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COLLEGIUM INTERNATIONALE NEURO-PSYCHOPHARMACOLOGIUM (CINP) SYMPOSIUM: NEUROBIOLOGICAL BASE OF STIMULANT-INDUCED SENSITIZATION: FROM ANIMALS TO MAN

Prior exposure to a behaviorally sensitizing regimen of d-methamphetamine does not alter the striatal dopaminergic damage induced by a neurotoxic regimen

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Abstract

Repeated exposure to psychostimulant drugs such as d-methamphetamine (d-METH) and cocaine can be associated with extremely long-lived changes in dopamine systems at the behavioral, cellular and molecular level. Sensitization or an enhanced response to drug exposure is one such change. Investigations of these phenomena at the cellular and molecular levels are being conducted in the hope that this will aid in understanding how such adaptations might contribute to drug addition. Repeated exposure to certain amphetamines can also result in damage to dopaminergic pathways. Although some of the same molecular adaptations and mechanisms are suspected to occur or play a role in the neurotoxic sequelae associated with psychostimulant exposure, there has been little attempt to examine the relationship among these phenomena. Here we utilized C57BL/6J female mice to examine whether exposure to a sensitizing regimen of d-METH would impact the degree of neural injury induced by a subsequent exposure to a neurotoxic regimen of the same psychostimulant. Every other day exposure to d-METH (1.0 or 2.0 mg/kg) for 11 days produced a behavioral sensitization, as evidenced by a significant increase in the degree of locomotor activity induced by each subsequent exposure to d-METH. Following a 5-day period of no drug exposure sensitized mice were given a neurotoxic regiment of d-METH (a total of four injections of 10.0 mg/kg, one every 2 hours) and striatal tissue examined 72 hours later. All groups, whether drug-naive or sensitized previously to d-METH, showed exactly the same degree of dopaminergic striatal damage induced by a neurotoxic regimen. This was evidenced by equivalent reductions in dopamine and elevations in GFAP protein, a marker of astrocytic response to injury, GFAP. The inability of a sensitizing regimen to either exacerbate or lessen the neurotoxic actions of the same compound suggests that the molecular and cellular control of these two aspects of psychostimulant exposure may differ.

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Introduction

Determination of the sensitizing and the neurotoxic properties of psychostimulant drugs are active areas of research but are not often considered in relationship to one another. The former is more often approached from a clinical or drug abuse perspective, while the latter is most often considered in conjunction with the development of models utilized in understanding the mechanisms involved in neurodegenerative diseases.[1,2] The neural changes evoked by sensitizing and neurotoxic regimens of stimulant exposure have been studied in different brain areas (nucleus accumbens and striatum, respectively). However, both regimens involve repeated exposure and enhanced binding of the chronic FRAs have been observed in both.[3] Further, the neural changes observed in both have been linked to signaling in dopaminergic and glutamatergic pathways.[4,5] We have shown previously that decreasing signaling in the nigral-striatal DA pathway by elimination of the postsynaptic protein, DARPP–32, can blunt the striatal neurotoxicity of d-METH.[6] Here, we investigated whether a sensitizing regimen of a psychostimulant would make the striatum more vulnerable to a subsequent neurotoxic regimen.

Repeated intermittent exposure to the same low dosages of certain psychoactive compounds, including the substituted amphetamines and cocaine can result in a "reverse tolerance" or sensitization to what are referred to as the psychostimulant effects of the compound.[7] That is, a given dosage will produce a greater stimulation or enhancement after repeated exposure rather than the lessening of response or tolerance that normally accompanies repeated exposure to pharmacologically active compounds. Sensitization is well described and documented from a behavioral as well as a neurochemical perspective and cannot be explained completely by the pharmacokinetic or metabolic changes that can be engendered by repeated exposure.[8] The enduring behavioral (e.g. enhanced motor activity) and neurochemical (e.g. increased DA release) changes that accompany repeated exposure are due probably to long-lasting but incompletely described cellular/molecular changes occurring in the neural substrates controlling these actions.[9] Such changes are of interest clinically as they may explain how drug-induced psychotic episodes can occur at lower dosages in drug addicts than naive subjects.[10] Such enhanced responses may indicate an increased vulnerability to other actions of these compounds (e.g. neurotoxicity).

Exposure to psychostimulants such as d-methamphetamine (d-METH), albeit at dosages higher than those producing sensitization but still well below lethal dosages, can be neurotoxic to the striatum of several species. This damage is evidenced by long-term changes in markers indicative of terminal degeneration. These include depletion of DA, loss of tyrosine hydroxylase (TH) protein, elevations in glial fibrillary acidic protein (GFAP) indicative of an injury-provoked astrocytic hypertrophy and the presence of argyophilia detected by silver degeneration stains.[11–14]

To determine if exposure to a sensitizing regimen of a pyschostimulant can alter the vulnerability of the striatum to a subsequent neurotoxicant insult C57BL/6J female mice were repeatedly exposed to a dosage of d-METH (1.0 or 2.0 mg/kg) known to produce behavioral sensitization in mice.[15] Sensitized and drug-naive mice were then exposed to a known neurotoxic regimen of d-METH.[14,16] Sensitized mice, relative to drug-naive mice, showed no change in the striatal damage induced by subsequent exposure to a neurotoxic regimen of d-METH. It appears that the cellular/molecular changes underlying the sensitization of certain neural substrates to psychostimulants may be different from those mediating the degenerative changes induced by these same compounds. Alternatively, the changes induced by sensitization may not be of sufficient magnitude to enhance neurotoxicity.

Methods

Materials

The following drugs and chemicals were obtained from the sources indicated: d-METH (Sigma Chemical Co., St Louis, MO, USA), bicinchoninic acid protein assay reagent and bovine serum albumin (Pierce Chemical Co., Rockford, IL, USA) and reagents used for HPLC were of HPLC grade (Burdick and Jackson, Muskegon, MI, USA). The materials used in the GFAP assay have been described in detail.[17]

Subjects

All studies were conducted with female C57 BL/6J mice (approximately 8–9 weeks of age)

Table 1. *Experimental design*

Group	Test Day 1	2	3	4	5	6	7	8	9	10	11	12
SAL												
Inj	S	S	S	–	S	–	S	–	S	–	S	S
Test	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	Y
METH 1.0 mg/kg												
Inj	S	S	M	–	M	–	M	–	M	–	M	S
Test	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	Y
METH 2.0 mg/kg												
Inj	S	S	M	–	M	–	M	–	M	–	M	S
Test	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	Y

All tests lasted 15 min. Testing began immediately after an i.p. injection of drug (METH 1.0 or 2.0 mg/kg) (M) or 0.9% saline (S). On day 16 one-half of each group received a proven neurotoxic regimen of METH (four injections of 10.0 mg/kg, one given s.c. every 2 hours) or 0.9% SAL on the same schedule. All injections were in a volume of 100 ml/kg. Yes = Y, No = N, S = saline, M = d-METH.

obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed in the CDC-NIOSH animal care facility (approved by the American Association for Accreditation of Laboratory Animal Care) for several weeks prior to use. Mice were group-housed (four per cage) and allowed access to food (Purina rat/mouse chow) and water *ad libitum*. The rooms where the studies were conducted are temperature- and humidity-controlled (21 ± 1°C and 50% ± 10%, respectively). All studies were conducted under protocols approved by the Animal Care and Use Committee of CDC-NIOSH.

Sensitization regimen for d-METH

Mice were assigned randomly to treatment groups and sensitized using a published dosing regimen shown to produce sensitization in female C57BL/6J mice.[15] This strain and gender of mice was utilized as we have previously described extensively the neurotoxicity of d-METH in these mice.[14] To aid in clarity, the dosing and testing schedule for sensitization is presented in Table 1. Mice were weighed, received either saline (Sal) or d-METH i.p. and within 10 min were placed in the monitoring cages for a duration of 15 min. Total activity and ambulations were monitored utilizing a San Diego Instruments (San Diego, CA, USA) activity system. Monitors were maintained in a HEPA filtered BioClean Shelving system (Baker Co., Inc, Sandford, MA, USA) which maintained a low level of "white" noise (~ 10 db) during testing. Test cages with monitoring frames were isolated from adjacent units by opaque plastic panels to prevent visual contact. Test cages (46 cm long × 24 cm wide × 20 cm high) contained no bedding and were larger than the home cages (28 cm long × 17 cm wide × 5 cm high); they were cleaned thoroughly between test sessions with disinfectant, soap and water.

Neurotoxicity regimen for d-METH

Mice received a s.c. injection of d-METH (10.0 mg/kg) or vehicle (0.15 M NaCl) every 2 hours for a total of four injections. This neurotoxic regimen reliably produces damage to striatum as evidenced by a marked depletion of DA, reduction in tyrosine hydroxylase protein level and an elevation of GFAP.[14]

Brain dissection and tissue preparation

At 72 hours following the last injection of d-METH, striatal tissue was obtained. Mice were decapitated, whole brains were removed and striatum was dissected free-hand on a thermoelectric cold plate (Model TCP–2, Aldrich Chemical Co., Milwaukee, WI, USA) using a pair of fine curved forceps (Roboz, Washington, DC, USA). Striatum from the left side of the brain was weighed, frozen on dry ice and stored at −70°C for subsequent analysis of DA by HPLC. Striatum from the right side was weighed and kept

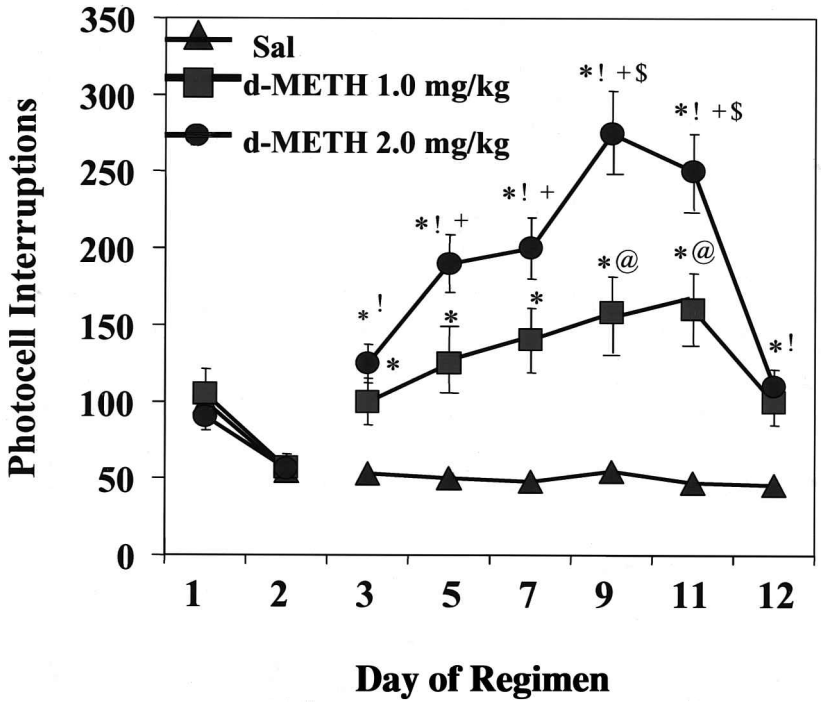

Figure 1. *The effect of repeated, intermittent i.p. injection of vehicle (0.9% Sal) or d-METH) (1.0 or 2.0 mg/kg as the base) on the total ambulations (i.e. photocell interruptions) of C57BL/6J female mice recorded in activity chambers. Mice received vehicle or d-METH on days 3, 5, 7, 9 and 11 and were placed in the activity chambers within 10 minutes for a 15-minute recording session. All groups received a Sal injection prior to sessions 1, 2 and 12. Mice receiving the 2.0 mg/kg dosage of d-METH on day 3 showed sensitization, as evidenced by a significant increase in photocell interruptions engendered by this same dosage on days 5, 7, 9 and 11. Further, the photocell interruptions on days 9 and 11 were significantly greater than those engendered by this dosage on days 5 and 7. Intermittent exposure to a dosage of 1.0 mg/kg of d-METH also engendered sensitization, as evidenced by a significant increase in photocell interruptions engendered by this same dosage on days 9 and 11. Each value represents the mean ± SEM of 12 mice; the SEM is not shown if it is smaller than the radius of the point. * Significantly different from Sal* ($p < 0.05$); *! significantly different from METH: 1.0 mg/kg* ($p < 0.05$). *+ significantly different from d-METH: 2.0 mg/kg day 3* ($p < 0.05$); *$ significantly different from d-METH 2.0 mg/kg – days 5 and 7* ($p < 0.05$); *@ significantly different from d-METH 1.0 mg/kg – day 3* ($p < 0.05$).

frozen at −70°C until assay. At this time the striatum was homogenized with an ultrasonic probe (model XL−2005, Heat Systems, Farmingdale, NY, USA) in 10 volumes of hot (90−95°C) 1% SDS and refrozen. Samples were assayed within 1 week for total protein and concentration of GFAP.

Biochemical assays

The method of Smith *et al.*[18] was used to determine total protein. GFAP was assayed according to modifications of a previously described sandwich ELISA.[17] DA and metabolites were analyzed by HPLC with electrochemical detection.[14]

Statistics

Data analysis was conducted utilizing the Statistical Analysis System (SAS Institute Inc., 1986). Individual variables were evaluated by analysis of variance followed by Duncan's Multiple Range Test for mean comparisons. The alpha level used to determine significance was 0.05 in all cases.

Results

Sensitization to d-METH

Mice given d-METH on an intermittent schedule (Table 1) at a dosage of 1.0 mg/kg or 2.0 mg/kg showed a progressive enhanced behavioral response, as evidenced by significant increases in ambulations (Fig. 1) and total activity counts

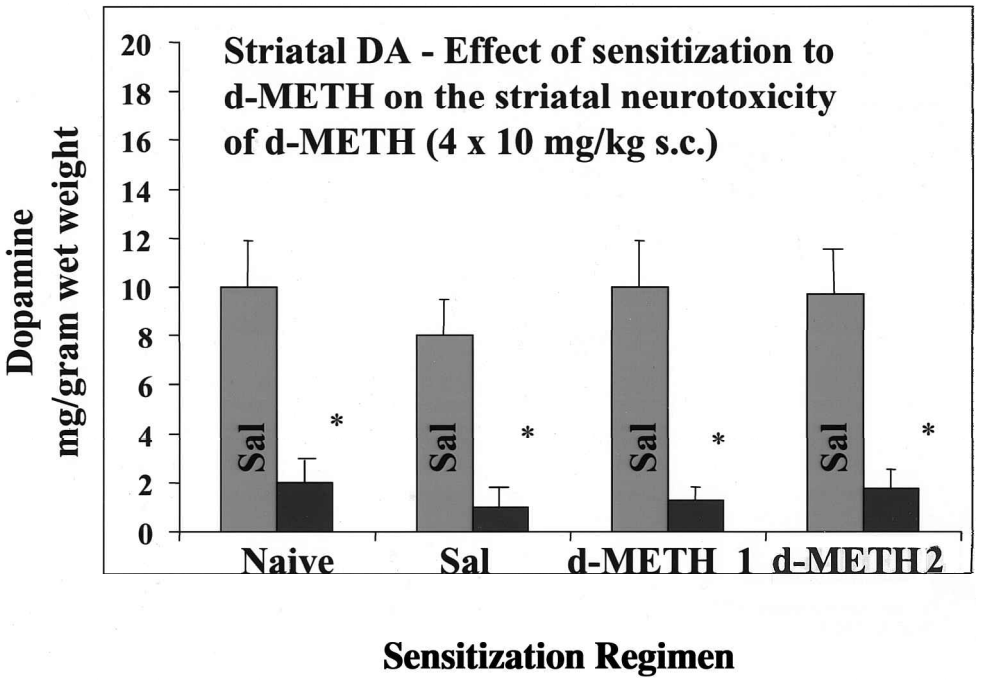

Figure 2. *The effect of repeated, intermittent i.p. injections of vehicle (0.9% Sal) or d-METH (1.0 or 2.0 mg/kg as the base) on the striatal DA depletion induced by a neurotoxic regimen of d-METH (four injections, one given s.c. every 2 hours). Each value represents the mean ± SEM of six mice.* * *Significantly different from Sal* ($p < 0.05$).

(data not shown). Further, this sensitization was dose-related; mice receiving the 2.0 mg/kg dosage of d-METH displayed significantly greater sensitization than those receiving 1.0 mg/kg.

Neurotoxicity of d-METH

Despite clear evidence of behavioral sensitization mice that were sensitized displayed the same degree of striatal injury (Figs 2 and 3) as naive mice or mice that received Sal during the sensitization regimen. A neurotoxic regimen of d-METH resulted in ~ an 80% depletion in striatal DA (Fig. 2) and an ~ 300% of control increase in GFAP (Fig. 3) for all groups receiving the psychostimulant.

Discussion

In accordance with previous reports, repeated intermittent exposure to d-METH produced a behavioral sensitization.[15,19] Female C57BL/6J mice repeatedly receiving either 1.0 or 2.0 mg/kg d-METH showed a progressive increase in both ambulation and total activity with continued exposure, although the higher dosage produced a greater sensitization. Although the sensitization regimen clearly produced a demonstrable behavioral change this prior exposure had no effect on the dopaminergic striatal neurotoxicity induced by subsequent exposure to d-METH. Rather, mice that were sensitized to either dosage of d-METH showed neither an increase nor a decrease in the degree of striatal damage induced by a neurotoxic regimen of d-METH.

The development of behavioral sensitization following repeated exposure to a psychostimulant has been interpreted as a clear indication of changes at the cellular/molecular level. Indeed, alterations in a number of brain extracellular, cytoplasmic and nuclear signaling pathways have been described.[20,21] For example, chronic stimulation of both mesolimbic and nigrostriatal dopaminergic pathways by psychostimulant drugs results in increased dopaminergic transmission and a prolonged expression of Fos-related proteins.[22] Striatal injury induced by psychostimulants is also accompanied by long-term adap-

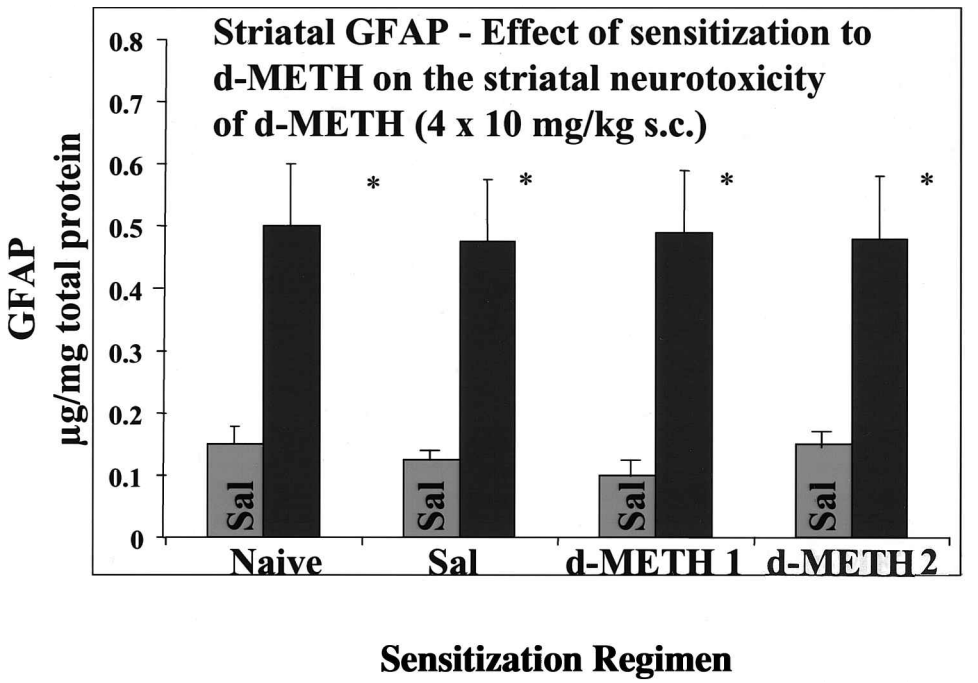

Figure 3. *The effect of repeated but intermittent i.p. injections of vehicle (0.9% Sal) or d-METH (1.00 or 2.0 mg/kg as the base) (see Table 1 for dosage schedule) on the striatal GFAP elevation induced by a neurotoxic regimen of d-METH (four injections, one given s.c. every 2 hours). Each value represents the mean ± SEM of six mice. * Significantly different from Sal* ($p < 0.05$).

tive changes at the cellular and molecular level. A prolonged expression of Fos-related antigens has also been described in striatum following neurotoxic regimens of psychostimulants.[3] Further, altering dopaminergic transmission by manipulating glutamatergic or dopaminergic pathways will also alter psychostimulant-induced damage. Also, mice null for the DARPP–32 protein display phenotypic alterations in DA neurotransmission suggestive of decreased efficancy in this pathway, and are protected from the striatal depletion in DA and elevation in GFAP that accompany exposure of the wild-type mouse to neurotoxic regimen of d-METH.[6] As long-term adaptive changes accompany DA neurotransmission changes as well as injury to DA systems we reasoned that the adaptive changes accompanying a behavioral sensitization to d-METH would probably alter the striatal injury induced by subsequent exposure to a neurotoxic regimen of d-METH. However, neither an increase nor decrease in striatal damage occurred in sensitized mice, suggesting that the sensitization does not result in adaptive changes in the nigral–striatal area or that any adaptive changes induced by this regimen or not of sufficient magnitude to impact the susceptibility of the striatum to subsequent neurotoxic insult by psychostimulants. Although ventral tegmental–nucleus accumbens is the brain dopaminergic pathway mentioned most often as the neural substrate altered in sensitization regimens, there is evidence that the nigral–striatal pathway is also altered. For example, the locomotor activity sensitization engendered by repeated administration of d-amphetamine is accompanied by increased dopamine release from dopaminergic nerve terminals in striatum.[23,24] As the current study did not determine the adaptive molecular/cellular or dopamine transmission changes associated with the induction of behavioral sensitiziation, it is difficult to speculate as to the failure of sensitization to influence subsequent neurotoxicity. Future studies should determine the types and time–course of sensitization-induced changes in the nigral–striatal pathway and determine the similarity of these changes

to those previously shown to affect the neurotoxicity of the substituted amphetamines.[6]

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