]PK-11195 binding in the same brain regions (compare Figs. 4 and 5; see also below). It is possible to suggest, nevertheless, that the increase in SERT binding in the CPu could reflect hypertrophy of existing 5-HT-ergic terminals secondary to DA depletion, as has been reported after 6-hydroxydopamine-induced lesions when decrease in DA levels is greater than 90% (Commins et al., 1989; Zhou et al., 1991). This is probably not the case in the present study because there was only a 48% depletion of DA in the CPu (Fig. 1).

In the CNS, MBBS are primarily localized on nonneuronal elements such as astrocytes and microglia (Myers et al., 1991; Banati et al., 1997). Neuronal damage produced by a variety of insults is associated with the proliferation of reactive astrocytes and microglia. Thus, increases in MBBS binding has been used as a marker for reactive gliosis because a good correlation seems to exist between neuronal damage, gliotic response, and increases in [³H]PK-11195 binding (Kuhlmann and Guilarte, 1999). Because METH neurotoxicity is also associated with reactive gliosis (Deng et al., 1999), our demonstration that METH-induced increase in MBBS binding is inhibited in IL-6^{-/-} mice, provide further support for a role for the participation of this cytokine in the toxicity of this drug.

It is of interest that astrocytes as well as microglia can be activated by variety of stimulus to secrete IL-6. For example, stimulation of astrocytes by IL-1 β caused an increase in IL-6 synthesis (Benveniste et al., 1990). Thus, the demonstrated increase in IL-1 β in the hypothalamus of METH-treated animals (Yamaguchi et al., 1991) suggests a scenario in which IL-1 β might lead to initial glial secretion of IL-6 followed by an autostimulatory IL-6 feedback mechanism. This idea is supported by observation that IL-6 can induce astrocytic proliferation both in vitro (Guillemin et al., 1996) and in vivo (Chiang et al., 1994).

In conclusion, this study documents for the first time that METH-induced damage to DA and 5-HT terminals, apoptotic cell death, and reactive gliosis are attenuated in IL-6 knockout mice. Although the role of IL-6 in METH-induced damage remains to be fully clarified, the protection afforded by the IL-6 null genotype, suggests that the cytokine might be an important component of the toxic cascade caused by the drug. When taken together with the accumulated literature, a picture of METH neurotoxicity is developing that seems to involve much more complex phenomena than hitherto imagined. This is exemplified by the recent observation that this illicit drug can cause apoptosis (Cadet et al., 1997; Deng et al., 1999), itself a very complicated pathophysiological event.

Acknowledgments

We thank the staff of the Animal Care Facility at the Division of Intramural Research of the National Institutes of Health/National Institute on Drug Abuse for the impeccable care of the animals. We also thank Ms. Bonnie Koepl for her kind assistance with statistical analysis.

References

- Andrews AM, Ladenheim B, Epstein CJ, Cadet JL and Murphy DL (1996) Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2'-NH₂-MPTP on serotonergic and noradrenergic nerve terminals. *Mol Pharmacol* 50:1511–1519.
- Banati RB, Myers R and Kreutzberg GW (1997) PK ("peripheral benzodiazepine")-binding sites in the CNS indicate early and discrete brain lesions: Microautoradiographic detection of [³H]PK-11195 binding to activated microglia. *J Neurocytol* 26:77–82.
- Bel N, Figueras G, Vilaro MT, Sunol C and Artigas F (1997) Antidepressant drugs inhibit a glial 5-hydroxytryptamine transporter in rat brain. *Eur J Neurosci* 9:1728–1738.
- Benveniste EN (1998) Cytokine actions in the central nervous system. *Cytokine Growth Factor Rev* 3–4:259–275.
- Benveniste EN, Sparacio SM, Norris JG, Grenett HE and Fuller GM (1990) Induction and regulation of interleukin-6 gene expression in rat astrocytes. *J Neuroimmunol* 30:201–212.
- Cadet JL and Brannock C (1998) Free radicals and the pathobiology of brain dopamine systems. *Neurochem Int* 32:117–131.
- Cadet JL, Ordóñez SV and Ordóñez JV (1997) Methamphetamine induces apoptosis in immortalized neural cells: Protection by the protooncogene, bcl-2. *Synapse* 25:176–184.
- Cadet JL, Sheng P, Ali S, Rothman R, Carlson E and Epstein C (1994) Attenuation

- of methamphetamine-induced neurotoxicity in copper/zinc superoxide dismutase transgenic mice. *J Neurochem* **62**:380–383.
- Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MB and Mucke L (1993) Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin-6. *Proc Natl Acad Sci USA* **90**:10061–10065.
- Chai S, Gatti S, Toniatti C, Poli V and Bartfai T (1996) Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: A study on IL-6-deficient mice. *J Exp Med* **183**:311–316.
- Chiang CS, Stadler A, Samimi A and Campbell IL (1994) Reactive gliosis as a consequence of interleukin-6 expression in the brain: Studies in transgenic mice. *Dev Neurosci* **16**:212–221.
- Choi C, Park JY, Lee J, Lim J-H, Shin E-C, Ahn YS, Kim C-H, Kim S-J, Kim J-D, Choi IS and Choi I-H (1999) Fas ligand and Fas are expressed constitutively in human astrocytes and the expression increases with IL-1, IL-6, TNF- α or IFN- γ . *J Immunol* **162**:1889–1895.
- Clausing P, Gough B, Holson RR, Slikker W Jr and Bower JF (1995) Amphetamine levels in brain microdialysate, caudate/putamen, substantia nigra and plasma after dosage that produces either behavioral or neurotoxic effects. *J Pharmacol Exp Ther* **274**:614–621.
- Clement MV and Pervaiz S (1999) Reactive oxygen intermediates regulate cellular response to apoptotic stimuli: An hypothesis. *Free Radic Res* **30**:247–252.
- Commins DL, Shaughnessy RA, Axt KJ, Vosmer G and Seiden LS (1989) Variability among brain regions in the specificity of 6-hydroxydopamine (6-OHDA)-induced lesions. *J Neural Transm* **77**:197–210.
- Deng X and Cadet JL (1999) Methamphetamine administration causes overexpression of nNOS in the mouse striatum. *Brain Res* **851**:254–257.
- Deng X, Ladenheim B, Tsao L-I and Cadet JL (1999) Null mutation of c-fos causes exacerbation of methamphetamine-induced neurotoxicity. *J Neurosci* **19**:10107–10115.
- Gadient RA and Otten U (1995) Interleukin-6 and interleukin-6 receptor mRNA expression in rat central nervous system. *Ann N Y Acad Sci* **762**:403–406.
- Gijbels K, Van Damme J, Proost P, Put W, Carton H and Billiau A (1990) Interleukin-6 production in the central nervous system during experimental autoimmune encephalomyelitis. *Eur J Immunol* **20**:233–235.
- Grau GE, Frei K, Piguet PF, Fontana A, Heremans H, Billiau A, Vassalli P and Lambert PH (1990) Interleukin-6 production in experimental cerebral malaria: Modulation by anticytokine antibodies and possible role in hypergammaglobulinemia. *J Exp Med* **172**:1505–1508.
- Gruol DL and Nelson TE (1997) Physiological and pathological roles of interleukin-6 in the central nervous system. *Mol Neurobiol* **15**:307–339.
- Guillemin G, Boussin FD, Le Grand R, Croitoru J, Coffigny H and Dormont D (1996) Granulocyte macrophage colony stimulating factor stimulates in vitro proliferation of astrocytes derived from simian mature brains. *Glia* **16**:71–80.
- Hirata H and Cadet JL (1997) p53-Knockout mice are protected against the long-term effects of methamphetamine on dopaminergic terminals and cell bodies. *J Neurochem* **69**:780–790.
- Hirata H, Ladenheim B, Carlson E, Epstein C and Cadet JL (1996) Autoradiographic evidence for methamphetamine-induced striatal dopaminergic loss in mouse brain: Attenuation in Cu/Zn superoxide dismutase transgenic mice. *Brain Res* **714**:95–103.
- Hirst WD, Price GW, Rattray M and Wilkin GP (1998) Serotonin transporters in adult rat brain astrocytes revealed by [3 H]5-HT uptake into glial plasmalemmal vesicles. *Neurochem Int* **33**:11–22.
- Itzhak Y, Gandia C, Huang PL and Ali SF (1998) Resistance of neuronal nitric oxide synthase-deficient mice to methamphetamine-induced dopaminergic neurotoxicity. *J Pharmacol Exp Ther* **284**:1040–1047.
- Jayanthi S, Ladenheim B and Cadet JL (1998) Methamphetamine-induced changes in antioxidant enzymes and lipid peroxidation in copper/zinc-superoxide dismutase transgenic mice. *Ann N Y Acad Sci* **844**:92–102.
- Jayanthi S, Ordonez S, McCoy MT and Cadet JL (1999) Dual mechanism of Fas-induced cell death in neuroglial cells: A role for reactive oxygen species. *Mol Brain Res* **72**:158–165.
- Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H and Kohler G (1994) Impaired immune and acute-phase responses in interleukin-6 deficient mice. *Nature (Lond)* **368**:339–342.
- Kuhlmann AC and Guilarte TR (1999) Regional and temporal expression of the peripheral benzodiazepine receptor in MPTP neurotoxicity. *Toxicol Sci* **48**:107–116.
- Lan KC, Lin YF, Yu FC, Lin CS and Chu P (1998) Clinical manifestations and prognostic futures of acute methamphetamine intoxication. *J Formos Med Assoc* **97**:528–533.
- LaVoie MJ and Hastings TG (1999) Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: Evidence against a role for extracellular dopamine. *J Neurosci* **19**:1484–1491.
- McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF and Ricaurte GA (1998) Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathione users: Evidence from positron emission tomography studies with [11 C]WIN-35,428. *J Neurosci* **18**:8417–8422.
- Melega WP, Cho AK, Schmitz D, Kuczenski R and Segal DS (1999) L-methamphetamine pharmacokinetics and pharmacodynamics for assessment of in vivo deprenyl-derived l-methamphetamine. *J Pharmacol Exp Ther* **288**:752–758.
- Miller DB and O'Callaghan JP (1994) Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. *J Pharmacol Exp Ther* **270**:741–751.
- Myers R, Manjil LG, Cullen BM, Price GW, Frackowiak RS and Cremer JE (1991) Macrophage and astrocyte populations in relation to [3 H]PK 11195 binding in rat cerebral cortex following a local ischaemic lesion. *J Cereb Blood Flow Metab* **11**:314–322.
- O'Callaghan JP and Miller DB (1994) Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mice. *J Pharmacol Exp Ther* **270**:741–751.
- Schiefer J, Topper R, Schmidt W, Block F, Heinrich PC, Noth J and Schwartz M (1998) Expression of interleukin 6 in the rat striatum after stereotaxic injection of quinolinic acid. *J Neuroimmunol* **98**:168–176.
- Schmidt CJ, Black CK and Taylor VL (1991) L-DOPA potentiation of the serotonergic deficits due to a single administration of 3,4-methylenedioxymethamphetamine, p-chloroamphetamine or methamphetamine to rats. *Eur J Pharmacol* **203**:41–49.
- Schmidt CJ, Ritter JK, Sonsalla PK, Hanson GR and Gibb JW (1985) Role of the dopamine in the neurotoxic effects of methamphetamine. *J Pharmacol Exp Ther* **233**:539–544.
- Schobitz B, Voorhuis DAM and De Kloet ER (1992) Localization of interleukin-6 mRNA and interleukin-6 receptor mRNA in rat brain. *Neurosci Lett* **136**:189–192.
- Sheng P, Cerruti C, Ali S and Cadet JL (1996a) Nitric oxide is a mediator of methamphetamine (METH)-induced neurotoxicity. In vitro evidence from primary cultures of mesencephalic cells. *Ann N Y Acad Sci* **801**:174–186.
- Sheng P, Ladenheim B, Moran TH, Wang X-B and Cadet JL (1996b) Methamphetamine-induced neurotoxicity is associated with increased striatal AP-1 DNA binding activity in mice. *Mol Brain Res* **42**:171–174.
- Villemagne V, Yuan J, Wong DF, Dannals RF, Hatzidimitiou G, Mathews WB, Ravert HT, Musachio J, McCann UD and Ricaurte GA (1998) Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: Evidence from [11 C]WIN-35,428 positron emission tomography studies and direct in vitro determinations. *J Neurosci* **18**:419–427.
- Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Rieber G, Anthony RM, Schmunk GA, Shannak K, Haycock JW and Kish SJ (1996) Striatal dopamine nerve terminals markers in human, chronic methamphetamine users. *Nat Med* **2**:699–703.
- Wrona MZ, Yang Z, McAdams M, O'Connor-Coates S and Dryhurst G (1995) Hydroxyl radical-mediated oxidation of serotonin: Potential insights into the neurotoxicity of methamphetamine. *J Neurochem* **64**:1390–1400.
- Xiao B-G and Link H (1998) Immune regulation within the central nervous system. *J Neurol Sci* **157**:1–12.
- Yamaguchi T, Kuraishi Y, Minami M, Nakai S, Hirai Y and Satoh M (1991) Methamphetamine-induced expression of interleukin-1 beta mRNA in the rat hypothalamus. *Neurosci Lett* **128**:90–92.
- Yu ACH and Lau LT (2000) Expression of interleukin-1 alpha, tumor necrosis factor alpha and interleukin-6 genes in astrocytes under ischemic injury. *Neurochem Int* **36**:369–377.
- Zhao B and Schwartz JP (1998) Involvement of cytokines in normal CNS development and neurological diseases: Recent progress and perspectives. *J Neurosci Res* **52**:7–16.
- Zhou FC, Bledsoe S and Murphy J (1991) Serotonergic sprouting is induced by dopamine-lesion in substantia nigra of adult rat brain. *Brain Res* **556**:108–116

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