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Original Research

Effects of Perinatal MeHg and/or 2, 2', 4, 4', 5, 5'- Hexachlorobiphenyl (PCB153) Exposure on Adult Vulnerability to Amphetamine in Rats

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Abstract

Methylmercury (MeHg) and polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental pollutants and known neurotoxicants. It has been recognized that dietary exposure to neurotoxic substances during pregnancy and breast feeding may affect the development of the child's nervous system and result in various neurological and neurobehavioural alterations later in life. One of the suspected consequences of such exposure may be an increased propensity to psychostimulant abuse and psychostimulant addiction. Data from animal studies indicate that behavioural sensitivity to psychostimulants is a good predictor of the propensity to psychostimulant self-administration – an animal model of drug abuse in humans. The aim of this study was to find out whether and how perinatal exposure to MeHg and/or PCB153 determines behavioural sensitivity and sensitizability to the psychostimulant amphetamine (AMPH) in adulthood. The subjects were adult rats, Wistars, born to mothers exposed, via drinking water, to MeHg (CH_3HgCl) at 0.5 mg/kg/day; or PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) at 5.0 mg/kg/day by gavage, or jointly MeHg (0.5 mg/kg/day, and PCB153 (5.0 mg/kg/day), from day 7 of pregnancy to day 21 post partum. The testing started at the age of 3 months. It consisted in measuring the behavioural response to a test dose of AMPH (0.5 mg/kg. i.p.) twice: 1) before a sensitization treatment in order to assess the rat's "normal" sensitivity to the psychostimulant, and 2) three weeks after the sensitizing treatment. The sensitization treatment consisted in a repeated (once a day for five consecutive days) administration of AMPH at 2.5 mg/kg. Results: 1) Before the sensitization treatment there were no differences between the exposed and the control rats in the response to the psychostimulant. 2) Three weeks after the sensitizing treatment the response to an AMPH challenge was increased in all rats. However, in males exposed perinatally to MeHg alone, this increase was significantly more pronounced than in males of the control group. A similar effect was not present in MeHg-exposed females as well as males or females exposed to PCB153 alone or in combination with MeHg. Perinatal exposure to MeHg may result in an increased susceptibility to psychostimulant sensitization in the male progeny. Co- exposure to PCB153 may compromise this effect of MeHg-exposure.

Keywords: MeHg, PCB153, rat, behavioural sensitivity, sensitization

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Introduction

Quite a number of chemicals polluting the environment, e.g. pesticides, heavy metals, and petroleum products, are neurotoxic. The vulnerability of the nervous system to neurotoxic insults is highest during fetal development and early childhood. Therefore, exposure during these periods, via maternal blood and/or milk, even at levels posing no risk for the mother, may result in neurological and neurobehavioral disorders in the progeny [1]. The variability of type and severity of the disorders which may result from prenatal or perinatal neurotoxic exposures is quite large, from cerebral palsy to retarded or delayed intellectual and emotional development or even accelerated ageing [2]. Some also suspect a link between developmental exposures to neurotoxic substances, e.g. lead, and a propensity for drug abuse and drug addiction [3].

Methylmercury (MeHg) is one neurotoxic pollutant that causes much concern to toxicologists and governmental agencies dealing with environmental safety. Environmental MeHg is a product of bacterial methylation of inorganic mercurials released from anthropogenic and natural sources. In aquatic systems MeHg enters the food chain and is accumulated and biomagnified in successive steps. The highest concentration of MeHg can be encountered in soft tissues of fish, especially large predator fish, and sea mammals [4].

The presence of MeHg in fish creates a dilemma. Fish, especially fatty fish, are important source of omega-3 polyunsaturated fatty acids, which are essential for optimum neural development. Therefore, eating fish meat should be recommended, especially for pregnant women. On the other hand, the epidemic outbreaks of mass MeHg poisoning, e.g. the Minamata or Niigata disease, demonstrate the tragic consequences in children that may result from eating MeHg-contaminated food by their mothers during pregnancy [5-7]. Studies of the victims have also indicated that the MeHg LOAEL (assessed on the basis of Hg concentration in maternal hair) for child effects was nearly five times lower than the LOAEL for the effects in mothers [8]. It is worth stressing, however, that in fish from Minamata Bay, which was polluted with Hg from industrial wastes, the MeHg concentration reached 50 ppm whereas in ocean fish it rarely exceeds 0.5 ppm [8]. How safe, then, is fish consumption? (*Based on the investigations on prenatally exposed children, the U.S. Environmental Protection Agency set, as the maximum daily intake of MeHg, a dose of reference (RfD) of 0.1 µg/kg/body weight/day. To keep up with this safety value, it is recommended that fish consumption by pregnant women should not exceed 340 g/week*.) The two most frequently cited studies on children from islander populations relying mainly on seafood, the Faroe Islands and the Seychelles, present contrasting results. The Faroese study yielded results suggesting that a fish diet may detrimentally affect the development of a child's nervous system: adverse effects on intellectual development (lower IQ), language, visual-spatial skills, gross motor skills, memory and attention have been detected [9]. Contrasting results (improvement in some tests) have been obtained in studies on children cohort from the Seychelles (the Seychelles Child Developmental Study) despite the fact that, as suggested by the maternal hair Hg concentration, MeHg exposure in the Seychelles was actually higher than in the Faroe Islands (6.8 vs. 4.27 ppm, respectively) [10, 11].

According to some authors, the inconsistency between the outcomes of the Faroese and the Seychellois studies may be due to differences in the kind of seafood in the diet and possible co-exposure to other neurotoxicants. Whereas the Seychellois population eats mainly fish, which contain MeHg, the mothers from the Faroe Islands eat also whale meat and blubber what is contaminated with polychlorinated biphenyls (PCBs).

PCBs are a family of 209 congeners characterized by high chemical stability. Up to the late 1970s they were used, usually in the form of mixtures, in the electrical industry but also as immersion and cutting oils and dispersants for pesticides. Owing to that, they become ubiquitous environmental pollutants. PCBs are lipophilic and accumulate and biomagnify in the food chain. Nowadays, PCBs and/or their metabolites can be detected in tissues of almost all aquatic and land species. As in the case of MeHg, the primary source of human exposure to PCBs is through consumption of fish and marine mammals. The existing evidence, gained from two mass poisonings, several cohort studies and numerous experiments on laboratory animals, leaves no doubt that PCBs are neurotoxic, especially for the developing brain [12-16].

The assumption that co-exposure to MeHg and PCBs may be the main factor responsible for the presence of adverse effects in children from the Faroe Islands but not in children from the Seychelles finds support in some in vitro studies indicating that PCB and MeHg may act synergistically in inducing dopamine (DA) and calcium release from neuronal tissue [17, 18]. A synergistic neurotoxic action of MeHg and PCB has also been suggested by results of some epidemiological studies [19, 20], and a few laboratory in vivo experiments [21].

To characterize the type of possible MeHg-PCB interaction in inducing developmental neurotoxicity was one of the main goals of the DEVNERTOX project (Specific Targeted Research Project, Priority 5, FOOD-CT-2003-506143) sponsored by the European Commission. The experiment presented in the present paper is a part of a broader study performed within the frame of this project and concerns the neurobehavioural consequences of a separate or combined maternal exposure to MeHg and a selected PCB congener in the offspring of laboratory rats. The PCB congener selected for the study was PCB153 [(2, 2', 4, 4', 5, 5'-hexachlorobiphenyl). It is one of the PCB congeners most commonly detected in biological tissues [22, 23].

In this paper we present results concerning the effect of separate and/or combined maternal exposure to MeHg and PCB 153 on the behavioural sensitivity to the psychostimulant amphetamine (AMPH) and susceptibility to behavioural AMPH sensitization in maturity. There are reasons to believe that behavioural sensitivity to psychostimulants

predicts the propensity to drug taking and susceptibility to develop psychostimulant dependence and addiction. Psychostimulant abuse and the rising number of psychostimulant addicts gives rise to serious medical and social problems in many countries. According to some authors, exposure to neurotoxins during intrauterine life and in early childhood can be one of the factors predisposing one to drug abuse and development of drug addiction later in life [24]. Looking from this perspective, determining whether and in what way perinatal exposure to MeHg, PCB or both these compounds affects sensitivity to AMPH and susceptibility to AMPH sensitization may provide some cues concerning the possible relation of such exposures to the spread of drug-taking behaviours in human populations.

There are several reports showing an increased sensitivity to dopaminergic agonists in animals exposed perinatally to MeHg, and this effect was ascribed to some alterations in the dopaminergic system (DA system) [25-29].

To out knowledge, behavioural sensitivity and susceptibility to behavioural psychostimulant sensitization were not assessed yet in adult progeny perinatally exposed to MeHg, PCB153 or jointly (MeHg and PCB153). This report presents the results of experiments involving the progeny of mothers exposed during pregnancy and lactation to MeHg at the daily doses of 0.5 mg/kg, to PCB 153 at the daily doses of 5.0 mg/kg, or to both neurotoxins at the daily doses indicated above. The dose of MeHg used in these studies was the same as that employed by other authors [28]. MeHg given to pregnant mothers at 0.5 mg/kg/day produces Hg concentration of about 500 ng/g in the brain of newborn rat pups [26], i.e. a level comparable to that found in the brains of human infants from fish-eating populations [30]. What concerns the dose of PCB153 (5.0 mg/kg/day), was identical to that described in Holene et al. [31].

Material and Methods

The experiment was run in three parts performed during three consecutive years. In part one, we investigated the effects of MeHg exposure. The effect of the PCB 153 exposure was studied in the second part. In the last part the effects of combined maternal exposure to MeHg and PCB were assessed.

Chemicals

The following chemicals were used: methylmercury [(CH3HgCl) from SIGMA (Germany), CAS REG - 115-09-3, purity: >98%], PCB153 [(2,2', 4,4',5,5'-hexachlorobiphenyl) from SIGMA (Germany) (CAS REG -35065-27-1, purity > 99%] and D-amphetamine [(d-amphetamine sulphate – AMPH, from SIGMA (Germany) CAS REG -51-63-8].

Animals

Adult (90- to 96-day) white Wistar rats, both genders, born to females exposed during pregnancy and breast feeding to the selected neurotoxicants were used as the material for the experiment. To achieve that aim, female rats were exposed from day 7 of pregnancy up to day 21 after delivery to MeHg (CH_3HgCl, CAS No: 115-09-3, from ALDRICH, Cat. No. 442534-5G-A) in drinking water, or PCB 153 (2, 2', 4, 4', 5, 5'-Hexachlorobiphenyl, CAS No: 35065-27-1, supplied by Fluka, Cat. No. 35602) by gavage, or to both these neurotoxicants in combination, at predetermined daily doses. Females of the control groups were treated with appropriate vehicles. After weaning (on postnatal day - PND 21), the animals were kept undisturbed (except for the routine procedures, including weekly weighing and change of bedding material) in cages (4 animals per cage) until about the 75th day of life. After that time, the rats were relocated into single rat cages and allowed 14 days to acclimate to the new housing conditions. Body weight was measured weekly and before each injection. At the start of the experiment the animals were 90–96 days old. In the experiments described in the present paper the MeHg cohort consisted of 11 males and 11 females born to mothers of the control (unexposed) group and 11 females and 11 males born to mothers of the MeHg exposed group (0.5 mg/kg/day). The PCB153 cohort consisted of 14 females and 12 males born to mothers of the control group, and 11 females and 11 males born to mothers of the PCB exposure group (5.0 mg/kg/day). The MeHg+PCB cohort consisted of 11 females and 11 males born to mothers of the control group and 11 females and 11 males born to mothers exposed to PCB 153 (5.0 mg/kg/day) and MeHg (0.5 mg/kg/day). All experimental procedures were approved by the Local Ethical Commission and were carried out by suitably qualified personnel.

Toxicant Concentration in Fodder

Some kinds of diets commonly employed in laboratory animal research may contain organic Hg, most likely MeHg, at concentrations sufficient to directly affect the results of an MeHg experiment [32]. According to the written statement by the manufacturer (AGROPOL, Motycz, Poland), the Hg concentration in rat food pellets (Murigran) to fed our rats was less than 0.02 mg/kg. Independent analysis, conducted in the National Veterinary Research Institute (Pulawy, Poland), showed that the Hg concentration in the provided food sample was 0.015 mg/kg. The PCB153 concentration in food was not measured.

Exposure Control

Samples of pups from of each cohort (3 males and 3 females in each sample) were sacrificed at different time - points during postnatal development to assess toxicant concentrations in blood and brain tissue. The collected specimens (brains, erythrocytes and plasma) were sent to the Laboratory of Industrial Toxicology at the University of Parma, Italy (one of the Partners in the DEVNERTOX project) for analysis.

Brain and Blood Concentrations of the Toxicants in the Offspring

Results concerning the concentration of toxicants in blood and brain were presented by Poli et al. [33] and are contained in the final DEVNERTOX report (to be published soon). In rat offspring, brain Hg concentration rapidly decreased (about 94% during the weaning period): from about 10 μg/g at PND1 to about 0.6 μg/g of dry tissue at PND21. At PND90, i.e. at the start of the present experiment, the Hg concentration values in pups exposed developmentally to MeHg were comparable to those found in pups of the control group. Similar values were found in pups exposed jointly to MeHg and PCB153. The brain –blood ratio for Hg was at PND21 and PND90: 0.32 and 0.16, respectively. The same results were obtained in rats exposed to MeHg alone as well as those exposed jointly to MeHg and PCB153, indicating that the Hg level in a pup's brain was not modified by the presence of PCB153 and that the presence of PCB 153 did not influence the Hg level in blood. In contrast to Hg, the PCB153 concentration decreased slowly in brain during the weaning period (45%) from about 16 μg/g at PND1 to about 7.5 μg/g wet tissues in PND21. On PND90 the offspring of dams exposed to PCB153 still presented a slightly higher toxicant level than controls: 0.9 vs. 0.2 μg/g, respectively. During different developmental ages the plasma PCB concentration values were lower than those observed in brain: 12.4 vs. 7.5 μg/g on PND21 and 0.3 vs. 0.9 μg/g on PND90. The brain-to-blood ratio for PCB153 was at PND21 and PND90: 1.42 and 4.89, respectively. No differences between serum and brain PCB concentration were observed in pups exposed to PCB153 alone as well as those exposed jointly to MeHg and PCB153, indicating that MeHg was devoid of any kinetic effect on PCB153 in the offspring brain and blood. In general, both toxicants presented a time–dependent reduction in their target tissues, although with different elimination kinetics.

Experimental Approach

The experimental approach consisted in measuring the behavioural arousal induced by a test dose of AMPH (0.5 mg/kg) before and after subjecting the animal to a procedure known to induce behavioural hypersensitivity to the psychostimulant. The level of behavioural arousal was assessed by measuring the rat locomotor activity in an open field.

Apparatus and Basic Test Procedure

The rat locomotor activity was assessed with a computerized 4-unit set of activity cages (open fields), located in a room neighboring the animal rooms and illuminated with white luminescent bulbs located on the ceiling. Each activity cage consisted of a clear acrylic box (63 x 63 x 40 cm), with no ceiling, equipped with 2 tiers of infrared motion sensors spaced 2.5 cm apart. The first and second tiers of sensors were 4.0 cm and 15.0 cm from the cage floor, respectively. Each cage was equipped with a calculating system which transformed the beam interruptions into the location of the animal within the cage five times per second. Raw data were stored in the cage memory. After the end of a test session the cage memory content was downloaded to a computer memory for further analysis. The basic test procedure comprised of placing the animal in the activity cage where it remained undisturbed for a predetermined period of time. The following data were extracted from the cage memory:

i) number of ambulatory movements (horizontal shifts of the rat body, equal to or longer than 4 cm),
ii) traveled distance in meters,
iii) short-distance movements (shifts shorter than 4 cm), and
iv) number of rearings. (interruption of at least one beam of the upper tier was counted as a rearing episode).

Test Protocol

The test protocol consisted of four steps:
step 1 – habituation,
step 2 – testing behavioural sensitivity to amphetamine,
step 3 – sensitization inductions, and
step 4 – sensitization assessment.

In step 1 the rats were placed in the activity cages and their motor activity was measured for 1h. Step 2 (occurring two days after Step 1) consisted of three separate 60. min measurements: a preinjection measurement and two postinjection measurements, of which the first was preceded by injecting the rat with 0.9% NaCl, (SAL) and the second by injecting the rat with 0.5 mg/kg of AMPH dissolved in SAL. All solutions were prepared directly before use and given intraperitoneally (i.p.) at 1.0 ml/kg volume. The AMPH dose was the same as that used in our earlier experiment [34]. The interval between successive measurements was no longer than 2-5 min. The postinjection measurements started immediately after the injections. Step 3 (sensitization induction) was started one day after Step 2. For this purpose each rat was repeatedly injected with AMPH at 2.5 mg/kg b.w. (one injection/day for five days). Step 4 was performed on day 21 after the last sensitizing AMPH dose. The procedure in step 4 was exactly the same as in step 2. The preinjection and postinjection measurements in step 2 and 4 included: the number of ambulation episodes, ambulation distance, the number of rearings and the number of short, nonambulatory movements.

Statistics

In step 1, comparisons between groups were made using one-way ANOVA (Kruskal-Wallis test) and detailed comparisons between groups were performed with the use of the Scheffe test [35]. Statistical evaluation of the results of preinjection (before SAL administration) and postinjection scores (after SAL and AMPH administration) in step 2 and 4 were performed with the use of a parametric two-way ANOVA (groups x measurements). In case of significant interaction, differences between groups within successive

measurements and between measurements within groups were estimated with the use of one-way ANOVA and Tukey's test. In the case of non-homogeneity of covariance, an approximation procedure, which avoids assumption about equal covariances, was applied. In this procedure, the degrees of freedom used in finding the critical values are reduced, which makes the test more conservative. Differences were regarded as significant when the probability of the null hypothesis was 5% or less [35].

Results

Behavioural Effects Noted before Weaning

Observations during the period from birth until weaning did not see overt changes in the offspring of groups exposed to MeHg (0.5 mg/kg/day) alone, or to MeHg (0.5 mg/kg/day) and PCB 153 (5.0 mg/kg/day). Some effects, such as accelerated growth rate but reduced muscular strength and endurance, were noted only in the male pups born to females exposed to PCB 153 (5.0 mg/kg/day) alone. On the day of the selection, no significant differences in body mass were noted between groups within each gender, irrespective of exposure type.

Behavioural Effects Noted in Adult Progeny

Part 1: Effect of perinatal exposure to MeHg.

Step1 – Habituation.

No significant differences in the walked distance were found between groups. There were no significant differences between groups within each gender in the number of short-distance movements, number of ambulation episodes and number of rearings (not shown).

Steps 2 and 4 - Response to AMPH before and after AMPH treatment.

Behavioural response to 0.5 mg/kg AMPH consisted mainly in an increase of locomotor activity (i.e. the number of ambulation episodes and the length of the walked distance). In Step 2, the groups within each gender did not differ in the magnitude of this response. In Step 4, in each group within each gender the locomotor response to the

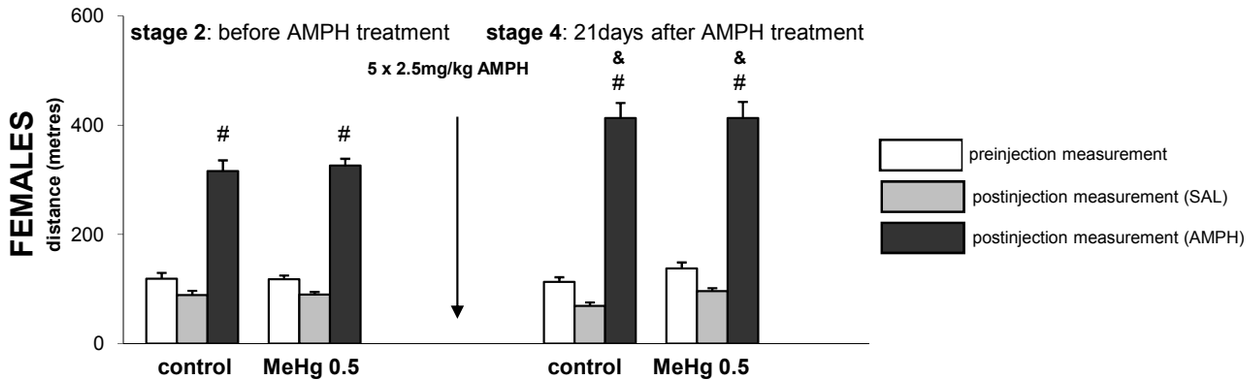

Fig. 1. A. Locomotor activity: walked distance in metres.
ANOVA: group effects: NS, session effects: F(1.20)=78.72, P<0,0001, interaction: NS.
- P<0.05 - compared to post SAL measurement;
&- P<0.05 - compared to corresponding measurement before sensitization.

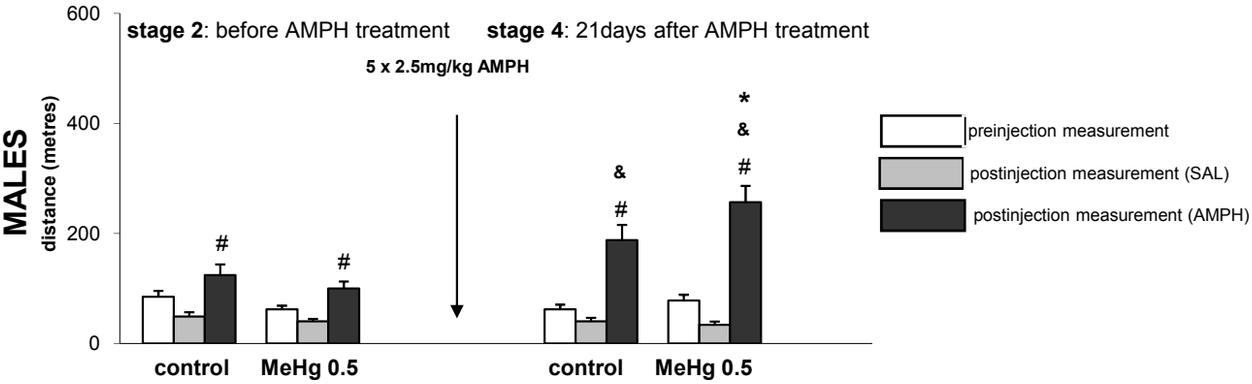

Fig. 1. B. Locomotor activity: walked distance in metres.
ANOVA: group effects: NS, session effects: F(1.20)=50.97, P<0,0001, interaction: F(1.20)=6.43, P<0,08.
- P<0.05 - compared to post SAL measurement;
* - P<0.05 - compared to control in the same session;
&- P<0.05 - compared to corresponding measurement before sensitization.

AMPH challenge was significantly increased compared to that in Step 2, which suggests an increased behavioural sensitivity to AMPH. In the MeHg 0.5 group, however, the distance walked after the AMPH test challenge dose was significantly longer than in control group. Similar differences between groups were also found in the number of ambulation episodes (not shown). These results suggest an increased susceptibility to AMPH sensitization in adult male progeny of mothers exposed to MeHg during gestation and lactation (Fig 1.A and 1.B).

Part 2: Effect of perinatal exposure to PCB.

Step 1 – Habituation.

Comparisons between groups showed that in the female exposed group the number of ambulation episodes recorded during the 1 h stay in the activity cages was significantly larger than in the female progeny of the control group. In the male progeny there were no differences between groups in the number of ambulation episodes and the length of the walked distance (not shown).

Step 2 and 4 - Response to AMPH before and after AMPH treatment.

In Steps 2 and 4 there were no significant differences between groups within each gender in the number of ambulation episodes and in the length of the walked distance during the first (before SAL) and the second (before AMPH) measurements. In Step 2, the challenge with 0.5 mg/kg of AMPH resulted, in all groups, in a significant increase in the number of ambulations and the length of the walked distance. There were no significant differences between groups within each gender in any of the two measurements as well as the number of rearings (not shown). This suggests that the maternal exposure to PCB153 had no effect on the behavioural sensitivity to AMPH in the adult progeny. In Step 4, in all groups within each gender the response to the AMPH challenge, i.e. the increase in the number of ambulations and the length of the walked distance, was significantly larger than in Step 2. However, in the male as well as in the female progeny, the groups did not differ significantly in any of the two measurements. This result suggests that perinatal exposure to 5.0 mg/kg/day of PCB153 did not affect the vulnerability to sensitization by repeated AMPH treatment (Fig 2.A and 2.B).

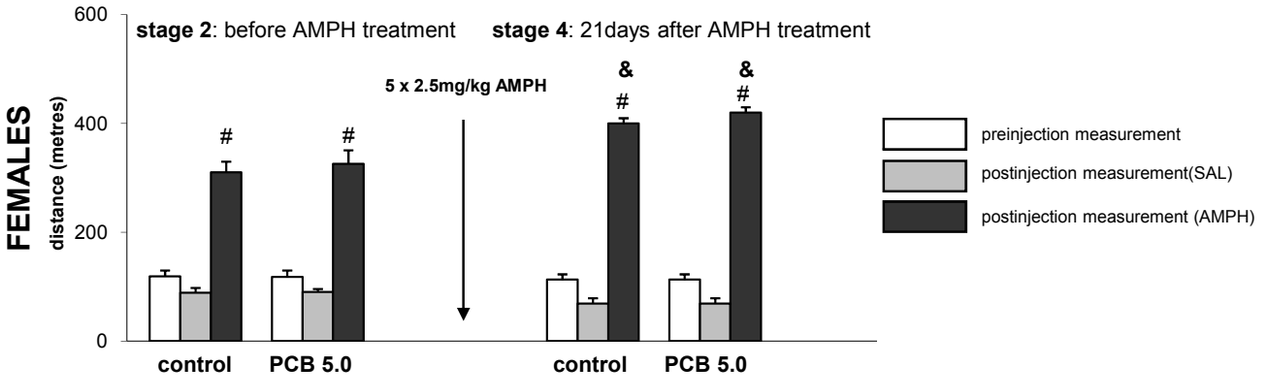

Fig. 2. A. Locomotor activity: walked distance in metres.
ANOVA: group effects: NS, session effects: F(1,19)=194.98, P<0,0000, interaction: NS.
- P<0.05 - compared to post SAL measurement;
&- P<0.05 - compared to corresponding measurement before sensitization.

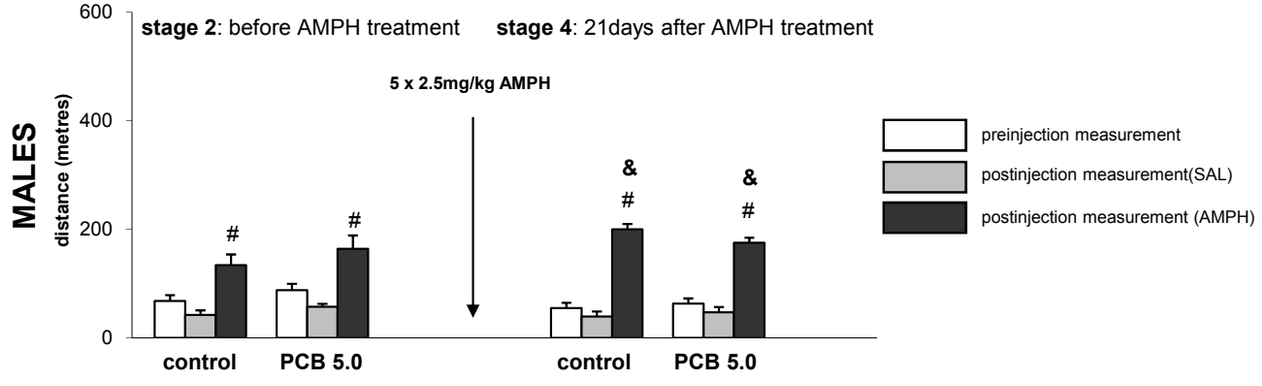

Fig. 2. B. Locomotor activity: walked distance in metres.
ANOVA: group effects: NS, session effects: F(1,21)=81.11, P<0,001, interaction: NS.
- P<0.05 - compared to post SAL measurement;
&- P<0.05 - compared to corresponding measurement before sensitization.

Part 3: Effects of combined perinatal exposure to MeHg and PCB153.

Step 1 – Habituation.

Comparisons between groups, within each gender separately, showed no significant differences between groups in the distance traveled during a 1 h stay in the open field. No differences between groups were also found in the number of ambulation episodes, number of rearings and number of nonambulatory short-distance movements (not shown).

Steps 2 and 4 - Response to AMPH before and after AMPH treatment.

In Stages 2 and 4 there were no significant differences between groups within the female as well as the male progeny in the distance traveled, number of ambulation episodes, number of rearings and number of short-distance (nonambulatory) movements. In all groups, the locomotor activity (walked distance and the number of ambulation episodes) increased markedly after the challenge with 0.5 m/kg of AMPH. In Stage 4, in all groups this response was significantly augmented compared to that in Stage 2, but the groups did not differ in the magnitude of this effect. These observations indicate that exposure did not affect an offspring's behavioural sensitivity to AMPH nor its susceptibility to develop hypersensitivity to the psychostimulant (Fig 3.A and 3.B).

Discussion

The results can be summarized as follows. Firstly, none of the perinatal exposures had an overt effect on the morphological development and general health status in maturity. This is suggested by the absence – in all three experiments – of differences in body weight between the exposed and control groups. Secondly, none of the exposures resulted in an overt alteration of the behavioral sensitivity to AMPH in maturity, which is suggested by the fact that in all three experiments, the rats of the exposed groups did not differ from their respective controls in the magnitude of the behavioural response to the first AMPH challenge. Thirdly, perinatal exposure MeHg, unlike exposure to PCB 153 or joint exposure to both these neurotoxicants, may result in an increased susceptibility to develop behavioral sensitization to psychostimulants in maturity. This effect, however, occurs only in males, and its presence is indicated by a

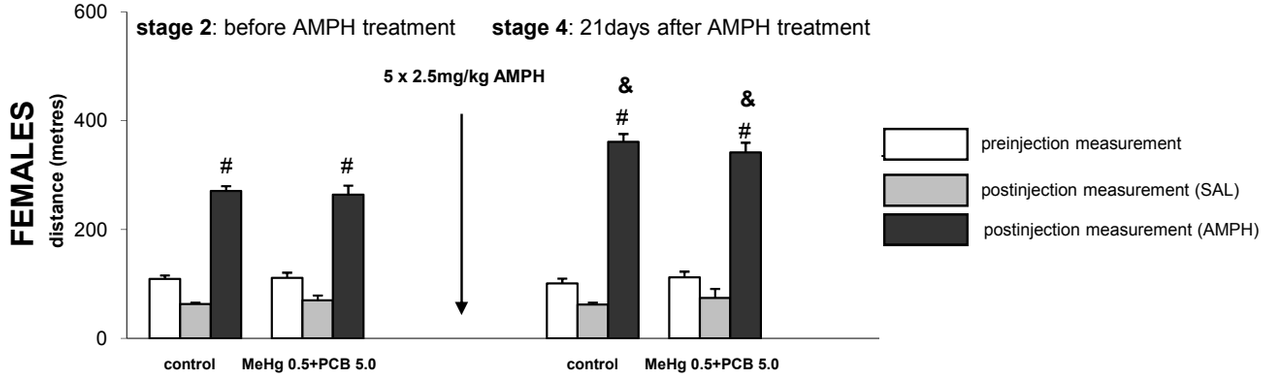

Fig. 3. A. Locomotor activity: walked distance in metres
ANOVA: group effects: NS, session effects: F(1.21)=182.23, P<0,0001, interaction: NS.
- P<0.05 - compared to post SAL measurement;
&- P<0.05 - compared to corresponding measurement before sensitization.

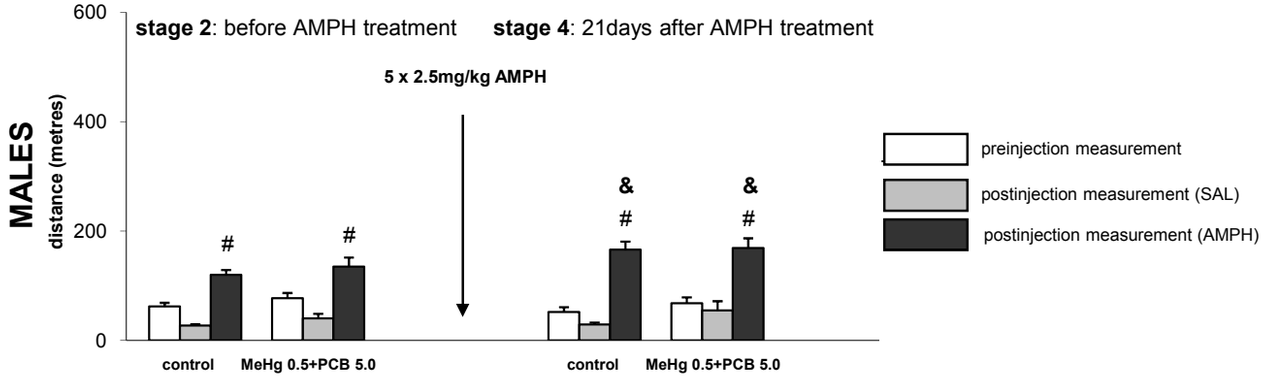

Fig. 3. B. Locomotor activity: walked distance in metres.
ANOVA: group effects: NS, session effects: F(1.21)=47.27, P<0,0001, interaction: NS.
- P<0.05 - compared to post SAL measurement;
&- P<0.05 - compared to corresponding measurement before sensitization.

significantly higher increase in the behavioural response to AMPH after the repeated (sensitizing) AMPH treatment. The basic mechanism of the psychostimulant AMPH action concerns the dopaminergic systems and consists in stimulation of DA release and inhibition of DA intake [36-38]. The DA system is also the main locus of the neuroadaptations developing after a sensitizing treatment and conditioning the post-treatment hypersensitivity to the psychostimulant [39]. Thus, the result of Experiment 1 simply suggests that in the adult male progeny of the MeHg exposed mothers, the development of such neuroadaptations is facilitated. The fact that those changes occurred only in males is somewhat puzzling. It has been demonstrated, however, that estrogen attenuates the degree of striatal DA depletion by MPTP, 6-OHDA and methamphetamine [40]. Thus, in the female offspring sex hormones may be the factor protecting the DA system from MeHg toxicity.

Information published heretofore on the effects of perinatal MeHg exposure on the DA system functional state is scarce. An in vitro study by Götz et al. [41] showed that the DA neurons exposed to MeHg demonstrated a striking decrease in the number of neurites, indicative of cytoskeletal alteration. In addition, a significant increase in the number of neurons with nuclei characterized with chromatin condensation was found. Based on these results it is concluded that MeHg is highly toxic to primary DA neurons. Neurochemical test results suggest possible changes (in the concentration of amines and monoaminooxidase (MAO) in some regions of the brain), but this is valid only for exposure levels above 0.5 mg/kg/day [4, 42, 43]. Besides, the experiments were finished shortly after weaning and, therefore, it is not clear whether the changes could persist until maturity. The prevalence of some functional changes in the DA system after perinatal MeHg exposure is evidenced mainly indirectly by behavioural responses to DA agonists and/or antagonists. Thus, e.g. in Cagiano et al. [25] experiments, single MeHg administration at 8 mg/kg on GD15 resulted in a stronger motoric reaction to AMPH in the 14-day-old offspring. A stronger reaction to DA agonists was recorded also by Cuomo et al. [44] and Dare et al. [28]. The changes described by the authors were noted only during the preweaning period of life. In some cases, however, increased sensitivity to DA agonists was noted also in adulthood. For example, Rossi et al. [26] reported an augmented AMPH response in adult (6-month) progeny of mothers exposed to MeHg (0.5 mg/kg/day) with drinking water since GD7 till PND7. The authors concluded that perinatal MeHg exposure results in long-term alterations of the DA system. An increased behavioural sensitivity to AMPH in adult (4- to 6-month) progeny of rat mothers exposed during pregnancy and lactation to MeHg at 0.5 or 6.6 ppm was reported also by Rasmussen and Newland [27]. Recently, the same effect has been reported by Wagner et al. [29] in adult male mice exposed to 2 or 4 mg/kg MeHg during their early post-natal period. The observations quoted above indicate that exposure to MeHg early in life may result in increased sensitivity to psychostimulants in adulthood.

In Experiment 1 of the present work, the rats (males) exposed perinatally to MeHg did not differ from controls in the response to the first AMPH challenge, which seems to be in contrast with the observations quoted above. However, differences in the AMPH response appeared in the test performed after the sensitizing treatment. As already stated, the functional state of the DA system seems to be the main determinant of both: the behavioural response to the psychostimulant and the susceptibility to the psychostimulant sensitization [39]. Thus, it is likely that, both in the experiments by the

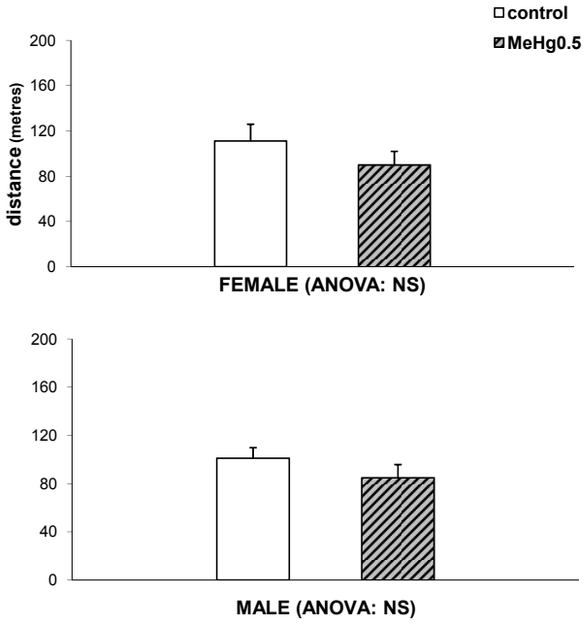

Fig. 4. Step 1: habituation - walked distance in animals (90 – 96 days old) born to mothers of the control (unexposed) group and born to mothers of the MeHg exposed group (0.5 mg/kg/day).

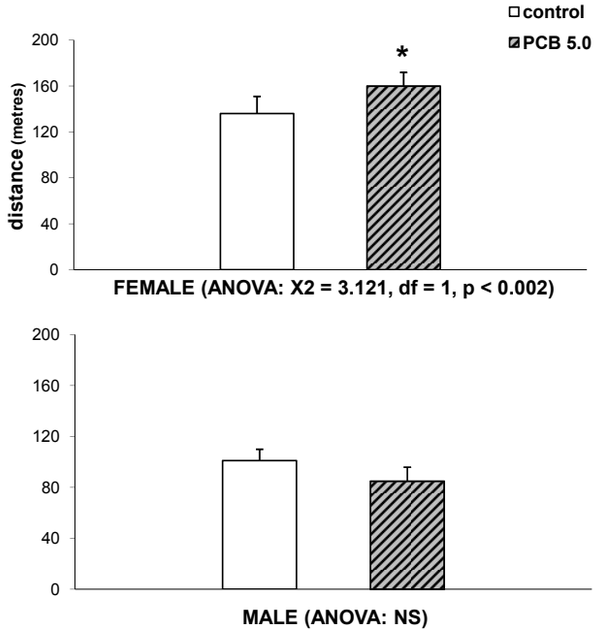

Fig. 5. Step 1: habituation - walked distance in animals (90 – 96 days old) born to mothers of the control (unexposed) group and born to mothers of thePCB exposed group (5.0 mg/kg/day).

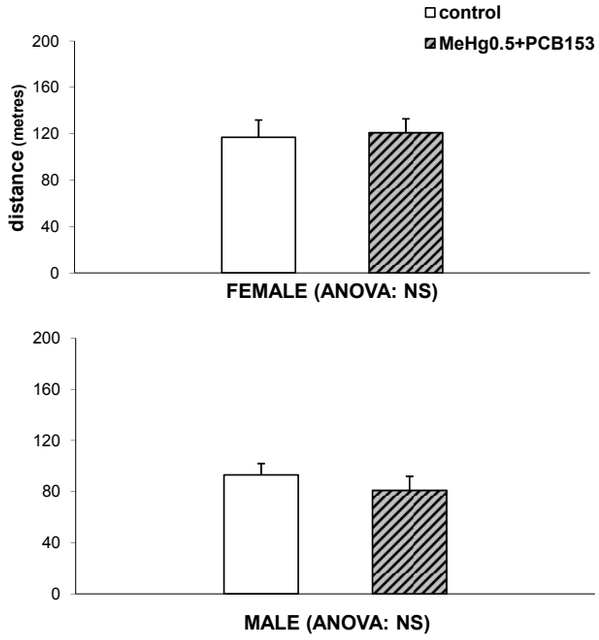

Fig. 6. Step 1: habituation - walked distance in animals (90 – 96 days old) born to mothers of the control (unexposed) group and born to mothers exposed to MeHg (0.5 mg/kg/day) and PCB 153 (5.0 mg/kg/day).

authors quoted above and in Experiment 1 of this work, the changes induced in the CNS by MeHg exposure were similar qualitatively, but they differed in magnitude (i.e. in our experiments they were probably weaker). According to some authors there is a relationship between the sensitivity to psychostimulants and development of drug addiction and dependence [3]. Thus, by extrapolating the data quoted above and the results of Experiment 1 to the human population one may assume that perinatal exposure to MeHg may be a factor promoting drug-taking behaviour and drug addiction in adulthood. A similar assumption has been put forward in relation to lead [3].

If the higher susceptibility to AMPH sensitization in the rats perinatally exposed to MeHg is proof of an effect on the DA system then, by analogy, the absence of similar changes in the rats perinatally exposed to PCB 153 in Experiment 2 may be taken as proof of no effect of exposure on this system. However, such a conclusion would be difficult to accept in view of the existing literature data and results of Experiment 3. What concerns the literature data is that most of the authors have found that adult exposure [13], or perinatal exposure with maternal blood or milk [45] to ortho-substituted PCBs results in lower DA content in various regions of the brain [46-49]. One may expect that a reduced DA level may result in a significant change in the sensitivity to DA agonists and antagonists. Unfortunately, in the reports quoted above the data on the reaction to psychostimulants are missing. The only exception is the Bushnell et al. [16] work reporting changed sensitivity to cocaine in rats exposed to PCB. However, no correlation between the effect and the level of exposure was found, and the result was regarded to be accidental.

Also, the results of our Experiment 3 suggest an effect of the perinatal exposure to PCB 153 on the DA system. If the elevated susceptibility to psychostimulant sensitization in male rats exposed perinatally to MeHg is related to some change in the DA system, then the "normal" susceptibility in animals in Experiment 3 may indicate that the exposure to PCB somehow makes the DA system resistant to MeHg activity or, to be more precise, makes the system less susceptible to the development of hypersensitivity.

To sum up, the results of the reported experiments suggest that perinatal exposure to MeHg may act to increase the susceptibility of the male progeny to psychostimulant sensitization. On the other hand, they do not support the assumption about the synergistic or additive activity of MeHg and PCB 153 in the CNS. Quite the contrary, they suggest that co exposure to PCB 153 may protect from at least some of the effects of MeHg exposure. It is worth noting that neither synergism nor additivity was noted for the activity of MeHg and PCB 153 in other studies performed under the DEVNERTOX Project and concerning the monoaminergic [4] and cholinergic [50] transmission. Besides, in an *in vitro* study on PC12 cells, it has been demonstrated, using cell viability as end-point that, at least at some concentration combinations, the toxic potential of MeHg is lower in the presence of PCB 153 and vice versa [51]. Those observations indicate that the protective activity of PCB 153 against the effects of MeHg observed in our work is not an epiphenomenon. Therefore, further studies are necessary to see whether other PCB congeners show a similar activity and to precisely determine whether, and if so - to what extent, the observed protective effect depends on the level and proportion of both neurotoxicants concentrations.

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