Université de Montréal

Exposition subaiguë au mercure méthylique chez le rat juvénile: effets sur le comportement, l'apprentissage et la mémoire et rôle protecteur du glutathion et de la nifédipine

par

Stéphane Beaudin

Département de psychologie

Faculté des Arts et des Sciences

Thèse présentée à la Facultés des études supérieures

en vue de l'obtention du grade Philosophiae Doctor (Ph.D.)

en psychologie option neuropsychologie expérimentale

Juillet 1998

Université de Montréal

Cette thèse intitulée:

Exposition subaiguë au mercure méthylique chez le rat juvénile: effets sur le comportement, l'apprentissage et la mémoire et rôle protecteur du glutathion et de la nifédipine

présentée par:

Stéphane Beaudin

a été évaluée par un jury composé des personnes suivantes:

Présidente du jury:	Dr. I. Peretz
Représentant du Doyen:	Dr. T. Reader
Directeurs de thèse:	Dr. M. Ptito, Dr. S. Chakrabarti
Examinateur interne:	Dr. M. Arguin
Examinatrice externe:	Dr. G. Muckle

Thèse acceptée le:

Sommaire

Le mercure méthylique (MeHg) est une toxine très répandue dans l'environnement. Les émanations naturelles et antropogéniques de mercure inorganique (Hg^{++} ou mercurique) contribuent à sa production. À la base de celle-ci interviennent des bactéries méthanogéniques qui, suite à l'addition d'un atome de carbone sur l'atome mercurique, méthylise le Hg^{++} après l'avoir digéré. Une fois libéré le MeHg se distribue rapidement en se liant aux protéines de la faune aquatique. De là, il remonte la chaîne alimentaire (e.i par bioamplification) pour s'accumuler préférentiellement dans les espèces prédateurs et les espèces plus anciennes. L'histoire des victimes et des habitants de Minamata et de Niigata, au Japon, est un exemple tragique d'une contamination environnementale par le MeHg suite a la consommation de poissons. Aujourd'hui, le MeHg est considere plus toxique que le plomb. Bien que les effets d'une exposition prénatale au MeHg aient fait l'objet de nombreuses études comportementales, les effets d'une exposition postnatale restent moins bien connus. Plusieurs indices neurochimiques et histopathologiques suggèrent pourtant que l'exposition postnatale au MeHg pourrait perturber le comportement, l'apprentissage et la mémoire.

La présente étude comporte deux objectifs. Le premier objectif est d'évaluer chez le rat juvénile dans quelle mesure l'atteinte de fonctions comportementales de base, comme la coordination motrice et la locomotion, et de fonctions cognitives, comme l'apprentissage et la mémoire, peut servir de signe précoce d'intoxication par

le MeHg. Le deuxième objectif consiste à étudier les effets protecteurs potentiels d'un anti-oxydant, le glutathion, et d'un blocqueur des canaux calciques, la nifédipine contre la neurotoxicité du MeHg.

À l'aide de tests comportementaux et cognitifs variés, différents groupes d'animaux sont évalués pendant et après une période d'exposition à des doses faibles ou modérées de MeHg et également traités avec du glutathion ou de la nifédipine.

L'étude des effets d'une exposition juvénile à des doses quotidiennes de 1, 2, 4 ou 6 mg/kg de MeHg pendant 10 jours révèle que même en l'absence d'une perte nette de poids corporel et de troubles moteurs typiques d'intoxication, la performance des animaux peut être affectée dans certains tests comportementaux. L'exploration d'objets disposés dans un champ ouvert (open field), mais non l'exploration horizontale et verticale de cet espace, est affectée à une dose de 4 mg/kg (article 1) quand les objets sont permutés à l'intérieur d'une configuration familière. La coordination motrice, telle que mesurée dans le test du rotarod ainsi que l'apprentissage d'un lieu d'échappement fixe dans le labyrinthe aquatique de Morris ne sont perturbés qu'à la dose la plus élevée de 6 mg/kg de MeHg (article 2). L'acquisition et la performance dans le DMTP aquatique de Means sont, quant à eux, affectés à des doses de 2, 4 et 6 mg/kg (articles 1 et 2). Ce test de mémoire de travail apparaît comme la procédure expérimentale la plus sensible aux effets d'une exposition juvénile au MeHg.

L'étude des effets protecteurs de 0,05 mg/kg de glutathion et de 5 mg/kg de nifédipine indique, quant à elle, que l'action anti-oxydative du glutathion et que le blocage des canaux calciques via l'administration de la nifédipine contrecarrent le déficit de mémoire de travail induit par une dose de 6 mg/kg de MeHg dans le DMTP aquatique. De plus, le glutathione limite le ralentissement du gain pondéral, un effet que ne produit pas la nifédipine (article 3).

Dans l'ensemble, les résultats de la présente thèse démontrent que certains tests comportementaux et cognitifs peuvent servir de procédure expérimentale pour identifier les signes précoces de l'intoxication par le mercure méthylique dans le cas d'une exposition chez le rat juvénile. Ils suggèrent également que l'utilisation de thérapeutiques spécifiques peut protéger les systèmes mnésiques et prévenir un déficit d'apprentissage successif à une exposition au MeHg. Par ailleurs, la transposition des procédures expérimentales et des tests comportementaux employés dans cette étude, à l'évaluation des déficits chez des sujets humains est maintenant possible grâce à la mise au point récente de tâches d'apprentissage et de mémoire équivalentes à celles utilisées pour les animaux.

Table des matières

Liste des Tableaux

[1]article 2.1

Liste des figures

[1]article 2.1 [2]article 2.2 [3]article 2.3

Liste des sigles et des abbréviations

Ach:	acétylcholine
BPC:	biphényles polychlorés
Ca^{++}:	ions calciques
CBTS:	Collaborative Behavioral Teratological Study
ChAT:	choline acétyltransferase
cm:	centimètre
DA:	dopamine
DMTP:	delayed matching-to-position
g:	gramme
GSH:	glutathion
h:	heure
hg^{0}:	mercure élémentaire ou vaporeux
hg^{++}:	mercure inorganique
i.p.:	intra-péritonial
kg:	kilogramme
MeHgCl:	methylmercury chloride
MeHg:	mercure méthylique
mg:	milligramme
min:	minute
NFD:	nifédipine

OMS:	Organisation Mondiale de la Santé
po:	per os
rpm:	rotation par minute
sec:	seconde
SOD:	superoxyde dismutase
W:	watts

Remerciements

Je tiens tout d'abord à remercier mes directeurs de thèse, Monsieur Maurice Ptito, Ph.D. et Monsieur Saroj Chakrabarti, Ph.D., pour m'avoir accordé confiance et autonomie tout au long de ce projet. Je tiens également à exprimer ma reconnaissance envers Monsieur François Doré, Ph.D. pour son assistance lors de la préparation de cette thèse ainsi que pour m'avoir si bien initié à l'étude comparée de la cognition animale.

J'aimerais remercier Monsieur Robert Lalonde, Ph.D. pour son support et ses encouragements répétés ainsi que pour ses conseils lors de la préparation de ce document. J'adresse mes remerciements aux membres professeurs(es) et étudiants(es) du groupe de recherche en neuropsychologie expérimentale (GRENE) et à ceux et celles qui m'ont encouragé et soutenu tout long de ces années à l'Université de Montréal.

Je tiens tout spécialement à exprimer mes saluations amicales et mes remerciements à Monsieur Chengiang Bai pour son aide et sa disponibilité lors du traitement des animaux. Je remercie également Marcel Kovana Loua pour toutes les discussions que nous avons eues au sujet de la neurotoxicité du mercure.

Enfin et surtout, je tiens à souligner du plus profond de mon coeur ma gratitude envers mon épouse, Pascale, et mon fils, Siméon, pour leurs multiples encouragements

et pour m'avoir épaulé même dans les instants les plus difficiles de mon cheminement académique. La patience et l'amour de Pascale lors des longs mois d'expérimentation en laboratoire ont rendu ce travail possible. Ainsi en est-il de mes parents et de mon frère sans qui le désire et la motivation d'aller toujours plus loin m'auraient déjà abandonné. Mercie également aux familles Basque, Berthiaume, Lapierre et Valois pour leur compréhension et amitié et tout spécialement à Patrick Berthiaume pour sa fidélité et son amitié sincère.

Dédicace

À mon père François, ma mère Jacqueline et mon frère Martin

CHAPITRE 1

INTRODUCTION GÉNÉRALE

Étendue du problème en neurotoxicologie

L'histoire de l'humanité a été marquée par de nombreux épisodes d'empoisonnement à différentes toxines présentes dans l'environnement. Hippocrate observe le premier les effets de la toxicité du plomb chez les travailleurs de l'industrie minière en l'an 370 a. J.-C (Klaassen, 1986). Aujourd'hui, selon les études cliniques, épidémiologiques et expérimentales, il existerait près de 25 substances chimiques reconnues pour leurs effets dévastateurs sur le système nerveux des humains et des animaux (Anger, 1986; Anger et Johnson, 1985). Dans le milieu industriel, 28% des 590 substances chimiques en utilisation courante sont considérées neurotoxiques (Anger, 1984). Cependant, le nombre exact de neurotoxines dans l'environnement est difficile à estimer puisque parmi les 70 000 produits déjà commercialisés, seulement quelques-uns ont fait l'objet d'une évaluation quant à leur potentiel neurotoxique. La situation apparaît encore plus critique si on considère qu'aux États-Unis, le potentiel neurotoxique de plusieurs composés commercialisés avant 1976 reste entièrement inconnu, ayant échappé à la surveillance mise en place par le Décret sur le Contrôle des Substances Toxiques (Toxic Substances Control Act, 1976). Résultat, plus de 23 milliards de dollars ont été consacrés en 1982 aux soins des personnes souffrant d'une atteinte neurologique causée, dans plusieurs cas, par l'exposition à une neurotoxine de l'environnement ou par l'utilisation d'une drogue légale (Rice, Hodgson et Kopstein, 1985). Cependant, les conséquences réelles de ces accidents ne s'expriment pas uniquement en frais de santé. Elles s'expriment aussi dans la réduction des capacités

cognitives qui empêchent les membres d'une société de mener une existence saine et productive.

Les maladies neurologiques ou psychiatriques d'origine environnementale, qui résultent de l'exposition aux toxines naturelles et synthétiques, de la consommation de nourriture ou de produits pharmaceutiques, surviennent à n'importe quel moment de la vie et se manifestent à tous les âges. Les effets d'une exposition prénatale ou périnatale peuvent apparaître très tôt ou au contraire, se manifester beaucoup plus tard au cours du développement ou du vieillissement par suite d'une neurotoxicité différée (Rice, 1996). Selon certains, l'exposition aux toxines de l'environnement et, en particulier aux métaux lourds (plomb, mercure, manganèse, cadmium), contribuerait aux maladies neurodégénératives de type Alzheimer, Parkinson ou de la sclérose amyotrophique latérale (Calne, Eisen, McGeer et Spencer, 1986).

La faible capacité régénératrice des neurones, leur dépendance par rapport au glucose et à l'oxygène ainsi que la multitude de circuits neurochimiques susceptibles d'être perturbés rendent le système nerveux, les processus cognitifs et la coordination du comportement particulièrement vulnérables à la présence de substances toxiques. La prévention des atteintes neurologiques est donc un objectif de première importance pour la médecine, la santé publique et les politiques sociales. La première forme de prévention est bien sûr la réduction à zéro de tout risque d'exposition aux toxines de l'environnement. Toutefois, à court et à moyen terme, un tel objectif est irréaliste et ne pourra être atteint que dans plusieurs générations, quand de nouveaux modes de vie

auront remplacé des traditions millénaires. En effet, la consommation de poissons, qui est l'une des principales voies d'intoxication par le mercure, les BPC et plusieurs autres composés, est pour plusieurs populations non seulement une source nutritive importante mais elle s'accompagne aussi d'activités profondément ancrées dans la culture et l'organisation sociale (Wheatley, 1996). Parallèlement aux efforts pour éliminer les neurotoxines de l'environnement, il faut donc perfectionner les instruments visant à détecter les signes précoces d'intoxication, à interrompre la dégénérescence neurale dans les cas connus et à empêcher un dommage irréversible au système nerveux.

Le principal objectif de cette thèse de doctorat est d'évaluer chez le rat dans quelle mesure l'atteinte de fonctions comportementales de base, comme la coordination motrice et la locomotion, et de fonctions cognitives, comme l'apprentissage et la mémoire, peut servir de signe précoce d'exposition à une toxine particulière et très répandue, le mercure méthylique. De plus, la thèse examine les effets protecteurs potentiels contre ce traitement d'un anti-oxydant, le glutathion, et d'un bloqueur de canaux calciques, la nifédipine.

Mercure, environnement et neurotoxicité

Le mercure méthylique (MeHg) est une toxine très répandue dans l'environnement. Selon l'Organisation Mondiale de la Santé (OMS), près de 15,000 tonnes de mercure seraient émis dans l'environnement à chaque année en provenance

de sources anthropogéniques et naturelles. À l'échelle mondiale, l'industrie minière produit annuellement près de 10,000 tonnes de mercure tandis qu'entre 2,700 et 6,000 tonnes de cette substance proviennent d'émissions naturelles dues à l'évaporation des cours d'eau, aux émanations gazeuses de la croûte terrestre et aux éruptions volcaniques (Lindberg, Stokes, Goldberg et Wren, 1987). Une fois émis dans l'atmosphère sous forme élémentaire ou vaporeuse (Hg^0), le mercure est converti sous forme soluble (Hg^{++}) et retourne à la surface de la terre par l'eau de pluie. C'est à ce moment du cycle de production que le mercure inorganique est transformé, par la présence dans l'eau de bactéries méthanogéniques, en mercure méthylique (MeHg) et remonte la chaîne alimentaire en s'accumulant dans les poissons et autres organismes marins qui intoxiqueront éventuellement les consommateurs humains et animaux de la faune aquatique.

En 1940, Hunter, Bomford et Russel (syndrome Hunter-Russel) publie la première description détaillée des effets toxiques du mercure chez quatre travailleurs de l'indiustrie. Ce n'est qu'avec les contaminations environnementales, cependant, que le mercure méthylique a été reconnu officiellement comme neurotoxine.

La maladie de Minamata est le premier cas connu, et sûrement le plus grave, de contamination environnementale (Harada, 1995; Marsh, 1994). Elle a été causée par le déversement de mercure méthylique dans les eaux usées d'une usine et par la contamination des poissons. Bien que les premiers patients intoxiqués aient été identifiés en 1956, ce n'est qu'en 1968 que le gouvernement japonais a officiellement

reconnu que cette maladie était causée par le mercure méthylique produit dans cette usine. Les recherches sur les patients adultes souffrant de la maladie de Minamata ont permis de découvrir que le développement intra-utérin est particulièrement sensible à l'intoxication par le mercure méthylique. La symptomatologie est très diversifiée. Dans les cas les plus légers, les symptômes sont non spécifiques: fatigue, pertes de mémoire, troubles mentaux légers, maux de tête, mouvements lents et engourdissement, tremblement des lèvres et des doigts. À un niveau un peu plus avancé, l'intoxication se manifeste par de l'ataxie, de la dysarthrie, le rétrécissement du champ visuel, la perturbation des mouvements oculaires et la dysmétrie oculaire. Les cas cliniques graves présentent des tremblements, un dérèglement des sensations, de l'ataxie, de la dysarthrie, des troubles auditifs, un rétrécissement du champ visuel et des anomalies de la sensibilité visuelle aux contrastes spatiaux. Enfin, les cas les plus sévères d'intoxication conduisent à la mort ou à un handicap permanent qui se distingue par un syndrome incluant notamment des troubles mentaux.

Deux autres cas célèbres d'intoxication par le mercure ont fait l'objet d'études systématiques et fouillées. En 1965, une situation analogue à celle de Minamata a été identifiée à Niigata où l'exposition au MeHg avait été cependant plus courte et moins intense. Les recherches sur les habitants de cette localité ont montré des troubles des sensations périphériques, de l'ataxie, de la surdité, de la dysarthrie, un rétrécissement du champ visuel et des pertes de mémoire (Marsh, 1994). Le pattern de détérioration de ces patients suggérait une amélioration possible des symptômes (Inskip et Piotrowski, 1985). L'autre cas célèbre a été identifié en Irak en 1971. Les recherches

sur ces patients ont démontré que la consommation de poissons n'est pas la seule voie d'intoxication par le MeHg et qu'une farine traitée avec un fongicide et servant à la fabrication du pain domestique en est une autre (Marsh, 1994).

Au Canada et au Québec, les populations autochtones sont particulièrement à risque à cause de leur consommation élevée de poissons. Les études cliniques et épidémiologiques suggèrent que les niveaux d'exposition, tels que mesurés par l'analyse d'échantillons sanguins et capillaires, sont généralement inférieurs à ceux de Niigata et de l'Irak (WHO, 1990).

Exposition prénatale au MeHg et comportement

La première démonstration expérimentale de l'intoxication prénatale par le MeHg a été fournie par Spyker, Spaber et Goldberg (1972) dans des travaux sur la souris. Depuis ce temps, les données se sont multipliées à un rythme accéléré et nous nous limiterons ici aux études en tératologie comportementale.

Le plus grand nombre de données sur le comportement des animaux exposés au MeHg durant la période intra-utérine provient du Collaborative Behavioral Teratological Study ou CBTS (Buelke-Sam et al., 1985). L'objectif principal du CBTS était d'évaluer la fiabilité et la sensibilité de plusieurs tests comportementaux dans des conditions standards à l'intérieur d'un même laboratoire et dans différents laboratoires. Des rates gestantes étaient exposées à des doses de 2 ou 6 mg/kg des jours 6 à 9 de

gestation. Les mêmes études étaient dupliquées dans cinq laboratoires indépendants. L'ensemble de ces travaux a mis en évidence des relations dose-effet pour plusieurs des mesures comportementales.

Chez les jeunes ratons soumis à la dose la plus élevée, l'amplitude des réponses lors de l'habituation du sursaut à un son augmente et certains laboratoires observent le même effet à la dose plus faible. Des relations dose-effet apparaissent aussi pour les mesures d'activité durant les premières heures et pour l'activité lors d'un test pharmacologique avec de la d-amphetamine dans un labyrinthe en huit. Ces deux mesures augmentent avec la durée d'exposition au MéHg. Dans une tâche de discrimination visuelle, les intervalles inter-essais sont plus longs et le nombre de réponses correctes est inférieur chez les ratons exposé à la dose la plus élevée. Parallèlement aux travaux du CBTS, Vorhees (1985) a effectué une recherche avec une batterie étendue de tests. Ses résultats démontrent que chez le groupe ayant reçu la dose la plus élevée, l'ontogénèse du réflexe de redressement et de la natation est retardée; le temps passé dans le labyrinthe aquatique est plus élevé et les échappements réussis moins nombreux. Comme dans l'étude du CBTS, le développement physique est modifié. À la dose prénatale la plus élevée, on note une accélération de l'émergence des incisives et de l'ouverture des yeux de même qu'un retard dans l'ouverture du vagin. De plus, les études du CBTS et de Vorhees rapportent toutes deux une diminution de poids chez les femelles gestantes et chez les ratons à 60 jours. Avec une durée d'exposition plus longue (des jours 6 à 15) et une dose quotidienne de 2.5 mg/kg, Geyer, Butcher et Fite (1985) trouvent des effets similaires

pour la plupart des mesures ainsi que des effets sur la géotaxie négative et le pivotement. Des souris exposés durant la période intra-utérine à des doses plus élevées ont un taux de survie faible et les survivants sont hypoactifs, les réflexes de redressement et les postures étant anormales (Inouye, Murao et Kajiwara, 1988).

D'autres études chez les rongeurs démontrent des effets sur la natation chez les souris (Spyker et al., 1972), sur l'activité dans l'open field chez les souris (Spyker et al., 1972; Su et Okita, 1976) et sur la réactivité aux drogues chez les rats (Eccles et Annau, 1982; Hughes et Sparber, 1978). Quelques recherches ont analysé les effets de l'exposition prénatale au MeHg sur la performance dans des tâches d'apprentissage. L'apprentissage d'évitement et un apprentissage appétitif (Eccles et Annau, 1982; Hughes et Annau, 1976; Shalock, Brown, Kark et Menon, 1981) ainsi que le conditionnement opérant avec un programme de renforcement du débit lent (DRL) (Bornhausen, Müsch et Greim, 1980; Müsch, Bornhausen, Kriegel et Greim, 1978) sont affectés.

À tous les niveaux d'intoxication, même les plus faibles, on note chez les rongeurs une diminution de la taille cérébrale et une diminution du nombre de neurones. Une intoxication cérébrale modérée produit en plus des dommages au cortex et au cervelet ainsi qu'une perte de myéline. Une forte intoxication entraîne des dommages additionnels aux ganglions de la base et à l'hippocampe, une dilatation des ventricules et une orientation anormale des cellules (Burbacher, Rodier et Weiss, 1990).

Chez les primates, les effets les plus marqués d'une exposition prénatale et périnatale au MeHg se manifestent au niveau des fonctions sensorielles comme chez l'humain (Rice, 1996): déficits de la vision spatiale (Rice et Gilbert, 1990); des seuils de détection normaux pour les basses fréquences mais une perte de sensibilité auditive pour les hautes fréquences (Rice et Gilbert, 1992); augmentation du seuil dans un test de sensibilité tactile (Rice et Gilbert, 1995). Par contre, les effets sur le fonctionnement cognitif sont beaucoup moins robustes et clairs que sur les fonctions sensorielles. Dans une tâche de recherche d'un objet disparu, des macaques (Macaca fascicularis) âgés de deux semaines ont de la difficulté à retrouver l'objet invisible mais ils ont aussi de la difficulté à l'atteindre lorsqu'il est visible (Burbacher, Grant et Mottet, 1986). À un mois, le temps de fixation visuelle d'un nouveau stimulus dans le test de Fagan (Fagan et McGrath, 1981), qui est un indice de la mémoire de reconnaissance, est inférieur à celui de sujets normaux (Gunderson, Grant, Burbacher, Fagan et Mottet, 1986; Gunderson, Grant-Webster, Burbacher et Mottet, 1988). Toutefois, ce résultat est contradictoire avec l'absence ultérieure de déficit chez les mêmes singes dans le non-appariement différé de l'échantillon (delayed nonmatching-to-sample) (Rice, 1996) qui est pourtant aussi une tâche de mémoire de reconnaissance. Dans un conditionnement opérant avec programme de renforcement à intervalle fixe, les singes exposés au MeHg produisent plus de réponses au début de l'intervalle, démontrant ainsi une plus faible capacité de discrimination temporelle (Rice, 1992). Par ailleurs, aucun déficit n'a été observé dans des tâches de discrimination visuelle et d'inversion de discrimination (Rice, 1992).

Exposition postnatale au MéHg et comportement

Bien que l'intoxication par le MéHg puisse survenir à n'importe quelle période du développement et de la maturation, les données sur l'exposition postnatale sont beaucoup moins nombreuses. Dans le cas des primates, les résultats sont très similaires à ceux obtenus à la suite d'une exposition prénatale ou périnatale (Rice, 1996).

Chez les rongeurs, la perte de poids ou le retard dans la croissance pondérale est considéré comme un signe précoce d'intoxication par le MéHg (Magos et al., 1985). À des doses élevées d'exposition postnatale, le croisement des pattes postérieures quand l'animal est soulevé par la queue (Magos et al., 1981, 1985) et la rotation de la queue (Ohi et al., 1978) constituent des indices moteurs d'intoxication reliés à des changements morphologiques des neurones. Certaines modifications comportementales, antérieures aux signes moteurs et observées lors d'une exposition à des doses modérées, ont été attribuées à une dysfonction cholinergique (Kobayashi et al., 1981). Ainsi en est-il de l'extension accrue des pattes postérieures, de la diminution de l'activité motrice spontanée et de la coordination motrice réduite dans le test du rotarod. Quant aux effets sur l'apprentissage et sur la mémoire, les données sont très rares et peu récentes. Des ratons exposés de façon répétée après le sevrage à des doses faibles de MéHg démontrent un déficit d'apprentissage dans un labyrinthe aquatique en T (Zenick, 1974). À la suite d'une exposition aiguë à une dose très élévée (25 mg/kg), des rats juvéniles mettent plus d'essais à atteindre le critère d'apprentissage dans une discrimination tactile et présentent des signes d'hypoactivité dans l'open-field en

termes d'exploration horizontale mais non en termes d'exploration verticale (Post, Yang, King et Sanger, 1973). À part ces résultats, il n'y a à notre connaissance aucune autre étude pertinente à ces fonctions cognitives.

Le peu de recherches sur l'apprentissage et la mémoire chez les rongeurs à la suite d'une exposition postnatale et la prédominance des résultats négatifs chez les primates est quelque peu étonnante, compte tenu des régions cérébrales où s'accumule prioritairement le mercure méthylique. En effet, une étude récente (Chakrabarti, Loua et Durham, 1998) a montré que le striatum accumule la plus grande quantité de MéHg, suivi par le cortex et le cervelet; l'intoxication de l'hippocampe est plus élevée que celle de l'hypothalamus et du tronc cérébral. Le striatum et le cervelet, en plus de leur fonction motrice bien connue, jouent un rôle important dans l'apprentissage et la mémoire (Kimura, 1995; Thompson et Kim, 1996; Thompson et Krupa, 1994; White, 1997; Wise, 1996). Quant à l'hippocampe et aux régions corticales qui l'entourent, elles sont mises en jeu dans une variété de tâches spatiales et non spatiales d'apprentissage et de mémoire (Cohen et Eichenbaum, 1993; Eichenbaum, 1996). Comme les changements comportementaux et cognitifs sont considérés comme des précurseurs d'une intoxication par le MéHg (Burbacher et al., 1990; Kobayashi et al., 1981), ce qui semble confirmé par la symptomatologie observée à Minamata et Niigata, ces changements pourraient être utilisés comme signes précoces de toxicité.

Agents protecteurs contre l'intoxication par le MeHg

Dans les années 1960 et 1970, on a découvert que le sélénium et la vitamine E contrecarrent jusqu'à un certain point la toxicité du mercure méthylique (Ganther et al., 1972; Kasuya, 1975; Parizek et Ostadalova, 1967; Welsh, 1979). Les effets bénéfiques du sélénium ont toutefois été remis sérieusement en question quand des études ultérieures n'ont pu rèproduire le retard antérieurement rapporté dans les signes d'intoxication (Inskip et Piotrowski, 1985; Ohi, Seki, Maeda et Yagyu, 1975). De plus, à forte concentration, cette substance a des effets neurotoxiques contraires. Néanmoins, l'hypothèse selon laquelle l'oxydation cérébrale jouerait un rôle déterminant dans la pathogénèse de l'intoxication par le MeHg, est demeurée viable et la fonction protectrice des anti-oxydants a même connu un regain d'intérêt depuis quelques temps.

Plusieurs mécanismes peuvent limiter le stress oxydatif causé par le MeHg. Parmi ceux-ci, figure le glutathion qui catalyse la réduction des radicaux libres. Comme le stress oxydatif fait partie de toute activité métabolique, le cerveau possède des mécanismes anti-oxydants endogènes grâce au superoxyde dismutase (SOD) et au glutathion. Toutefois, en présence de MeHg, ces mécanismes endogènes sont supprimés et l'état d'oxydation du cerveau augmente par suite de l'accroissement des radicaux libres. Par conséquent, en stimulant de façon exogène le niveau de glutathion, il est possible de réduire ou de supprimer les effets oxydants induits par le MeHg et de protéger les neurones contre une éventuelle détérioration. Le glutathion est une

thérapeutique particulièrement intéressante car, contrairement au sélénium, il accélère l'excrétion de MéHg de la cellule en se complexant directement avec le MéHg (Kromidas, Trombetta et Jamall, 1990). L'efficacité du glutathion pour bloquer la neurotoxicité induite par le MéHg a été récemment démontrée *in vitro* (Park, Lim, Chung et Kim, 1996).

Le MéHg n'augmente pas seulement les réactions oxydatives, il perturbe aussi l'homéostasie du Ca^{++} en haussant la concentration de Ca^{++} intracellulaire, un mécanisme par lequel plusieurs substances neurotoxiques produisent la mort neuronale (Komulainen et Bondy, 1987). Des études neurochimiques *in vitro* suggèrent que les bloqueurs de canaux calciques pourraient avoir un rôle protecteur (Pauwesl, Leysen et Janssen, 1991; Yoshimura, Watanabe et Shibuya, 1993). Ces études montrent que l'augmentation intracellulaire de Ca^{++} consécutive à une exposition au MéHg est retardée par de fortes concentrations de nifédipine qui retardent également l'accès du MéHg à des composantes cellulaires comme les mitochondries. Il faut noter que les composés dihydropyridine, qui comprennent entre autres la nifédipine et le nimodipine, améliorent l'apprentissage et la mémoire chez des animaux âgés ou lésés (Finger et al., 1990; Izquierdo, 1990; Sandin, Jasmin et Levere, 1990).

Bien que les expériences *in vitro* suggèrent fortement que les anti-oxidants comme le glutathion et les bloqueurs de canaux calciques comme la nifédipine pourraient constituer des agents protecteurs contre la neurotoxicité du MéHg, cette fonction protectrice n'a pas été jusqu'à maintenant testée au niveau comportemental.

Objectifs et plan de la thèse

Tel que déjà mentionné, le principal objectif de cette thèse de doctorat est d'évaluer chez le rat dans quelle mesure l'atteinte de fonctions comportementales de base, comme la coordination motrice, et de fonctions cognitives, comme l'apprentissage et la mémoire, peut servir de signe précoce d'intoxication par le mercure méthylique. La thèse vise également à examiner les effets protecteurs potentiels contre cette intoxication du glutathion, et de la nifédipine.

Le premier article (2.1), présenté dans le chapitre 2 et soumis à Neurotoxicology and Teratology, analyse l'effet d'une exposition subaiguë postnatale au MeHg sur la coordination motrice dans le test du rotarod, sur l'exploration de l'espace et d'objets dans l'open-field, sur l'apprentissage spatial dans le labyrinthe aquatique de Morris et sur la mémoire de travail dans l'appariement différé de la position (DMTP) dans le labyrinthe aquatique de Means.

Le deuxième article (2.2) soumis à Behavioral Neuroscience, vise à reproduire les effets observés antérieurement avec certaines des doses utilisées mais aussi à examiner ces effets avec une dose supérieure. Encore une fois, la coordination motrice dans le test du rotarod et l'apprentissage spatial dans le labyrinthe aquatique de Morris sont testés. La mémoire dans le DMTP aquatique est toutefois mesurée différemment. Après une phase d'entraînement où les rats doivent atteindre un critère d'apprentissage,

leur performance est testée avec des délais plus longs de 5 et 15 minutes entre la présentation de l'échantillon et le choix.

Le troisième article (2.3) soumis à NeuroToxicology, compare l'apprentissage et la mémoire de rats exposés au MéHg, qui ont été ou non également traités avec du glutathion ou de la nifédipine, dans le DMTP aquatique.

Le chapitre 3 résume les principaux résultats obtenus et conclut sur la contribution des études comportementales à la neurotoxicologie.

CHAPITRE 2

ARTICLES DE RECHERCHE

2.1 Exposition postnatale au mercure méthylique: effets sur le comportement, l'apprentissage et la mémoire chez des rats juvéniles

Soumis à *Neurotoxicology and Teratology*

sous le titre de

Postnatal exposure to methylmercury: Effects on behavior, learning and memory in juveniles rats

Postnatal Exposure to Methylmercury: Effects on Behavior, Learning, and Memory in Juvenile Rats

Stéphane Beaudin[1], Saroj K. Chakrabarti[2], François Y. Doré[3], and Maurice Ptito[1]

[1]Groupe de recherche en neuropsychologie expérimentale, Département de psychologie, Université de Montréal, Montréal, Qué., Canada H3C 3J7

[2]Département de médecine du travail et d'hygiène du milieu, Faculté de médecine, Université de Montréal, Montréal, Qué., Canada H3C 3J7

[3]École de psychologie, Université Laval, Québec, Qué., Canada G1K 7P4

Correspondence: Stéphane Beaudin
Department of Nutritional Sciences and Psychology
Savage Hall Building
Cornell University
Ithaca, NY

Running Head: Behavioral Effects of Postnatal MeHg Exposure

Abstract

BEAUDIN, S., S.K., CHAKRABARTI, F.Y., DORÉ AND M. PTITO. Postnatal exposure to methylmercury: Effects on behavior, learning, and memory in juvenile rats. NEUROTOXICOL TERATOL - In the field of environmental toxicology, there is a need to identify early warning signs of MeHg toxicity before any irreversible damage to the nervous system could occur. This study examined the effects of postnatal subacute exposure to MeHg on motor coordination, exploration, and water maze learning tasks. Rats were treated po with 0, 1, 2, or 4 mg/kg/day of MeHg chloride for 10 consecutive days. Behavioral testing began after the fourth treatment and lasted 25 days. Weight gain was depressed but no crossing of hind legs was observed and motor coordination was intact. Object exploration was modified at a dose of 4 mg/kg whereas water maze Delayed Matching-to-Position (DMTP), but not performance in the Morris water maze, was impaired at doses of 2 and 4 mg/kg. The results suggest that some learning and memory tasks are likely to be impaired under the experimental conditions and hence, may serve as early signs, before any overt motor indication of severe intoxication.

INTRODUCTION

Methylmercury (MeHg) is known as an ubiquitous environmental pollutant and the detrimental effects of MeHg on the development of the nervous system and of behavior in humans and animals are well documented (6, 7, 22). In animal models, behavioral teratological studies have demonstrated that prenatal exposure to MeHg in monkeys and rodents impairs sensory, motor, and cognitive functions (for reviews see 3,41) as well as social development (4). Although individuals are at risk of intoxication at any age, the effects of postnatal exposure to MeHg on neural, behavioral, and cognitive functions have not been studied as extensively as the effects of prenatal exposure.

According to Rice (1996), methylmercury preferentially damages deep sulci in the adult human and monkey. Indeed, deficits on visual (2, 16, 17, 42), auditory (43), and somatosensory (13, 44, 49) functions have been observed after postnatal chronic exposure in monkeys. In rodents, depressed weight gain or even weight loss (25) is an early sign of MeHg toxicity. Crossing of hind legs on being held by the tail (25, 26) and rotating movements of the tail (35), which have been reported after postnatal exposure to high doses of MeHg (8 to 10 mg/kg), are considered typical motor indications of intoxication and of morphological changes in nerve cells of the brain. In adult mice exposed to 5mg/kg of MeHg for about 15 days, decreased spontaneous motor activity, impaired rotarod performance, and hypothermia preceded an abrupt loss of body weight and crossing of hind legs. These behavioral changes prior to the manifestation of the overt signs were attributed to biochemical changes, more

specifically to a central cholinergic dysfunction (21). Loss of body weight, increased hindlimb foot-spread, and impaired performance on the rotarod motor task were also reported when adult mice are exposed to 10, 20, or 40 ppm of MeHg in their drinking solution for 71, 22, and 11 days (for a total estimated dose of 114, 59 or 58 mg/kg), respectively (15). However, the sequence of overt signs and behavioral changes was dose-dependent. At the lowest dose level, increased hindlimb foot-spread preceded loss of body weight and rotarod impairment whereas at the other two dose levels, all three changes occurred at about the same time.

Whereas learning deficits have been reported in rodents (11, 14, 18, 47) and monkeys (33) after prenatal exposure to methylmercury, the effects of postnatal exposure on cognitive functions are much less clear. Yet, MeHg has been found to accumulate not only in sensory and motor areas but also in a number of cortical and subcortical structures that are especially involved in learning and memory. This accumulation in cognitive brain areas has been observed after postnatal exposure to high and low doses (10, 31). More recently, it has been found in our laboratory (5) that in juvenile rats exposed to 2, 4, and 6 mg/kg po during 10 days, the striatum accumulates the higher amount of total mercury, followed by the cortex and the cerebellum. The hippocampus accumulates more total Hg than the hypothalamus and the brain stem. The striatum and the cerebellum, which are well know for their motor function, also play a crucial role in learning and memory (20, 49, 50, 51 52). As to the hippocampus and related cortical and subcortical areas, they are involved in a variety of spatial and nonspatial learning and memory tasks (8, 12). Since behavioral and

cognitive changes associated with MeHg intoxication have been proposed as precursors of more obvious damage to the nervous system (3, 21), they could serve as early warning signs.

The purpose of the present experiment was to examine the effects of subacute postnatal exposure to MeHg on motor coordination, locomotion and exploration, as well as on learning and memory. In order to model behavioral and cognitive impairments without jeopardizing survival or essential sensory and motor functions, juvenile rats were exposed to low and moderate doses of methylmercury in four tasks. Motor coordination was tested in the rotarod task whereas locomotion and exploration were measured in an open field. Learning and memory were tested in the Morris water maze and in delayed matching-to-position (DMTP) in the water maze.

In behavioral toxicological studies, the rotarod task is a standard procedure to assess the involvement of the central nervous system in motor function (15). The open field task used in the present experiment was developed by Poucet (38). It measures not only locomotion and exploration of a new environment but also investigation of new objects.

The Morris water maze is recognized as a spatial learning task and is frequently used to test memory in hippocampectomized rodents (32). Rats have to swim in a circular pool and can escape the water by finding a hidden (submerged) platform. On each trial, the animal is placed into the pool from a different starting

location and there are no cues within the pool to guide the rat to the platform. The problem can be solved by acquiring a place strategy, that is, the location of the hidden platform can be found by learning the spatial relationships between extramaze cues. To control for perceptual, motor or motivational deficits, performance in the task with the hidden platform is generally compared with performance in the same task but with a visible plarform.

The water maze DMTP has been developed by Means (9, 28) to test working memory (Figure 1). A circular pool surrounded by extramaze cues is divided into radial thirds. A position at the center of the arc forming the perimeter of one section served as the starting point for all trials. The other two sections (goals) are separated from the starting section by doors. In the center of one of the goal sections, a platform is submerged. Each trial is divided into a sample run and a test run. The sample run is a forced choice: the door of the section containing the hidden platform is opened whereas the door of the other goal section is closed. The rat is placed into the water and allowed to swim until it reaches the platform. Then, the rat is removed from the pool and after a delay, the test run (free choice) is presented. The hidden platform is in the same section as in the sample run (DMTP) but this time the doors of the two goal sections are opened. Again the rat is placed in the pool and allowed to reach and climb onto the platform.

According to Means (29), in the water maze DMTP, rodents have a strong unlearned bias to return to the location where they escape on their last trial. Forced-

choice run (information or sample trial), a barrier between sections to prevent short cuts, and detention following incorrect test run choices are all necessary to counteract this bias. Although counterintuitive and inconsistent with findings in appetitive working memory procedures, performance on test runs given either immediately or after a delay longer than 1 min is better than on test runs with a delay of 1 min (29). The enhancing effect of longer retention intervals is explained by nonmemory hypotheses: longer escape from the water provides a more effective reward as well as the needed time to recover from the emotional effects of the sample run.

Water mazes were preferred to dry mazes because testing learning and memory in water has at least two advantages. One is that subjects are motivated by escape from an aversive milieu and do not have to be deprived; deprivation would increase health hazards in MeHg exposed animals since they already tend to lose weight. The other is that swimming is not as impaired as walking in animals with severe motor problems such as those resulting from cerebellar lesions (23, 36) or such as crossing of hind legs.

MATERIAL AND METHODS

Animals and Housing

Sprague-Dawley male rats (Charles River Canada Inc., St-Constant, Québec), approximately 60-day old and weighing 250 g at the start of the experiment, served as subjects. They were housed individually in a standard Plexiglass cage and placed in a room maintained at a constant temperature ($21\pm3^{o}C$) and on a standard 12:12

light/dark cycle. Purina laboratory chow and demineralized water were available *ad libitum*throughout the experiment.

Doses and Treatment

Methylmercury chloride (MeHgCl) (purity over 99%) was obtained from Johnson Matthey Company (Ward Hill, MA, USA). Naive rats were randomly assigned to three treatment groups and a placebo group (n = 10). Treatment groups received by gastric gavage a dose of 1, 2, or 4 mg/kg/day of MeHgCl for 10 consecutive days, for a total cumulative dose of 10, 20, or 40 mg/kg. The compound was dissolved in a 0.9% saline solution and administered in a volume of 4 ml/kg. The placebo group received an equal volume of vehicle only. Body weights were monitored at 2-day intervals. The animals were observed periodically for any crossing of hind legs.

Behavioral Measurements

Behavioral testing started on the 4^{th} day of treatment because steadily increasing accumulation of methylmercury in the brain is known to occur after 72 hours (34). The tasks were administered into two stages separated by a 3-day rest period. Testing of motor coordination in the rotarod and of spatial learning in the Morris water maze began on Day 4 and lasted 7 and 12 consecutive days, respectively. The rest period occurred from Day 16 to Day 18. Then, from Day 19 to Day 28, locomotion and exploration were tested in the open field whereas working memory was tested in the water escape DMTP. All tasks were administered between 11:00 and

20:00 h and subjects were individually tested in squads of five. Throughout the study, the experimenter was unaware of the group to which the subjects belonged.

Rotarod - Motor coordination was tested in the rotarod. The apparatus was made of a horizontal cylinder (diameter: 8.5 cm; ; length: 30 cm) driven by a DC motor; the rotation speed (20 rpm) was controlled by a speed motor driver and variable size gears. Wooden side walls served to confine the rat in an area of 20 cm in width. The apparatus was placed at a height of 90 cm from the cushion-covered floor. A trial always started by gently placing the rat on the rotarod in the direction opposite to the rotation and ended after a maximum of 60 sec. If the animal fell before the end of the trial, it was returned immediately to its home cage. Fall latency was measured with a stop watch. One daily trial was administered for 7 consecutive days.

Morris water maze - The usual procedure to hide the platform in the Morris water maze is to dilute powdered milk into the water. Instead, we used a black swimming pool and a black platform, the absence of contrast making the submerged platform invisible. The swimming pool was a circular plastic tank (200 cm in diameter with 60 cm high walls) and was filled to a height of 32 cm with 23° C water. It occupied the center of the experimental room. Four starting points around the perimeter of the pool were arbitrarily designated North, East, South, and West and on this basis, the pool was divided into four quadrants (NE, NW, SE, SW). The platform was a circular Plexiglas stand (9.0 cm in diameter and 30 cm high) covered with a grid to facilitate clinging and climbing. The platform, which was submerged 2 cm below

the surface of the water, occupied the center of the NW quadrant throughout testing. Extramaze cues were provided by the door of the experimental room, a cupboard, four posters, and the experimenter who was standing between the SE and SW quadrants. The task consisted of two training conditions: spatial learning with the hidden platform and control trials with the visible platform.

Spatial learning lasted 10 consecutive days with 2 trials/day for a total of 20 trials. The two daily trials were separated by an interval of 25 sec. This training regimen has been shown to be especially sensitive to lesion-induced deficits (27). A trial always started by lowering the rat in the water while facing the wall at the starting point. Four starting points (N, S, E, W) were used randomly over two days. Once the hidden platform was reached, the rat was allowed to remain on it for 15 sec. If the platform was not found within 60 sec, the rat was placed manually on the platform for 15 sec. Escape latency and the number of quadrants crossed were recorded.

In the two days following spatial learning, control trials with the visible platform were presented. The procedure was the same with two exceptions: 1) control trials lasted 2 days (4 trials) instead of 10; and 2) a white platform emerging 2 cm from the surface of the water was placed in the SE quadrant. Escape latency, the number of quadrants crossed and swim path errors were also recorded.

Open field - The apparatus was a black wooden square field (90 cm x 90 cm) with a transparent glass floor divided into 25 equal squares. The open field was

illuminated with one 40 W light bulb installed 100 cm above it and was surrounded by a black curtain which provided a visually uniform outside environment. A white noise generator masked external sounds. Three plastic objects of different shapes and colors were placed on the floor of the open field and occupied fixed locations forming a triangular arrangement. These objects were unfamiliar to the rat at the beginning of testing.

During the first nine daily sessions, the rats were individually tested for 3 min. They were gently placed on the floor of the field, always facing the same corner. Session 10 was aimed at assessing attention to environmental change. The same triangular arrangement was maintained but the locations of the three objects were switched. The animal's reaction to that change was recorded for 3 min. For all 10 sessions, the number of squares crossed (horizontal exploration), rearing (vertical exploration), and close contacts with the objects (object exploration) were measured. Entry in a square was recorded when the head and at least one forepaw crossed the line drawn on the floor. Rearing was represented by the number of times the rat stood upright on its hind legs with or without wall support. Finally, close contact was defined as sniffing, licking, gnawing, or climbing on one of the three objects. At the end of each session, the rat was returned to its home cage and the open field was cleaned before testing the next rat.

Water maze DMTP - Training was conducted in the same swimming pool as the one used in the Morris water maze task. A T-shaped opaque plastic insert (height:

51 cm; stem: 105 cm long; bar: 72 cm) divided the pool into a starting section (maximal width: 35 cm) and two equal goal sections (maximal width: 17.5 cm) on the left and right of the starting section. The doors to the goal sections were 20-cm wide. A black circular platform (diameter: 9 cm) was placed in the correct goal section and submerged 2 cm beneath the surface of water. The extramaze cues used in the Morris water maze task were removed and replaced at the exact same locations by four dim spotlights (two 75 and two 40 W bulbs). Therefore, rats had to learn the spatial relationships between new extramaze cues. Because water maze DMTP was expected to be especially difficult, these cues were made more salient.

Rats received 2 trials/day for 10 consecutive days, each trial consisting of a sample run and a test run. During the sample run, the rat was allowed 60 sec to enter the correct goal section and to escape onto the hidden platform where it was left for 15 sec. If the platform was not found within 60 sec, the rat was gently guided to it and allowed to remain on it for 15 sec. During the test run, the rat had also 60 sec to choose the correct goal section and escape. If it chose the wrong goal section, the door of this section was closed and re-opened after a 30-sec delay to allow a correct choice (correction procedure). At the beginning sample and test runs, the rat was placed in the pool facing the outside wall of the starting section. The sample run and the test run were separated by a 2-min retention interval during which the rat was returned to its home cage. The correct goal section was varied semirandomly so that it was never the same for more than two consecutive trials and overall, it was on the right and the left of the starting section an equal number of times. The two daily trials were separated by

3 hours during which the rat was returned to its home cage and room. On sample runs, escape latency was calculated from release in the water to climbing onto the hidden platform. First choice was recorded on test runs.

Statistics - ANOVAs (Treatment x Session or Block of trials) with repeated measures on the second factor were used for the rotarod, the Morris water maze, the open field and the water maze DMTP results. Simple main effects were analyzed when the interaction was significant and post hoc comparisons were made with the Newman-Keuls' test. One-way Anovas were used when appropriate.

RESULTS

General Health Parameters

Crossing of hind legs did not appear in any rat from the treatment groups at any stage of toxic exposure or during the following days of testing, thus confirming that the dose levels of MeHg used in the present experiment did not result in severe overt motor deficits.

Results for weight gain were divided into two stages: from the first day of toxic exposure to the day after the last gastric gavage (Day 1 to Day 11), and from the third day after the last gavage to the end of the experiment (Day 13 to Day 29).

As can be seen from Fig. 2a, although there was no weight lost during toxic exposure, weight gain was depressed in some of the treatment groups. The difference

between the Placebo group and the other groups began to appear after four days of exposure to MeHg. The ANOVA showed a significant effect of the factor Group ($F=3.31$, $df=3,36$, $p < .04$) and of the factor Day ($F=169.88$, $df=5.\ 180$, $p < .0001$), as well as a significant interaction ($F=4.16$, $df=15,\ 180$, $p < .0001$). The analysis of simple main effects (Table 1, Satterthwhaite's Mean Square of Error and df) revealed that the groups did not differ on Day 1, Day 3, and Day 5 but did differ on Day 7, Day 9, and Day 11. Post hoc comparisons ($p < .05$) revealed that the weight of Group 4 mg/kg was lower than the weight of the Placebo group on Day 7, 9, and 11 and lower than the weight of Group 1 mg/kg on Day 9 and 11. Finally, on Day 11, the weight of Group 2 mg/kg was lower than the weight of the Placebo group. These results indicate that depressed weight gain was apparent after 6 days in Group 4 mg/kg and after 10 days in Group 2 mg/kg. No difference was observed between the Placebo group and Group 1 mg/kg during toxic exposure.

It seems that the effect of toxic exposure on weight gain was short-lived (Fig. 2b) because in the days that followed the last gastric gavage, the groups did not differ. The ANOVA showed a significant effect of the factor Group ($F=2.99$, $df=3,\ 36$, $p < .05$) and of the factor Day ($F=201.09$, $df=\ 8,\ 288$, $p < .0001$) as well as a significant interaction ($F=3.38$, $df=24,\ 288$, $p < .0001$). However, the analysis of simple main effects and post hoc comparisons (Table 2, Satterthwhaite's Mean Square of Error and df) found no significant difference between groups on Day 13 to Day 19 and in the following days, the only significant differences were between the Placebo group and Group 2 mg/kg and/or Group 4 mg/kg.

Rotarod Task

None of the doses of exposure to MeHg resulted in a deficit of motor coordination as measured in the rotarod task (Fig. 3). The ANOVA showed that there was no significant differences either between the treatment groups and the Placebo group or between the treatment groups themselves ($F=0.39$, $df=3, 36$).

Morris Water Maze

Rats from all groups improved on the Morris water maze task in terms of escape latencies (Fig. 4a) as well as of the number of quadrants (Fig. 4b) they entered before reaching the hidden platform (Day 4 to Day 13). The ANOVA revealed no significant effect of the factor Group ($F=0.43$, $df=3, 36$) and no interaction ($F=0.73$, $df=27, 324$) for escape latencies but a significant effect of the factor Day ($F=63.42$, $df=9, 324$, $p < .0001$). Similarly, there was no effect of the factor Group ($F=1.25$, $df=3, 36$) and no interaction ($F=0.60$, $df=27,324$) for quadrant entries but a significant effect of the factor Day ($F=34.33$, $df=9.423$, $p < .0001$). Exposure to MeHg at any dose level did not impair water maze learning.

In addition, performance on the visible platform task (Day 14 and 15) showed that toxic exposure did not impair visuo-motor abilities. All groups improved on the second day on this task both in terms of escape latencies (Group: $F=2.80$, $df=3, 36$; Day: $F=33.81$, $df=1, 36$, $p < .0001$; Group x Day: $F=2.04$, $df=3, 36$) and quadrant entries (Group: $F= 1.16$, $df=3. 36$; Day: $F=17.47$, $df=1, 36$, $p < .005$; Group x Day: $F=2.16$, $df=3, 36$).

Open Field

We first compared the behavior of the four groups of rats on the first (Day 19) and on the last day (Day 27) of exploration of the open field, with the objects at fixed locations (Fig. 5). Horizontal exploration as measured by the number of squares crossed increased in all groups but was not affected by MeHg exposure. The ANOVA showed that the factor Day was significant ($F=21.60$, $df=1, 36$, $p < .0001$) but neither the factor Group ($F=.90$, $df=3, 36$) nor the interaction were significant ($F=2.06$, $df=3, 36$). Vertical exploration as measured by the number of rearings increased in all groups ($F=8.60$, $df=1, 36$, $p < .01$) and the factor Group ($F=3.73$, $df=3, 36$, $p < .05$), but not the interaction ($F=0.42$, $df=3, 36$), was significant. As to the frequency of contacts with the objects, it increased in all groups ($F=18.08$, $df=1, 36$, $p < .0001$) and again, the factor Group ($F=3.34$, $df=3, 36$, $p < .05$), but not the interaction ($F=0.11$, $df=3, 36$), was significant.

We also compared performance on the last day of exploration (Day 27) with performance on the next day (Day 28) when the objects were switched within the triangular arrangement they formed. Horizontal exploration was increased overall by switching the objects' locations. The ANOVA on the number of squares crossed indicated that the factor Day ($F=13.53$, $df=1, 36$, $p < .0005$) and the interaction ($F=3.76$, $df=3, 36$, $p < .02$) were significant whereas the factor Group was not ($F=0.05$, $df=3, 36$). However, post hoc comparisons did not find any significant increase of horizontal exploration, for any group. Vertical exploration, i.e. rearings, did not increase between the last day of open field exploration and the day after when the

locations of the objects were switched. Neither the factor Group (F=0.76, df=3.36), the factor Day (F=1.17, df=1, 36), or the interaction (F=0.81, df=3, 36) was significant. The stability of horizontal and vertical exploration is not surprising since only the locations of the objects, and not the open field itself, were modified. On the other hand, contacts with the objects did increase in all groups except Group 4 mg/kg. The ANOVA showed a significant effect of the factor Day (F=124.28, df=1.36, $p < .0001$) and of the interaction (F=17.61, df=3. 36, $p < .0001$) and no effect of the factor Group (F=.93, df=3, 36). The analysis of simple main effects confirmed that after switching the objects' locations, contacts significantly increased in the Placebo group (F=13.89, df=1, 36, $p < .001$), in Group 1 mg/kg (F=5.55, df=1, 36, $p < .05$), and in Group 2 mg/kg (F=5.00, df=1, 36, $p < .05$) but not in Group 4 mg/kg (F<0.005, df=1, 36).

Water Maze DMTP

On sample runs, escape latencies (Fig. 6) were recorded as an additional indication of visuo-motor abilities. The results confirmed that throughout the whole task, the groups did not differ on this basic behavioral measurement. The ANOVA showed that escape latencies during sample runs decreased across blocks of 2 days (F=45.19, df=4, 144, $p < .0001$) but the factor Group (F=0.28, df=3, 36) and the interaction (F=0.63, df=12, 144) were not significant.

As to the performance on the water maze DMTP task (Fig. 7), it was superior in all groups to the level expected by chance (Placebo: $t(9)=8.5$, $p < .001$; Group 1 mg/kg: $t(9)=9.7$, $p < .0001$; Group 2 mg/kg: $t(9)= 4.4$, $p < .002$; Group 4 mg/kg:

$t(9)=6.9$, $p < .0001$). However, the success rate was clearly affected by toxic exposure in some groups ($F=5.91$, $df=3, 36$, $p < .005$). Post hoc comparisons ($p < .05$) revealed that Group 2 mg/kg and Group 4 mg/kg were significantly impaired compared to the Placebo group.

DISCUSSION

The present experiment shows that behavioral and cognitive impairments can be detected in rodents after postnatal subacute exposure to low and moderate doses of MeHg and that they can serve as early signs of intoxication when the general health of the animal and basic sensory or motor functions are not overtly affected. None of our preexposed rats displayed crossing of hind legs which is the typical motor indication of a severe deficit. Depressed weight gain was observed at the two highest dose levels used, i.e. 2mg/kg and 4 mg/kg, but this effect was short-lived and disappeared in the days that followed the end of treatment.

The rotarod task was apparently especially demanding since the mean fall latency for Placebo rats was approximately 20 sec. Nevertheless, the treated groups did not differ from the Placebo group and therefore, motor coordination was not affected by toxic exposure. Moreover, as suggested by performance during control trials in the Morris water maze with the visible platform and in the sample runs of the water maze DMTP, visuo-motor abilities were not impaired in the treated groups. Therefore, deficits in basic brain functions required to perform the tasks did not seem to be responsible for the impairments observed in the open field and the water maze DMTP.

The Morris water maze is, with the radial arm maze, one of the most widely used procedures to assess spatial learning and memory in rodents. Deficits in this task are generally associated with a dysfunction of the hippocampal formation (19, 32). Navigational behavior and learning were not affected in any of the three treatment groups of the present study even though the hippocampal system is one of the brain areas where total Hg especially accumulates (5). However, it seems that it takes 16 and 20 days of exposure to MeHg before mercury can be detected in the entorhinal cortex and hippocampal formation, respectively (31). Since our rats were tested in the Morris water maze early in the exposure treatment (Day 4 to Day 13), it is possible that MeHg did not accumulate enough in these brain areas to impair performance. It is also possible that pyramidal cells, which are the main type of neurons found in the hippocampus, are more resistant to intoxication or more resilient than smaller cell types (39).

Horizontal and vertical exploration of the open field was unaffected by postnatal exposure to MeHg, which is consistent with an earlier report using similar dose regimen and exposure periods (1) but contrasts with other rodent studies using higher dose levels (24, 37). On the other hand, object exploration was affected by exposure to a moderate dose of MeHg. When the locations of the objects in the open field were switched, contacts with the objects increased in all groups except Group 4 mg/kg. This behavioral modification, which was also observed after lesions of the hippocampal formation or of the parietal cortex (45, 46, 48), could result from either a

memory impairment for the locations of objects or a lower attention to environmental changes.

In the present experiment, the most sensitive test of learning and memory was the water maze DMTP. An impairment was found at dose levels of 2 mg/kg and 4 mg/kg. Although this task is clearly a memory paradigm, it is still difficult to identify which memory system is involved. To our knowledge, no lesion studies have investigated the brain structures required to perform this task. A limited number of psychopharmacological experiments with the win-shift version of the task (29, 30) suggests, as in other working memory tasks, the involvement of the cholinergic system. Further investigations are however needed to better characterize the neural memory system underlying performance in the water maze DMTP. However, this task seems to be especially appropriate to reveal the behavioral and cognitive effects of early intoxication due to MeHg.

Acknowledgments

This article includes portions of a doctoral dissertation submitted by Stéphane Beaudin in partial fulfillment of the requirements for the doctoral degree. The research was supported in part, by Grants from the Medical Research Council of Canada (MRC), Hydro-Québec, Bay James Committee on MeHg and Canadian Electricity Association. Stéphane Beaudin was supported by Fonds pour la Formation des Chercheurs et l'Aide à la Recherche of Gouvernement du Québec (FCAR). The present research protocol was approved by the Comité de protection des animaux de laboratoire de l'Université de Montréal, in accordance with the rules of the Canadian Council on Animal Care. We thank Mr. Chengiang Bai for his assistance in gastric gavage.

References

1. Aschner, M. Changes in axonally transported proteins in the rat visual system following systemic methyl mercury exposure. Acta Pharmacol. Et Toxicol. 59: 151-157; 1986.

2. Berlin, M.; Nordberg, G; Hellberg, J. The uptake and distribution of methylmercury in the brain of Saimiri sciureus in relatin to behavioral and morphological changes. In Miller, M.W.; Clarkson, T.W., eds. Mercury, mercurials and mercaptans. Springfield, Ill: Thomas; 1973; 187-208.

3. Burbacher, T.M.; Rodier, P.M.; Weiss, B. Methylmercury developmental neurotoxicity: A comparison of the effects in humans and animals. Neurotoxicol. Teratol. 12: 191-202; 1990.

4. Burbacher, T.M.; Sackett, G.P.; Mottet, N.K. Methylmercury effects on the social behavior of Macaca fascicularis infants. Neurotox. Teratol. 12: 65-71; 1990.

5. Loua, K. M., Chakrabarti, S., Durham, H. Modulation of cholinergic system in different brain regions following repeated exposure of rats to methylmercury. Soc. Toxicol., Seattle, March 1998, p 193, 953.

6. Chang, L.W. Neurotoxic effects of mercury: A review. Environ. Res. 14: 329-373; 1977.

7. Chang, L.W. Mercury in experimental and clinical neurotoxicology. In: Spencer, P.S.; Schaumburg, H.H., eds. Experimental and clinical neurotoxicology. London: Williams and Wilkins; 1980: 508-526.

8. Cohen, N.J.; Eichenbaum, H. Memory, amnesia, and the hippocampal system. Cambridge, Mass.: MIT Press; 1993.

9. Comer, T.R.; Means, L.W. Overcoming unlearned response biases: Delayed escape following errors facilitates acquisition of a win-stay and win-shift water-escape tasks in rats. Behav. Neural Biol. 52: 239-250; 1989.

10. Diamond, S.S.; Sleight, S.D. Acute and subchronic methylmercury toxicosis in the rat. Toxicol. Appl. Pharmacol. 23: 197-207; 1972.

11. Eccles, C.U.; Annau, Z. Prenatal methyl mercury exposure: I. Alterations in neonatal activity. Neurobehav. Toxicol. Teratol. 4: 371-376; 1982.

12. Eichenbaum, H. Is the rodent hippocampus just for "place"? Curr. Op. Neurobiol. 6: 187-195; 1996.

13. Evans, H.L.; Laties, V.G.; Weiss, B. Behavioral effects of mercury and methylmercury. Federation Proc. 34: 1858-1867; 1975.

14. Geyer, M.A.; Butcher, R.E.; Fite, K. A study of startle and locomotor activity in rats exposed prenatally to methylmercury. Neurobehav. Toxicol. Teratol. 7: 759-765; 1985.

15. Gilbert, S.G.; Maurissen, J.P.J. Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice. J. Toxicol. Environ. Health 10: 31-41; 1982.

16. Grant, C.A. Pathology of experimental methylmercury intoxication: Some problems of exposure and response. In Miller, M.W.; Clarkson, T.W., eds. Mercury, mercurials and mercaptans. Springfield, Ill: Thomas; 1973; 294-310.

17. Hunter, D.; Bomford, R.R.; Russell, D.S. Poisoning by methylmercury compounds. Q. J. Med. 9: 193-213; 1940.

18. Inoye, M.; Murao, K.; Kajiwara, Y. Behavioral and neuropathological effects of prenatal methylmercury exposure in mice. Neurobehav. Toxicol. Teratol. 7: 227-232; 1985.

19. Jarrard, L. E. On the role of the hippocampus in learning and memory in the rat. Behav. Neural Biol. 60: 9-26; 1993.

20. Kimura, M. Role of basal ganglia in behavioral learning. Neurosc. Res. 22: 353-358; 1995.

21. Kobayashi, H.; Yuyama, A.; Matsuaka, N.; Takeno, K.; Yanagiva, I. Neuropharmacological effect of methylmercury in mice with special reference to the central cholinergic system. Japan J. Pharmacol. 31: 711-718; 1981.

22. Krigman, M.R. Neuropathology of heavy metal intoxication. Environ. Health Perspec. 26: 117-120; 1978.

23. Lalonde, R.; Botez, M.I. The cerebellum and learning processes in animals. Brain Res. Rev. 15: 325-332; 1990.

24. Lown, B.A.; Morganti, J.B.; Stineman, C.H.; Massaro, E.J. Differential effects of acute methylmercury poisoning in the mouse arising from route of administration: LD_{50} tissue distribution and behavior. Gen. Pharmac. 8: 97-101; 1977.

25. Magos, L.; Brown, A.W.; Sparrow, S.; Bailey, E.; Snowden, R.T.; Skipp, W.R. The comparative toxicity of ethyl- and methylmercury. Arch. Toxicol. 57: 260-267; 1985.

26. Magos, L.; Peristianis, G.C.; Clarkson, T.W.; Brown, A.; Preston, S.; Snowden, R.T. Comparative study of the sensitivity of male and female rats to methylmercury. Arch. Toxicol. 48: 11-20; 1981.

27. Mandel, R.J.; Gage, F.H.; Thal, L.J. Enhanced detection of nucleus basalis magnocellularis lesioon-induced spatial learning deficit in rats by modification of training regimen. Behav. Brain Res. 31: 221-229; 1989.

28. Means, L.W. Rats acquire win-stay more readily than win-shift in a water-escape situation. Anim. Learn. Behav. 16: 303-311; 1988.

29. Means, L.W. Working memory for water-escape loci in rodents. In Spear, N.E.; Spear, L.P.; Woodruff, M.L., eds. Neurobehavioral plasticity: learning, development, and response to brain insults. Hillsdale, NJ: Lawrence Erlbaum; 1995: 67-89.

30. Means, L.W.; Holsten, R.D.; Long, M.; High, K.M. Scopolamine- and morphine-induced deficits in water maze alternation: Failure to attenuate glucose. Neurobiol. Learn. Memory 66: 167-175; 1996.

31. Moller-Madsen, B. Localization of mercury in the CNS of the rat. II. Intraperitoneal injectin of methylmercury chloride (CH_3HgCl) and mercuric chloride ($HgCl_2$). Toxicol. Appl. Pharmacol. 103: 303-323; 1990.

32. Morris, R.G.M.; Garrud, P.; Rawlins, J.N.P.; O'Keefe, J. Place navigation impaired in rats with hippocampal lesions. Nature 297: 681-683; 1982.

33. Newland, M.C.; Yezhou, S.; Lögberg, B.; Berlin, M. Prolonged behavioral effects of in utero exposure to lead or methyl mercury: Reduced sensitivity to changes in

reinforcement contingencies during behavioral transitions and in steady state. Toxicol. Appl. Pharmacol. 126: 6-15; 1994.

34. Nielsen, J.B.; Andersen, O. Time dependent disposition of mercury after oral dosage. In Merian, E; Haerdi, W., eds. Metal compounds in environment and life, vol. 4. Delware: Science Reviews; 1991: 341-348.

35. Ohi, G.; Nishigaki, S.; Seki, H.; Tamura, Y.; Mizoguchi, I.; Yagyu, H.; Nagashima, K. Tail rotation, an early neurological sign of methylmercury poisoning in the rat. Environ. Res. 16: 353-359; 1978.

36. Pelligrino, L.J.; Altman, J. Effects of differential interference with postnatal neurogenesis on motor performance, activity level and maze learning of rats: a developmental study. J. Comp. Physiol. Psycho. 93: 1-33; 1979.

37. Post, E.M.; Yang, M.G.; King, J.A.; Sanger, V.L. Behavioral changes of young rats force-fed methyl mercury chloride. Proc. Soc. Exp. Biol. Med. 143: 1113-1116; 1973.

38. Poucet, B. Object exploration, habituation, and response to a spatial change in rats following septal or medial frontal cortical damage. Behav. Neurosci. 103: 1009-1016; 1989.

39. Racz, W.J.; Vandewater, L.J.S. Perspectives on the central nervous system toxicity of methylmercury. Can. J. Physiol. Pharmacol. 60: 1037-1045; 1982.

40. Rice, D.C. Delayed neurotoxicity in monkeys exposed developmentally to methylmercury. Neurotoxicology 10: 645-650; 1989.

41. Rice, D.C. Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents. Neurotoxicology 17: 139-154; 1996.

42. Rice, D.C.; Gilbert, S.G. Early chronic low-level methylmercury poisoning in monkeys impairs spatial vision. Science 216: 759-761; 1982.

43. Rice, D.C.; Gilbert, S.G. Exposure to methylmercury from birth to adulthood impairs high-frequency hearing in monkeys. Toxicol. Appl. Pharmacol. 115: 6-10; 1992.

44. Rice, D.C.; Gilbert, S.G. Effects of developmental methylmercury exposure or lifetime lead exposure on vibration sensitivity function in monkeys. Toxicol. Appl. Pharmacol. 134: 161-169; 1995.

45. Save, E.; Buhot, M.-C.; Foreman, N.; Thinus-Blanc, C. Exploratory activity and response to spatial change in rats with hippocampal or posterior parietal cortical lesions. Behavi. Brain Res. 47: 113-127; 1992.

46. Save, E.; Poucet, B.; Foreman, N.; Buhot, M.-C. Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. Behav. Neurosci. 106: 447-456; 1992.

47. Su, M.-Q.; Okita, G.T. Behavioral effects on the progeny of mice treated with methylmercury. Toxicol. Appl. Pharmacol. 38: 195-205; 1976.

48. Thinus-Blanc, C.; Save, E.; Poucet, B.; Buhot, M.-C. The effects of reversible inactivations of the hippocampus on expo=loratory activity and spatial memory. Hippocampus 1: 365-372; 1991.

49. Thompson, R.F.; Kim, J.J. Memory systems in the brain and localization of a memory. Proc. Natl. Acad. Sci. USA 93: 13438-13444; 1996.

50. Thompson, R.F.; Krupa, D.J. Organization of memory traces in the mammalian brain. Ann. Rev. Neurosci. 17: 519-549; 1994.

51. White, N.M. Menmonic functions of the basal ganglia. Current Op. Neurobiol. 7: 164-169; 1997.

52. Wise, S. P. The role of the basal ganglia in procedural memory. Neurosci. 8: 39-46; 1996.

Table 1 - The analysis of simple main effects on weights and post hoc comparisons during the days of toxic exposure.

	F (3, 72)	p <	Newman-Keuls
Day 1	0.24	ns	
Day 3	0.21	ns	
Day 5	2.13	ns	
Day 7	3.37	.05	Placebo > 4 mg/kg
Day 9	6.35	.001	Placebo > 2mg/kg
			Placebo > 4 mg/kg
			1 mg/kg > 4 mg/kg
Day 11	9.15	.001	Placebo > 2 mg/kg
			Placebo > 4 mg/kg
			1 mg/kg > 4 mg/kg

Table 2 - The analysis of simple main effects and post hoc comparisons in the days following toxic exposure.

	F (3, 46)	p <	Newman-Keuls
Day 13	2.26	ns	
Day 15	2.82	ns	
Day 17	3.17	.05	no differences
Day 19	2.56	ns	
Day 21	2.89	.05	Placebo > 2mg/kg
			Placebo > 4 mg/kg
Day 23	3.31	.05	no differences
Day 25	3.45	.05	Placebo > 2 mg/kg
			Placebo > 4 mg/kg
Day 27	3.58	.05	Placebo > 2 mg/kg
Day 29	3.24	.05	Placebo > 2 mg/kg

Figure Captions

Fig. 1. Schematic representation of the apparatus used in the water maze DMTP.

Fig 2. Body weight of the four groups during toxic exposure (A) and in the rest of the experiment (B).

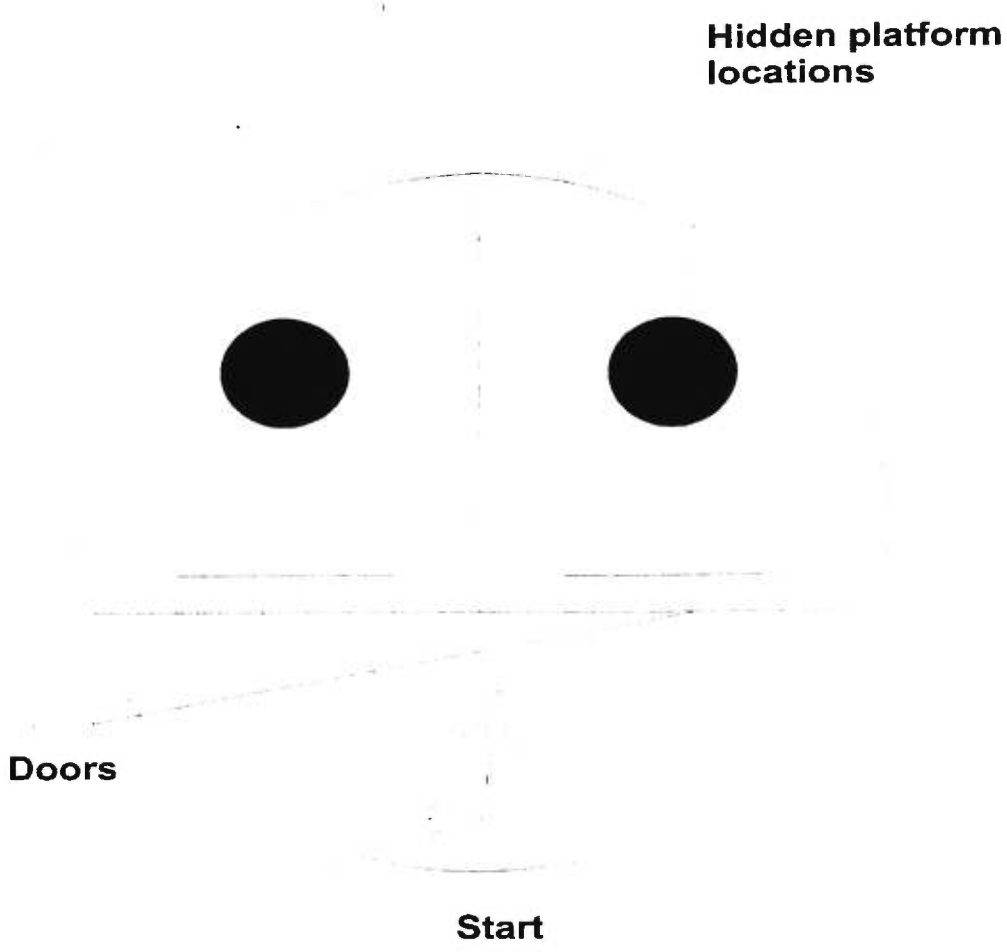
Fig. 3. Fall latency of the four groups on the rotarod task.

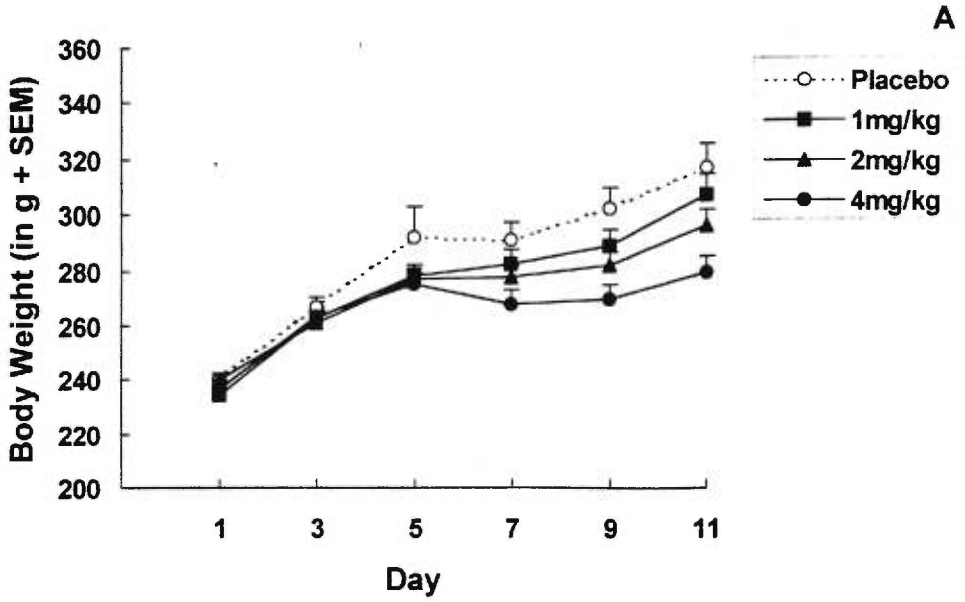
Fig 4. Performance of the four groups in the Morris water maze with the hidden platform (Day 4 to Day 13) and the visible platform (Day 14 and 15) in terms of escape latencies (A) and number of quadrant entries (B).

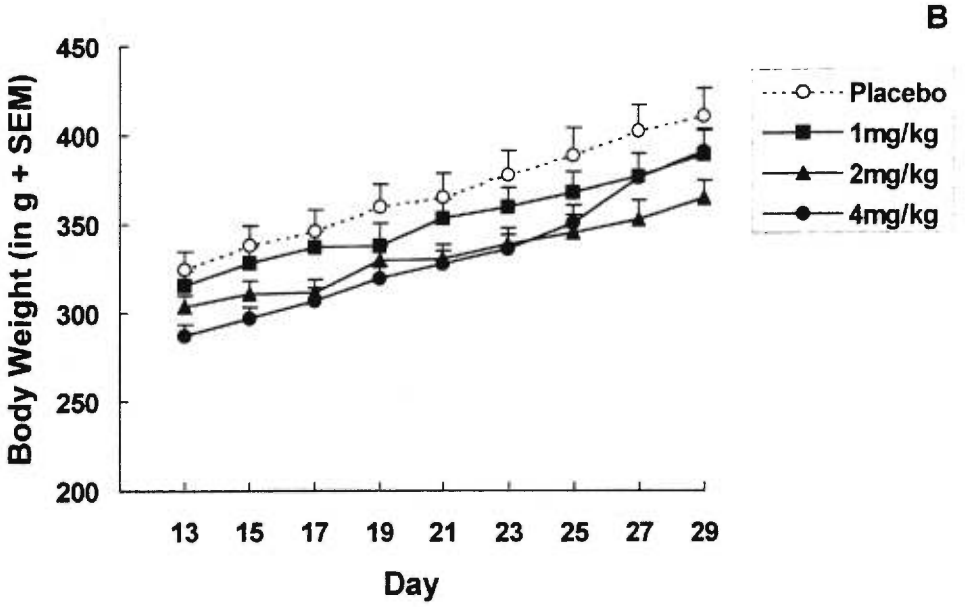
Fig. 5. Horizontal exploration (A), vertical exploration (B), and contacts with the objects (C) in the open field on the first and on the last day with the objects at fixed locations, and on the next day when objects' locations were switched.

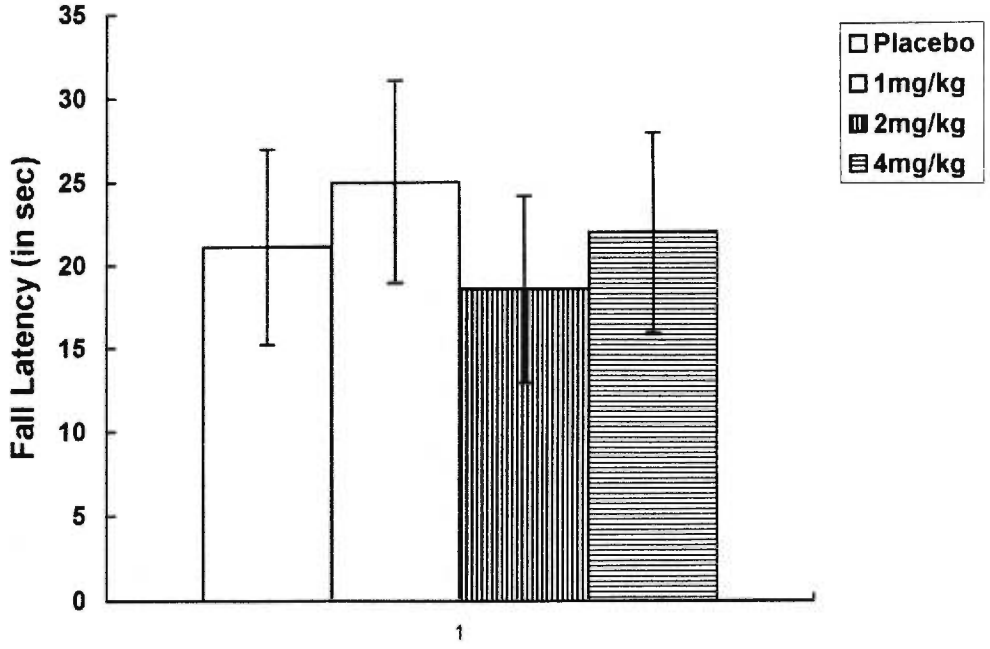
Fig. 6. Escape latencies of the four groups by blocks of 2 days during the sample runs of the water maze DMTP.

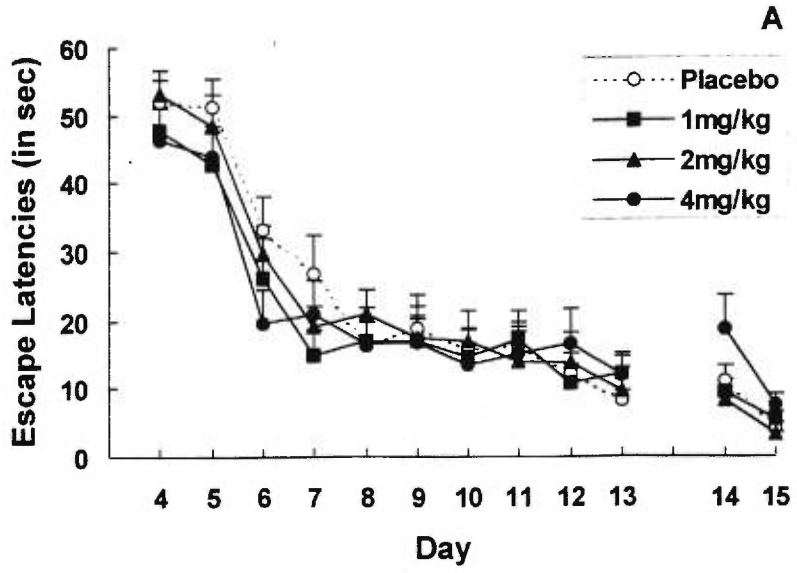
Fig. 7. Success rate of the four groups during test runs of the water maze DMTP.

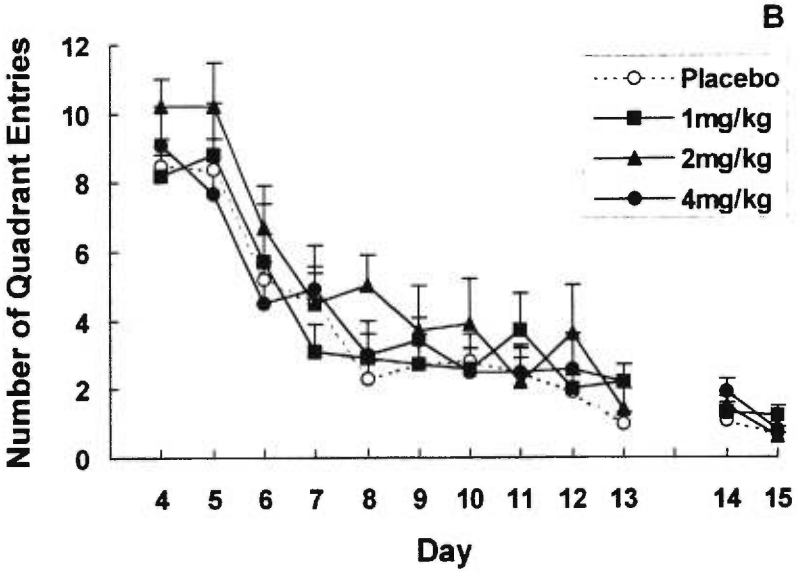

Hidden platform locations
Doors
Start

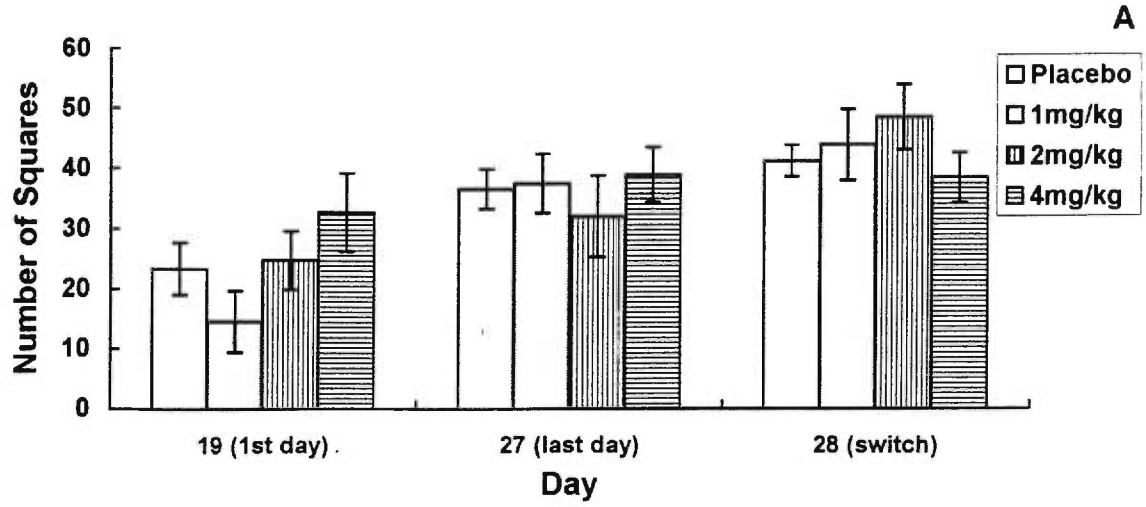

A
Body Weight (in g + SEM)
360
340
320
300
280
260
240
220
200
1
3
5
7
9
11
Day
Placebo
1mg/kg
2mg/kg
4mg/kg

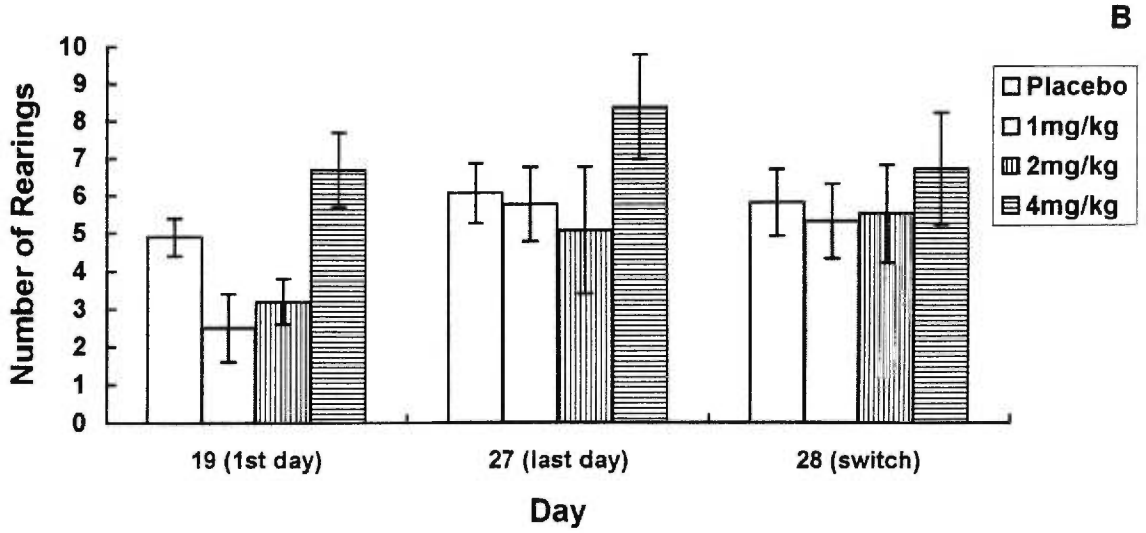

B
Body Weight (in g + SEM)
450
400
350
300
250
200
13
15
17
19
21
23
25
27
29
Day
Placebo
1mg/kg
2mg/kg
4mg/kg

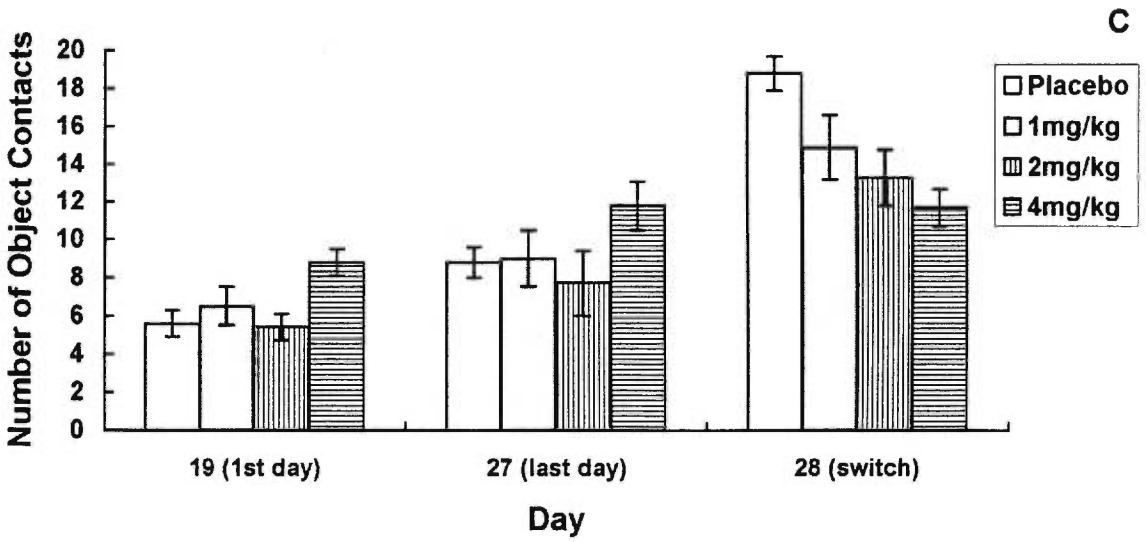

Fall Latency (in sec)
35
30
25
20
15
10
5
0
1
Placebo
1mg/kg
2mg/kg
4mg/kg

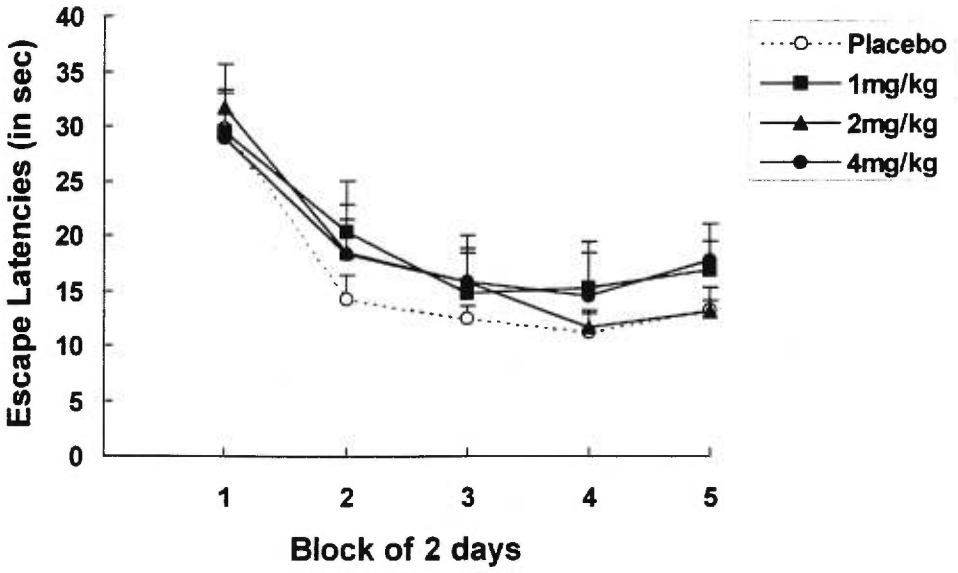

A
Escape Latencies (in sec)
60
50
40
30
20
10
0
Placebo
1mg/kg
2mg/kg
4mg/kg
4 5 6 7 8 9 10 11 12 13 14 15
Day

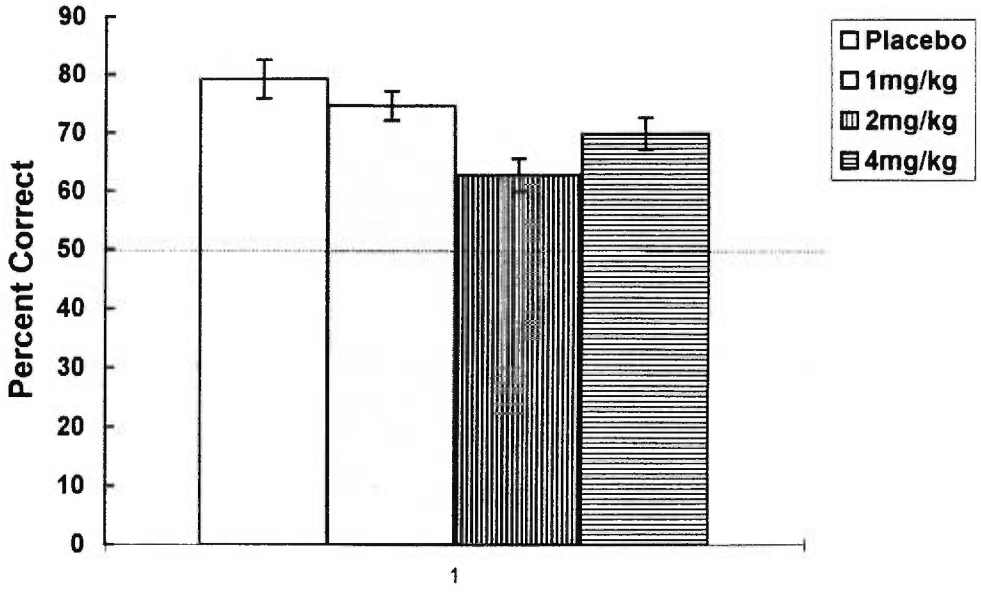

B
Number of Quadrant Entries
12
10
8
6
4
2
0
Placebo
1mg/kg
2mg/kg
4mg/kg
4 5 6 7 8 9 10 11 12 13 14 15
Day


A
Number of Squares
60
50
40
30
20
10
0
Placebo
1mg/kg
2mg/kg
4mg/kg
19 (1st day)
27 (last day)
28 (switch)
Day


B
Number of Rearings
10
9
8
7
6
5
4
3
2
1
0
Placebo
1mg/kg
2mg/kg
4mg/kg
19 (1st day)
27 (last day)
28 (switch)
Day


C
Number of Object Contacts
20
18
16
14
12
10
8
6
4
2
0
Placebo
1mg/kg
2mg/kg
4mg/kg
19 (1st day)
27 (last day)
28 (switch)
Day


Escape Latencies (in sec)
40
35
30
25
20
15
10
5
0
1
2
3
4
5
Block of 2 days
Placebo
1mg/kg
2mg/kg
4mg/kg


Percent Correct
90
80
70
60
50
40
30
20
10
0
1
Placebo
1mg/kg
2mg/kg
4mg/kg

2.2 L'exposition subaiguë postnatale au mercure méthylique perturbe la coordination motrice et des tâches d'apprentissage dans le labyrinthe aquatique chez des rats juvéniles

Soumis à *Behavioral Neuroscience*

sous le titre de

Subacute postnatal exposure to methylmercury impairs motor coodination and water maze learning tasks in juvenile rats

Subacute Postnatal Exposure to Methylmercury Impairs Motor Coordination and Water Maze Learning Tasks in Juvenile Rats

Stéphane Beaudin[1], Saroj K. Chakrabarti[2], François Y. Doré[3], and Maurice Ptito[1]

[1]Groupe de recherche en neuropsychologie expérimentale, Département de psychologie, Université de Montréal, Montréal, Qué., Canada H3C 3J7

[2]Département de médecine du travail et d'hygiène du milieu, Faculté de médecine, Université de Montréal, Montréal, Qué., Canada H3C 3J7

[3]École de psychologie, Université Laval, Québec, Qué., Canada G1K 7P4

Correspondence: Stéphane Beaudin
Department of Nutritional Sciences and Psychology
Savage Hall Building
Cornell University
Ithaca, NY

Running Head: Neurobehavioral Effects of Postnatal MeHg Exposure

Abstract

In the field of environmental toxicology, there is a need to identify early warning signs of MeHg toxicity before any irreversible damage to the nervous system could occur. This experiment examined the effects of postnatal subacute exposure to MeHg on motor coordination, Morris water maze, and water maze DMTP. Rats were treated po with 0, 2, 4, and 6 mg/kg/day of MeHg chloride for 10 consecutive days. Behavioral testing began after the 4^{th} treatment. Weight gain was depressed during exposure but no crossing of hind legs was noticed at any stage. Motor coordination and Morris water maze performance were impaired at a dose of 6 mg/kg whereas a clear deficit was observed at all dose levels on training to water maze DMTP. Post-acquisition of DMTP was also affected in MeHg-treated rats. These results are discussed with regard to the effects of MeHg intoxication on cholinergic and dopaminergic systems.

Methylmercury (MeHg) is probably the environmental neurotoxicant that has been the most studied for its detrimental effects on development. Since Spyker, Spaber & Goldberg's (1972) seminal article, animal models are used to test neurochemical, neuronal, and behavioral modifications resulting from prenatal exposure to MeHg. Consistent patterns of sensory and motor deficits have now been identified in rodents, primates, and humans (Burbacher, Rodier & Weiss, 1990; Rice, 1996; Satoh, 1991; Weiss & Elsner, 1996). Although individuals are at risk of intoxication at any age, the effects of postnatal exposure to MeHg on brain and behavior have not been studied as extensively as the effects of prenatal exposure. In rodents, the most salient effect is impairment of motor function. Crossing of hind legs on being held by the tail (Magos et al., 1981; 1985) and rotating movements of the tail (Ohi et al., 1978), which have been reported after exposure to high doses of MeHg (8 to 10 mg/kg), are considered typical signs of intoxication and of morphological changes in nerve cells. Decreased spontaneous motor activity (Kobayashi et al., 1981), impaired performance on the rotarod task (Berthoud, Garman & Weiss, 1976; Gilbert & Maurissen, 1982; Kobayashi et al., 1981), and increased hindlimb foot-spread (Gilbert & Maurissen, 1982) have also been reported.

The effects of prenatal or postnatal exposure to MeHg on learning and memory in rodents are not as well investigated as motor deficits and studies which found no effect of MeHg exposure on cognitive endpoints are preponderant (Rice, 1996). However, several lines of evidence from morphological studies suggest that exposure to MeHg might interfere with learning and memory processes. Cortex, hippocampus,

neostriatum, and cerebellum all undergo morphological changes after exposure to repeated doses of MeHg (Berthoud, Garman & Weiss, 1976; Burbacher et al., 1990; Diamond & Sleight, 1972; Magos et al., 1981). Neurochemical studies also support Kobayashi et al.'s hypothesis (1981) that behavioral changes observed after intoxication are related to hypofunctioning of the cholinergic system.

First, many of the cholinoceptive cells are cortical layer V pyramidal cells (Woolf, 1996) where MeHg accumulates (Moller-Madsen, 1990). Second, whereas acetylcholine release increases with learning (Park, Pappas, Murtha & Ally, 1992), inhibition of choline acetyltransferase (ChAT) has been repeatedly reported after acute, subacute, and chronic MeHg exposure with doses varying between 4 and 10 mg/kg (Dwidedi, Raghutan, Joshi & Foster, 1980; Okuda, Tsuzuki & Yamada, 1978; Omata et al., 1980). Third, MeHg has been found in vitro to inhibit muscarinic (Abd-Elfattah & Shamoo, 1981; Eldefrawi, Mansour & Eldefrawi, 1977; Von Burg, Northington & Shamoo, 1980) and nicotinic receptor (Eldefrawi et al., 1977) binding in brain tissue; it also preferentially associates with the M1 muscarinic receptor subtype (Castoldi et al., 1996). Fourth, inhibition of brain protein synthesis, which occurs during the latent stage of intoxication (Cavanagh & Chen, 1971; Yoshino, Mozai & Nakao, 1966), has been proposed as an essential step in the formation of long-term memory (Davis & Squire, 1984). Thus, because MeHg affects important brain structures and neurochemical processes that are involved in learning and memory, one can speculate that the preponderance of negative neurobehavioral results are due to a lack of test sensitivity and specificity. As acknowledged by Olton and

Markowska (1994), behavioral analysis of performance in appropriate mnemonic tasks is an important step in assessing the functional consequences of any neurotoxicant.

In a previous study (Beaudin, Chakrabarti, Doré & Ptito, submitted), the Morris water maze (Morris, Garrud, Rawling & O'Keefe, 1982) and the water-maze DMTP (Comer & Means, 1989; Means, 1988) were used to assess the effects of subacute postnatal exposure to MeHg on reference and working memory, respectively. Learning and memory tests in water have at least two advantages. One is that subjects do not have to be deprived, thus avoiding additional health hazards in MeHg exposed animals which already tend to lose weight. The other is that swimming, unlike walking, is not impaired in animals with severe motor problems such as ataxia (Lalonde & Botez, 1990; Pelligrino & Altman, 1979) or crossing of hind legs. When exposed to 2 and 4 mg/kg/day of MeHg po., but not to 1 mg/kg/day, juvenile rats were impaired on the water-maze DMTP but not on the Morris water maze. No crossing of hind legs was observed at these dose levels and motor coordination (rotarod task) as well as horizontal and vertical exploration in an open field were not affected by any of the three levels of exposure. These results suggested that MeHg might interfere with learning and memory processes, without overt motor signs.

A recent study (Loua, Chakrabarti & Durham, submitted) has shown that in juvenile rats exposed to MeHg at 2, 4, and 6 mg/kg/day for 10 days, the highest dose level decreased choline acetyltransferase (ChAT) activity significantly more than the other two doses. Also, exposure to MeHg at 6 mg/kg/day significantly depleted both

other two doses. Also, exposure to MeHg at 6 mg/kg/day significantly depleted both Ach and dopamine (DA) concentration whereas exposure to 4 mg/kg/day reduced only Ach concentration (Charkrabarti, Loua & Durham, submitted; Loua, et al., submitted). The present experiment was aimed at determining whether spatial learning in the Morris water maze would be impaired, like the water-maze DMTP, at a higher level of postnatal exposure to MeHg.

Method

Subjects

Sprague-Dawley male rats (Charles River Canada Inc., St-Constant, Québec), approximately 60-day old and weighing 250 g at the start of the experiment, served as subjects. They were housed individually in a standard Plexiglass cage and placed in a room maintained at a constant temperature ($21\pm3^{o}C$) and on a standard 12:12 light/dark cycle. Purina laboratory chow and demineralized water were available ad libitum throughout the experiment.

Neurotoxic Exposure

Methylmercury chloride (MeHgCl) (purity over 99%) was obtained from Johnson Matthey Company (Ward Hill, MA, USA). Naive rats were randomly assigned to three treatment groups and a placebo group (n = 10). Treatment groups received by gastric gavage a dose of 2, 4 or 6 mg/kg/day of MeHgCl for 10 consecutive days, for a total cumulative dose of 20, 40, or 60 mg/kg. The compound

was dissolved in a 0.9% saline solution and administered in a volume of 4 ml/kg. The placebo group received an equal volume of vehicle only. Body weights were monitored at 2-day intervals during MeHg exposure and every 5 days, thereafter. The animals were observed periodically for any crossing of hind legs.

Behavioral Testing

Behavioral testing started on the 4th day of treatment because steadily increasing accumulation of methylmercury in the brain is known to occur after 72 hours (Klaassen, 1986). The tasks were administered into two stages separated by a 3-day rest period. Testing of motor coordination in the rotarod task and of spatial learning in the Morris water maze began on Day 4 and lasted 7 and 12 consecutive days, respectively. The rest period occurred from Day 16 to Day 18. Then, from Day 19 to Day 49, acquisition and retention of the water-maze DMTP were tested. All tasks were administered between 11:00 and 20:00 h and subjects were individually tested in squads of five. Throughout the study, the experimenter was unaware of the group to which the subjects belonged.

Rotarod Task - Motor coordination was tested in the rotarod. The apparatus was made of a horizontal cylinder (diameter: 8.5 cm; ; length: 30 cm) driven by a DC motor; the rotation speed (20 rpm) was controlled by a speed motor driver and variable size gears. Wooden side walls served to confine the rat in an area of 20 cm in width. The apparatus was placed at a height of 90 cm from the cushion-covered floor. A trial always started by gently placing the rat on the rotarod in the direction opposite to the

trial, it was returned immediately to its home cage. Fall latency was measured with a stop watch. One daily trial was administered for 7 consecutive days.

Morris Water Maze - The usual procedure to hide the platform in the Morris water maze is to dilute powdered milk into the water. Instead, we used a black swimming pool and a black platform, the absence of contrast making the submerged platform invisible. The swimming pool was a circular plastic tank (200 cm in diameter with 60 cm high walls) and was filled to a height of 32 cm with 23° C water. It occupied the center of the experimental room. Four starting points around the perimeter of the pool were arbitrarily designated North, East, South, and West and on this basis, the pool was divided into four quadrants (NE, NW, SE, SW). The platform was a circular Plexiglass stand (9.0 cm in diameter and 30 cm high) covered with a grid to facilitate clinging and climbing. The platform, which was submerged 2 cm below the surface of the water, occupied the center of the NW quadrant throughout testing. Extramaze cues were provided by the door of the experimental room, a cupboard, four posters, and the experimenter who was standing between the SE and SW quadrants. The task consisted of two training conditions: spatial learning with the hidden platform and control trials with the visible platform.

Spatial learning lasted 10 consecutive days with 2 trials/day for a total of 20 trials. The two daily trials were separated by an interval of 25 sec. This training regimen has been shown to be especially sensitive to lesion-induced deficits (Mandel, Gage & Thal, 1989). A trial always started by lowering the rat in the water while

facing the wall at the starting point. Four starting points (N, S, E, W) were used randomly over two days. Once the hidden platform was reached, the rat was allowed to remain on it for 15 sec. If the platform was not found within 60 sec, the rat was placed manually on the platform for 15 sec. Escape latency and the number of quadrants crossed were recorded.

In the two days following spatial learning, control trials with the visible platform were presented. The procedure was the same with two exceptions: 1) control trials lasted 2 days (4 trials) instead of 10; and 2) a white platform emerging 2 cm from the surface of the water was placed in the SE quadrant. Escape latency and the number of quadrants crossed were also recorded.

Water maze DMTP - The water-maze DMTP has been developed by Means (1988; Comer & Means, 1989) to test working memory (Figure 1). A circular pool surrounded by extramaze cues is divided into radial thirds. A position at the center of the arc forming the perimeter of one section serves as the starting point for all trials. The other two sections (goals) are separated from the starting section by doors. In the center of one of the goal sections, a platform is submerged. Each trial is divided into a sample run and a test run. The sample run is a forced choice: the door of the section containing the hidden platform is opened whereas the door of the other goal section is closed. The rat is placed into the water and allowed to swim until it reaches the platform. Then, the rat is removed from the pool and after a delay, the test run (free choice) is presented. The hidden platform is in the same section as in the sample run

choice) is presented. The hidden platform is in the same section as in the sample run (DMTP) but this time the doors of the two goal sections are opened. Again the rat is placed in the pool and allowed to reach and climb onto the platform.

According to Means (1995), in the water maze DMTP, rodents have a strong unlearned bias to return to the location where they escaped on the last trial. Forced-choice run (information or sample trial), a barrier between sections to prevent short cuts, and detention following incorrect test run choices (free choice run) are all necessary to counteract this bias. Although counterintuitive and inconsistent with findings in appetitive working memory procedures, performance on test runs given either immediately or after a delay longer than 1 min is better than on test runs with a delay of 1 min (Means, 1995). The enhancing effect of longer retention intervals is explained by nonmemory hypotheses: longer escape from the water provides a more effective reward as well as the needed time to recover from the emotional effects of the sample run.

Training was conducted in the same swimming pool as the one used in the Morris water maze task. A T-shaped opaque plastic insert (height: 51 cm; stem: 105 cm long; bar: 72 cm) divided the pool into a starting section (maximal width: 35 cm) and two equal goal sections (maximal width: 17.5 cm) on the left and right of the starting section. The doors to the goal sections were 20-cm wide. A black circular platform (diameter: 9 cm) was placed in the correct goal section and submerged 2 cm beneath the surface of water. The extramaze cues used in the Morris water maze task

were removed and replaced at the exact same locations by four dim spotlights (two 75 and two 40 W bulbs). Therefore, rats had to learn the spatial relationships between new extramaze cues which were made more salient because water maze DMTP has proven to be especially difficult for rats exposed to MeHg (Beaudin et al., submitted).

Since normal rats can attain a criterion of 9 correct choices out of 10 consecutive trials in less than 20 trials (Means & Kennard, 1991), subjects first received 20 trials. Those that did not reach criterion (9 correct choices out of 10) were trained for a maximum of 20 additional trials. During the sample run, the rat was allowed 60 sec to enter the correct goal section and to escape onto the hidden platform where it was left for 15 sec. If the platform was not found within 60 sec, the rat was gently guided to it and allowed to remain on it for 15 sec. During the test run, the rat had also 60 sec to choose the correct goal section and escape. If it chose the wrong goal section, the door of this section was closed and re-opened after a 30-sec delay to allow a correct choice (correction procedure). At the beginning of sample and test runs, the rat was placed in the pool facing the outside wall of the starting section. The sample run and the test run were separated by a 2-min retention interval during which the rat was returned to its home cage. The correct goal section was varied semirandomly so that it was never the same for more than two consecutive trials and overall, it was on the right and the left of the starting section an equal number of times. The two daily trials were separated by 3 hours during which the rat was returned to its home cage and room. On sample runs, escape latency was calculated from release in the water to climbing onto the hidden platform; the 30-sec delay during which the rat

Animals that reached criterion within 40 trials were tested on post-acquisition for 10 consecutive days with 5- and 15-min intervals between the sample and the test run. The procedure was the same as in acquisition. Again, animals received 2 trials/day with one trial with each interval. The intervals were never the same for more than two consecutive days.

ANOVAs (Treatment x Session or Block of trials) with repeated measures on the second factor were used for the rotarod task, the Morris water maze, and the water maze DMTP data. Simple main effects were analyzed when the interaction was significant (Satterthwhaite's Mean Square of Error and df) and post hoc comparisons were made with the Newman-Keuls' test. One-way Anovas were used when appropriate. The level of significance was .05 unless specified otherwise.

Results

General Health Parameters

Three rats from Group 2mg/kg, one from Group 4 mg/kg, and one from Group 6 mg/kg died early in the toxic exposure stage of the experiment, probably as a result of bad intubation during gastric gavage. Crossing of hind legs did not appear in any rat from the treatment groups during exposure to MeHg or in the following days of testing, thus confirming that the dose levels used did not result in overt motor deficits.

Results for weight gain were divided into two stages: from the first day of toxic exposure to the day after the last gastric gavage (Day 1 to 11), and from the sixth day

after the last gavage to the end of the experiment (Day 16 to 51). During the period of toxic exposure (Figure 2a), the weights of Group 4 mg/kg and Group 6 mg/kg were stable and did not increase whereas Group 2 mg/kg gained weight but at a slower rate than the Placebo group. The ANOVA showed that the factor Group, $F(3, 31) = 7.66$ $p < .0006$, the factor Day, $F(5, 155) = 5.10$ $p < .0002$, and the interaction, $F(15, 155) = 5.72$ $p < .0001$, were significant. The analysis of simple main effects revealed that at the beginning of exposure, that is, on Day 1, $F(3, 66) = .34$, and on Day 3, $F(3, 66) = 1.55$, the groups did not differ. The effect of MeHg intoxication on body weight began to appear on Day 5, $F(3, 66) = 4.50$ $p < .01$, and this difference between the groups was also significant on the following days (Day 7: $F(3, 66) = 9.68$ $p < .001$; Day 9: $F(3, 66) = 13.43$ $p < .001$; Day 11, $F(3, 66) = 12.57$ $p < .001$). Post hoc comparisons revealed that the weights of Group 4 mg/kg and Group 6 mg/kg were lower than the weight of the Placebo group on Day 5, 7 and 11 whereas on Day 9, they were lower than the weights of Group 2 mg/kg and Placebo. Although the weights still differed five days after the last gastric gavage (Figure 2b), all groups gained weight in the following weeks (Group: $F(3, 31) = 10.35$ $p < .0001$; Day: $F(7, 217) = 437.90$ $p < .0001$; Group x Day: $F(21, 217) = 1.9$ $p < .05$). Results from the period of toxic exposure and from the following weeks suggest that depressed weight gain in treated rats is caused by gastric irritation and anorexia rather than by a general deterioration of physiological functions.

Rotarod Task

The rotarod task was especially sensitive because even in Placebo rats, the maximal fall latency did not exceed 80 sec (Figure 3). Except for Group 6 mg/kg, motor coordination improved across days. Although the factor group was not significant, $F(3, 31) = 2.04$, the factor Day, $F(6, 186) = 12.37$ $p < .0001$, and the interaction, $F(18. 186) = 1.92$ $p < .05$, were significant. The analysis of simple main effects showed that the groups significantly differed on Day 6, $F(3, 123) = 3.43$ $p < .05$, and on Day 7, $F(3, 123) = 4.15$ $p < .01$, but not on the preceding days. Post hoc comparisons indicated that only the fall latency of Group 6 mg/kg was inferior to the one of the Placebo group.

Morris Water Maze

On the spatial learning test with the hidden platform, the escape latency of all groups decreased across daily sessions (Figure 4a) from 50 sec to 10 sec. The ANOVA confirmed that performance improved across days, $F(9, 279) = 61.36$ $p < .0001$, but the factor Group, $F(3, 31) = 0.45$, and the interaction, $F(27, 279) = 0.72$, were not significant. Similarly, the number of quadrant entries decreased from 10 to 1 over the 10 days of testing. The ANOVA indicated that not only the factor Day, $F(9, 279) = 46.67$ $p < .0001$, but also the factor Group, $F(3, 31) = 4.79$ $p < .01$, were significant, with no Group x Day interaction, $F(27, 279) = 0.92$. Post hoc comparisons revealed that the number of quadrant entries was higher in Group 6 mg/kg than in the Placebo group. In other words, rats from Group 6 mg/kg crossed more quadrants than Placebo rats in the same amount of time. In fact, whereas Group Placebo, 2 mg/kg, and 4

mg/kg crossed 4.5, 4.4, and 5.5 quadrants/min, respectively, Group 6 mg/kg crossed 7.2 quadrants/min.

Performance on sessions with the visible platform (Day 14 and 15) showed that toxic exposure did not impair the basic visuo-motor abilities required to perform the Morris water maze. All groups improved on the second day on this task in terms of escape latencies (Group: $F(3, 31) = 2.35$; Day: $F(1, 31) = 43.67$ $p < .0001$; Group x Day: $F(3, 31) = .93$) as well as of quadrant entries (Group: $F(3, 31) = 1.11$; Day: $F(1, 31) = 37.42$ $p < .0001$; Group x Day: $F(3, 31) = 1.42$) but the factor Group and the interaction were not significant.

Water Maze DMTP

During the first 20 trials of training to criterion, escape latencies on sample runs (Figure 5) were recorded as an additional index of visuo-motor abilities. The results confirmed that the groups did not differ on this basic behavioral measurement. The ANOVA showed that escape latencies during sample runs decreased across blocks of 2 days, $F(4, 124) = 34.39$ $p < .0001$, but the factor Group, $F(3, 31) = 0.69$, and the interaction, $F(12, 124) = 0.54$, were not significant.

On training to water maze DMTP (Figure 6), Placebo rats generally reached criterion within the first 20 trials whereas MeHg-treated groups required additional training trials. The one-way ANOVA showed a significant group difference, $F(3, 31) = 5.20$ $p < .005$, in terms of the number of trials to reach a criterion of 9 successes out of

5.20 $p < .005$, in terms of the number of trials to reach a criterion of 9 successes out of 10 consecutive trials. Post hoc comparisons indicated that all three treated groups required more trials than the Placebo group. Whereas all Placebo rats reached criterion, only 5/7 rats from Group 2 mg/kg, 7/9 rats from Group 4 mg/kg, and 3/9 from Group 6 mg/kg were able to complete training.

Because all treated groups were impaired and because of the small number of treated rats which completed training, data from these groups were pooled in the analysis of the post-acquisition testing. Although MeHg rats reached criterion on training and their performance on post-acquisition testing was significantly superior to the level expected by chance (5-min delay: $t(14) = 3.9$ $p < .002$; 15-min delay: $t(14) = 2.6$ $p < .05$), they were clearly impaired when sample and test runs were separated by either a 5-min or a 15-min delay (Figure 7). The ANOVA confirmed that the factor Group, $F(1, 23) = 9.98$ $p < .005$, was significant but the factor Delay, $F(1, 23) = 0.49$, and the interaction, $F(1, 23) = 2.78$, were not. The comparison of MeHg rats at the 5- and 15-min did not reach the level of significance, $t(14) = 1.75$ $p = .051$, but it was very close.

Discussion

The present results have shown that behavioral, learning, and memory impairments can be detected after postnatal subacute exposure to low and moderate doses of MeHg. These impairments can serve as early signs of intoxication when the general health of the animal and basic sensory and motor functions are not overtly

affected. In fact, none of our exposed animals displayed crossing of hind legs, which is a typical motor indication of severe toxicity. Weight gain was depressed during toxic exposure, especially at doses of 4 and 6 mg/kg, but this effect was short-lived and was probably related to gastric irritation and anorexia as suggested by increased weight in all groups in the following weeks. Visuo-motor abilities did not seem to be impaired in the treated groups as shown by performance during control trials in the Morris water maze with the visible platform and in sample runs of training to water maze DMTP.

Basic motor coordination seemed intact in exposed rats: they did not differ from placebo rats in the first five sessions on the rotarod task. However, motor coordination in Group 6 mg/kg did not improve across sessions as much as in the other groups and therefore, their fall latencies were shorter on the last two sessions. This poorer performance coincided with the last two treatments. Whereas the maximal cumulative dosage received by the other groups did not exceed 40 (Group 4 mg/kg) and 20 (Group 2 mg/kg) mg, it reached 54 mg on Day 9 and 60 mg on Day 10 in Group 6 mg/kg. Thus, motor coordination was disturbed at high levels of MeHg in the organism which is consistent with an earlier report (Sakamato et al., 1993).

On the sole basis of escape latencies, spatial learning in the Morris water maze was not impaired by exposure to MeHg. However, Group 6 mg/kg crossed more quadrants than Group Placebo before finding the hidden platform and thus, they did not learn the optimal spatial strategy. Navigational deficits in place learning tasks such as the Morris water maze are generally associated with a dysfunction of the

hippocampal formation (Jarrard, 1993; Morris et al, 1982). In fact, the hippocampal system is one of the brain areas where total Hg especially accumulates (Chakrabarti, Loua & Durham, 1998). Neuronal degeneration and separation in the pyramidal fields were observed at high doses of MeHg (Diamond & Sleight, 1972). Also, according to Moller-Madsen (1994), mercury can be detected in the entorhinal cortex and hippocampal formation after 16 and 20 days, respectively, of exposure to a liquid dose of MeHg (20 mg/l). It seems that after exposure to 6 mg/kg during 10 days, the hippocampal areas begin to be affected and hippocampal function, although not fully impaired, is compromised.

In the present study, the most sensitive test of learning and memory was the water maze DMTP. All treated groups were impaired on acquisition of the task with a 2-min delay; Group 6 mg/kg was the most affected since only 3 rats out of 9 reached criterion, compared to 5 out of 7 in Group 2 mg/kg and 7 out of 9 in Group 4 mg/kg. A deficit in MeHg rats was also observed in post-acquisition testing, the deficit being more pronounced with a 15-min delay than a 5-min delay. These results are consistent with recent studies on the crucial role of cholinergic-dopaminergic interaction on spatial working memory (Kim & Levin, 1996; Levin, McGurk, Rose & Butcher, 1990; McGurk, Levin & Butcher, 1988), as well as with recent neurochemical analyses of the effect of MeHg on cholinergic and monoaminergic systems (Chakrabarti et al., submitted; Loua et al., submitted). At a dose level of 6 mg/kg, acetylcholine and dopamine are depleted in all brain areas whereas at a dose of 4 mg/kg, only acetylcholine is depleted. On the other hand, behavioral studies have shown that

systemic administration of the DA D2 agonist quinpirole potentiated the amnestic effect of the ACh antagonist mecamylamine on choice accuracy in the radial arm maze (Kim & Levin, 1996).

In conclusion, the present results and a prior report (Beaudin et al., submitted) have confirmed that sensitive and specific learning and memory tasks can potentially serve to identify early signs of MeHg intoxication. Although the direct relationship between the neural systems most affected by exposure to MeHg and learning and memory deficits remains to be exactly established, the results of the neurobehavioral experiments may have the potential to signal the progression of neural degeneration in identified cases of intoxication and hence, to prevent irreversible damage to the nervous system.

Acknowledgments

This article includes portions of a doctoral dissertation submitted by Stéphane Beaudin in partial fulfillment of the requirements for the doctoral degree. The research was supported in part, by Grants from the Medical Research Council of Canada (MRC), Hydro-Québec, Bay James Committee on MeHg and Canadian Electricity Association. Stéphane Beaudin was supported by Fonds pour la Formation des Chercheurs et l'Aide à la Recherche of Gouvernement du Québec (FCAR). The present research protocol was approved by the Comité de protection des animaux de laboratoire de l'Université de Montréal, in accordance with the rules of the Canadian Council on Animal Care. We thank Mr. Chengiang Bai for his assistance in gastric gavage.

References

Abd-Elfattah, A.-S. A. & Shamoo, A.E. (1981). Regeneration of a functionally active rat brain muscarinic receptor by d-penicillamine after inhibition with methylmercury and mercuric chloride. Molecular Pharmacology, 20, 492-497.

Beaudin, S., Doré, F.Y., Chakrabarti, S.K. & Ptito, M. (submitted). Postnatal exposure to methylmercury: Effects on behavior, learning, and memory in juvenile rats.

Berthoud, H.R., Garman, R.H. & Weiss, B. (1976). Food intake, body weight, and brain histopathology in mice following chronic methylmercury treatment. Toxicology and Applied Pharmacology, 36, 19-30.

Burbacher, T.M., Rodier, P.M. & Weiss, B. (1990). Methylmercury developmental neurotoxicity: A comparison of the effects in humans and animals. Neurotoxicology and Teratology, 12, 191-202.

Castoldi, A.F., Candura, S.M., Costa, P., Manzo, L. & Costa, L.G. (1996). Interaction of mercury compounds with muscarinic receptor subtypes in the rat brain. NeuroToxicology, 17, 735-742.

Cavanagh, J.B. & Chen, F.C.K. (1971). Amino acid incorporation in protein during the silent phase before organo-mercury and P-bromophenylacetylurea neuropathy in the rat. Acta Neuropathologica, 19, 216-224.

Comer, T.R. & Means, L.W. (1989). Overcoming unlearned response biases: Delayed escape following errors facilitates acquisition of a win-stay and win-shift water-escape tasks in rats. Behavioral and Neural Biology, 52, 239-250.

Davis, H.P. & Squire, L.R. (1984). Protein synthesis and memory: A review. Psychological Bulletin, 96, 518-559.

Durham, H., Loua, K. M. Chakrabarti, S. Modulation of neurotransmitter amines and their metabolites and monoamines oxidase activity in different brain regions following repeated exposure of rats to methylmercury. Toxicologist, 952, 1998.

Diamond, S.S. & Sleight, S.D. (1972). Acute and subchronic methylmercury toxicosis in the rat. Toxicology and Applied Pharmacology, 23, 197-207.

Dwidedi, C., Raghunathan, R., Joshi, B.C. & Foster, H.W. (1980). Effect of mercury compounds on cholinacetyl transferase. Research Communications in Chemical and Pathological Pharmacology, 30, 381-384.

Eldefrawi, M.E., Mansour, N.A. & Eldefrawi, A.T. (1977). Interactions of acetylcholine receptors with organic mercury compounds. Advances in Experimental Medical Biology, 84, 449-463.

Gilbert, S.G. & Maurissen, J.P.J. (1982). Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice. Journal of Toxicology and Environmental Health, 10, 31-41.

Jarrard, L. E. (1993). On the role of the hippocampus in learning and memory in the rat. Behavioral and Neural Biology, 60, 9-26.

Kim, J.S. & Levin, E.D. (1996). Nicotinic, muscarinic and dopaminergic actions in the ventral hippocampus and the nucleus accumbens: effects on spatial working memory in rats. Brain Research, 725, 231-240.

Kobayashi, H., Yuyama, A., Matsuaka, N., Takeno, K. & Yanagiva, I. (1981). Neuropharmacological effect of methylmercury in mice with special reference to the central cholinergic system. Japan Journal of Pharmacology, 31, 711-718.

Lalonde, R.& Botez, M.I. (1990). The cerebellum and learning processes in animals. Brain Research Review, 15, 325-332.

Levin, E.D., McGurk, S.R., Rose, J.E. & Butcher, L.L. (1990). Cholinergic-dopaminergic interactions in cognitive performance. Behavioral and Neural Biology, 54, 271-299.

Loua, K.M., Chakrabarti, S.K. & Durham, H. Modulation of cholinergic system in different brain regions following repeated exposure of rats to methylmercury. Toxicologist, 953, 1998.

Magos, L., Brown, A.W., Sparrow, S., Bailey, E., Snowden, R.T. & Skipp, W.R. (1985). The comparative toxicity of ethyl- and methylmercury. Archives of Toxicology, 57, 260-267.

Magos, L., Peristianis, G.C., Clarkson, T.W., Brown, A., Preston, S., & Snowden, R.T. (1981). Comparative study of the sensitivity of male and female rats to methylmercury. Archives of Toxicology, 48, 11-20.

Mandel, R.J., Gage, F.H. & Thal, L.J. (1989). Enhanced detection of nucleus basalis magnocellularis lesion-induced spatial learning deficit in rats by modification of training regimen. Behavioral Brain Research, 31, 221-229.

McGurk, S.R., Levin, E.D. & Butcher, L.L. (1988). Cholinergic-dopaminergic interactions in radial-arm maze performance. Behavioral and Neural Biology, 49, 234-239.

Means, L.W. (1988). Rats acquire win-stay more readily than win-shift in a water-escape situation. Animal Learning & Behavior, 16, 303-311.

Means, L.W. & Kennard, K.J.P. (1991). Working memory and the aged rat: Deficient two-choice win-stay water-escape acquisition and retention. Physiology & Behavior, 49, 301-307.

Moller-Madsen, B. (1990). Localization of mercury in the CNS of the rat. II. Intraperitoneal injectin of methylmercury chloride (CH_3HgCl) and mercuric chloride ($HgCl_2$). Toxicology and Applied Pharmacology, 103, 303-323.

Moller-Madsen, B. (1994). Localization of mercury in CNS of the rat. Pharmacology and Toxicology, 75, Supplement 1.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P. & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature, 297, 681-683.

Ohi, G., Nishigaki, S., Seki, H., Tamura, Y., Mizoguchi, I., Yagyu, H. & Nagashima, K. (1978). Tail rotation, an early neurological sign of methylmercury poisoning in the rat. Environmental Research, 16, 353-359.

Okuda, J., Tsuzuki, Y. & Yamada, T. (1978). Some neurochemical changes in the brain of rats with acute intoxication of methylmercury chloride. Japanese Journal of Legal Medecine, 32, 51-58.

Olton, D.S. & Markowska, A.L. (1994). Memory and hippocampal function as targets for neurotoxic substances. Neurotoxicology, 15, 439-444.

Omata, S., Hirakawa, E., Daimon, Y., Uchiyama, M., Nakashita, H., Horigome, T., Sugano, I. & Sugano, H. (1982). Methylmercury-induced changes in the activities

of neurotransmitter enzymes in nervous tissues of the rat. Archives of Toxicology, 51, 285-294.

Park, G.A.S., Pappas, B.A., Murtha, S.M. & Ally, A. (1992). Enriched environment primes forebrain choline acetyltransferase activity to respond to learning experience. Neuroscience Letters, 143, 259-262.

Pelligrino, L.J. & Altman, J. (1979). Effects of differential interference with postnatal neurogenesis on motor performance, activity level and maze learning of rats: a developmental study. Journal of Comparative and Physiological Psychology, 93, 1-33.

Rice, D.C. (1996). Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents. NeuroToxicology, 17, 139-154.

Sakamato, M., Nakano, A., Kajiwara, Y., Naruse, I. & Fujisaki, T. (1993). Effects of methyl mercury in postnatal developing rats. Environmental Research, 61, 43-50.

Satoh, H. (1991). Behavioral toxicology of mercury compounds. In T. Suzuki, (Eds.), Advances in Mercury Toxicology (pp. 367-379). New York: Plenum Press.

Spyker, J.M., Spaber, S.B. & Goldberg, A.M. (1972). Subtle consequences of methylmercury exposure: Behavioral deviation in offspring of treated mothers. Science, 18, 621-623.

Von Burg, R., Northington, F.K. & Shamoo, A. (1980). Methylmercury inhibition of rat brain muscarinic receptors. Toxicology and Applied Pharmacology, 53, 283-292.

Weiss, B. & Elsner, J. (Eds) (1996). Risk assessment for neurobehavioral toxicity. Environmental Health Perspectives, 104, Supplement 2.

Woolf, N.J. (1996). The critical role of cholinergic basal forebrain neurons in morphological change and memory encoding: A hypothesis. Neurobiology of Learning and Memory, 66, 258-266.

Yoshino, Y., Mozai, T. & Nakao, K. (1966). Distribution of mercury in the rat brain and its subcellular units in experimental organic mercury poisoning. Journal of Neurochemistry, 13, 397-406.

Figure Captions

Figure 1. Schematic representation of the swimming pool used in the water maze DMTP.

Figure 2. Body weights during toxic exposure (A) and in the rest of the experiment (B).

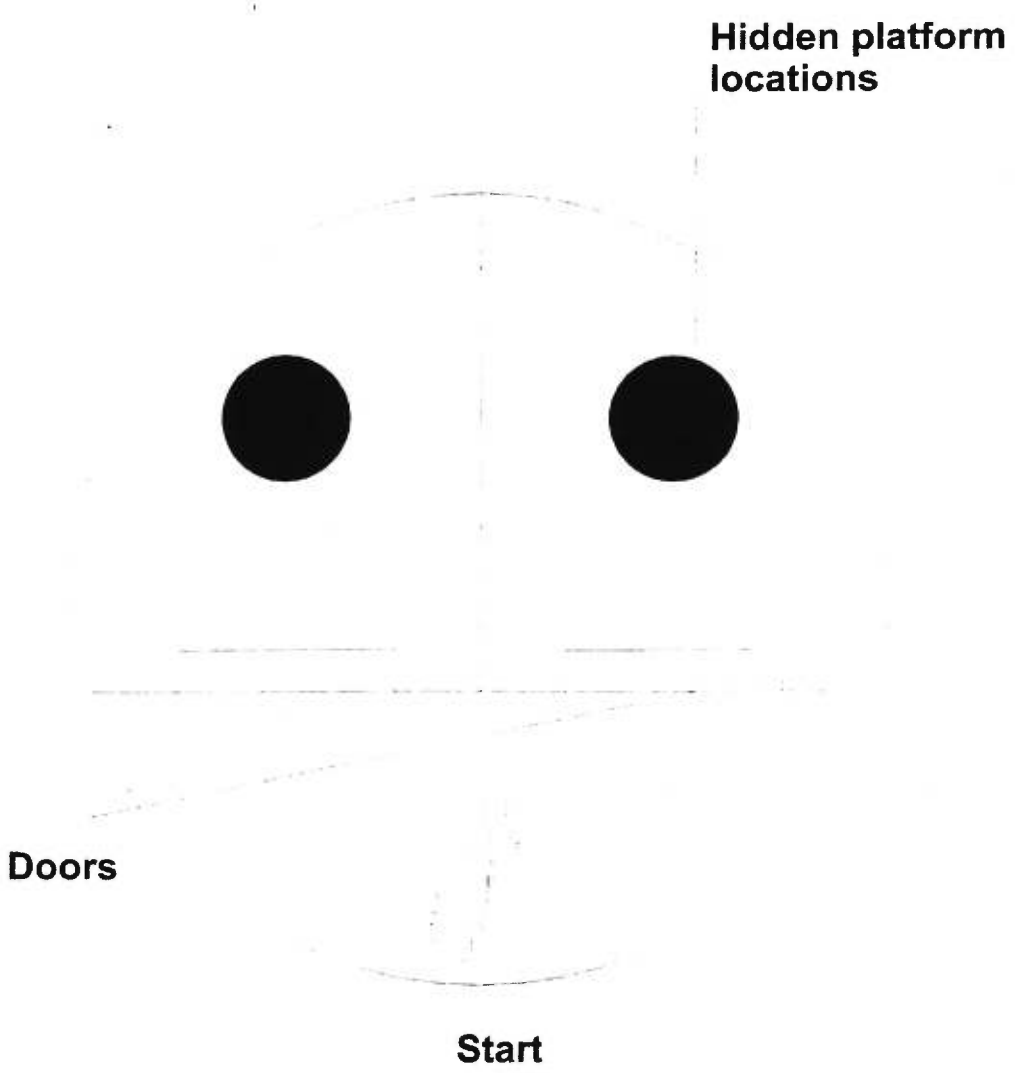
Figure 3. Fall latency on the rotarod task on each daily session.

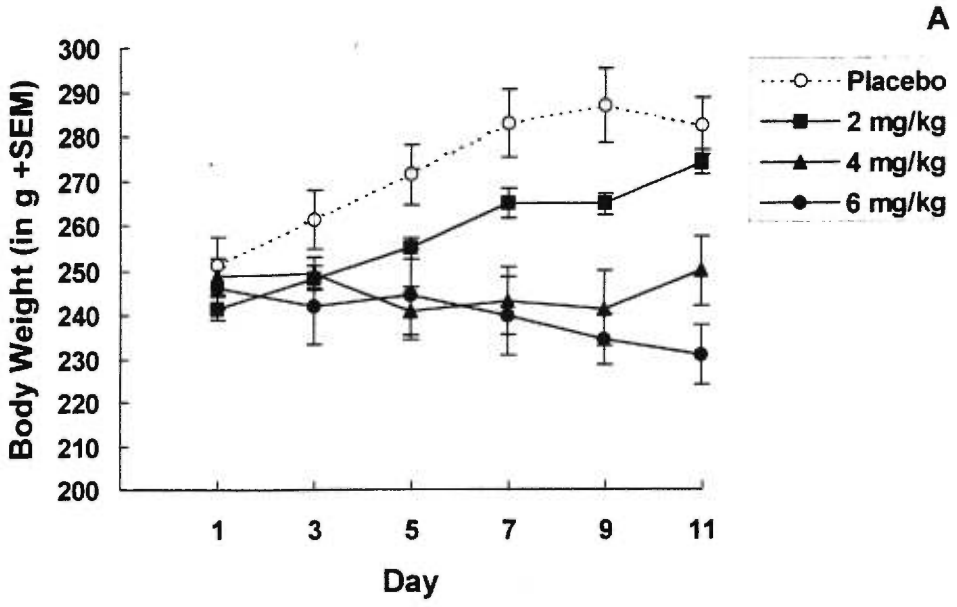
Figure 4. Escape latency (A) and number of quadrant entries (B) in the Morris water maze with the hidden platform (Day 4 to 13) and with the visible platform (Day 14 and 15).

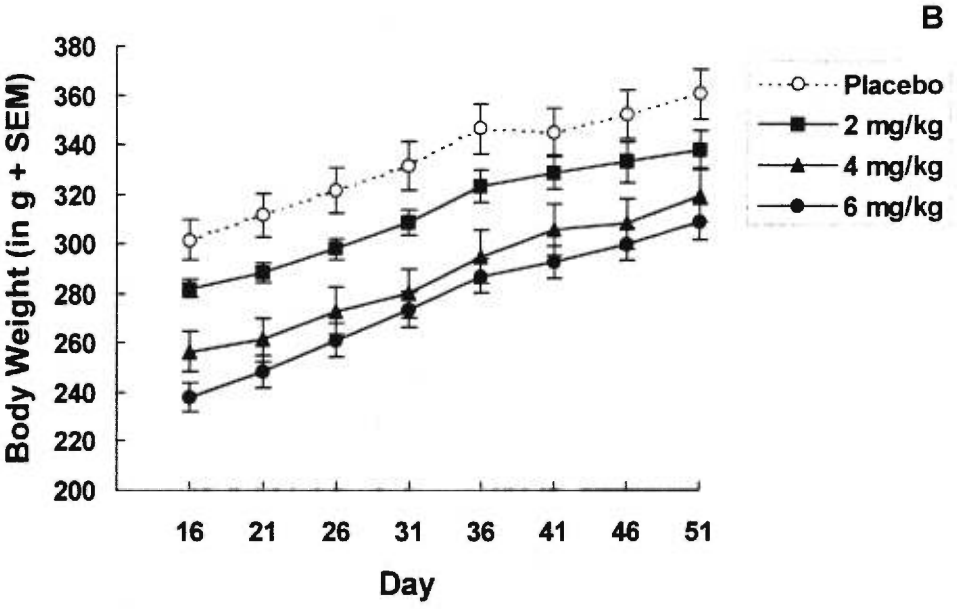
Figure 5. Escape latency during the sample runs of the first 20 trials of training on water maze DMTP.

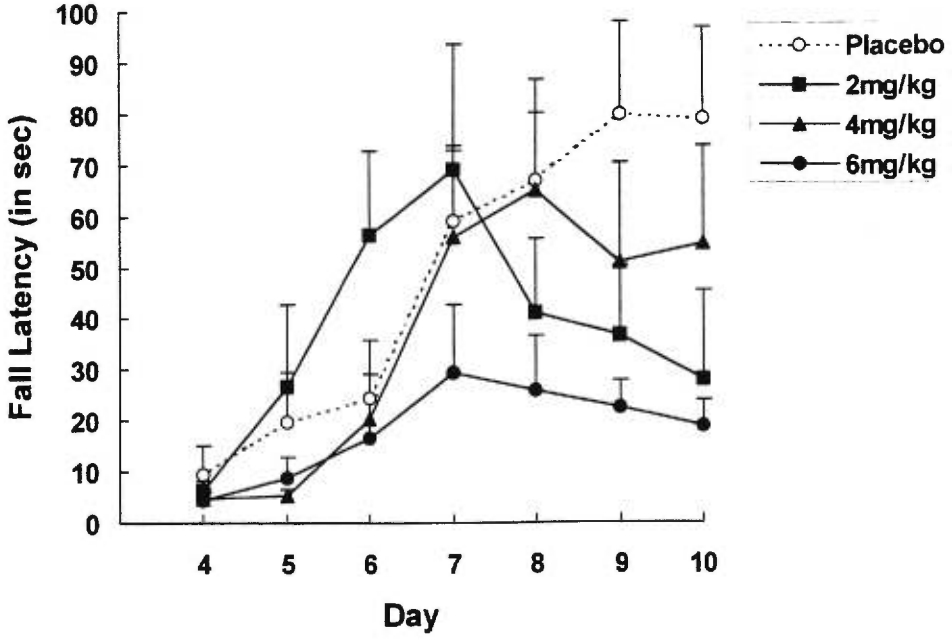
Figure 6. Number of trials to criterion in training on water maze DMTP.

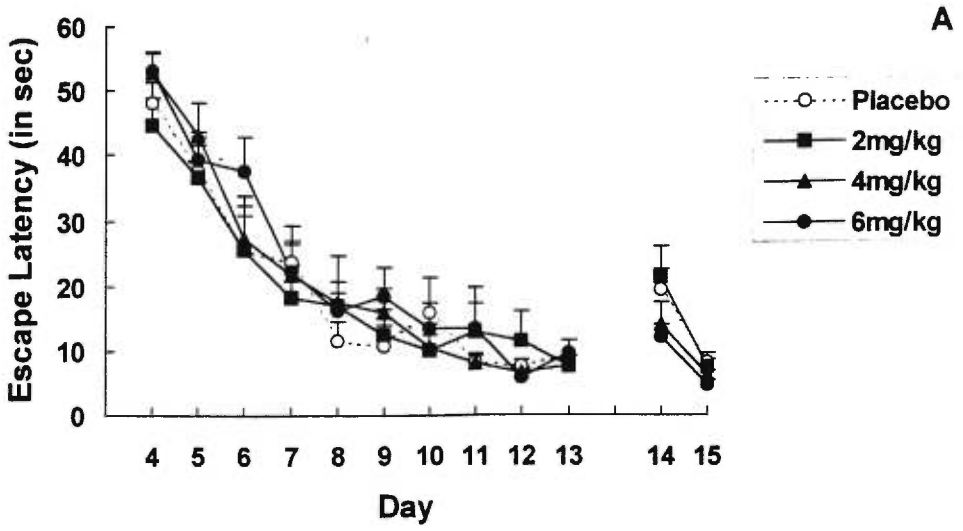
Figure 7. Success rate of Placebo and MeHg rats at 5-min and 15-min delays during postacquisition testing of water maze DMTP.

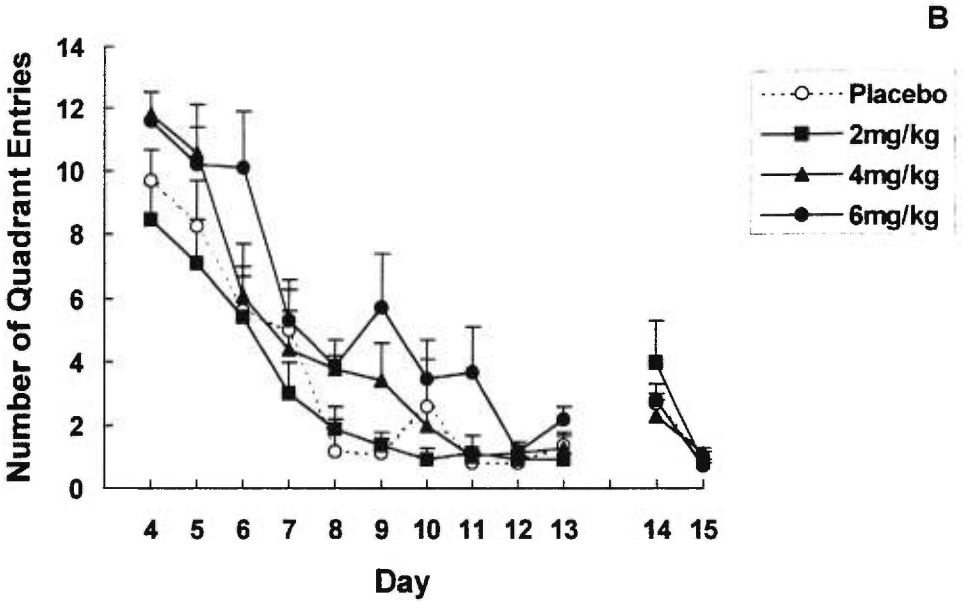

Hidden platform locations
Doors
Start

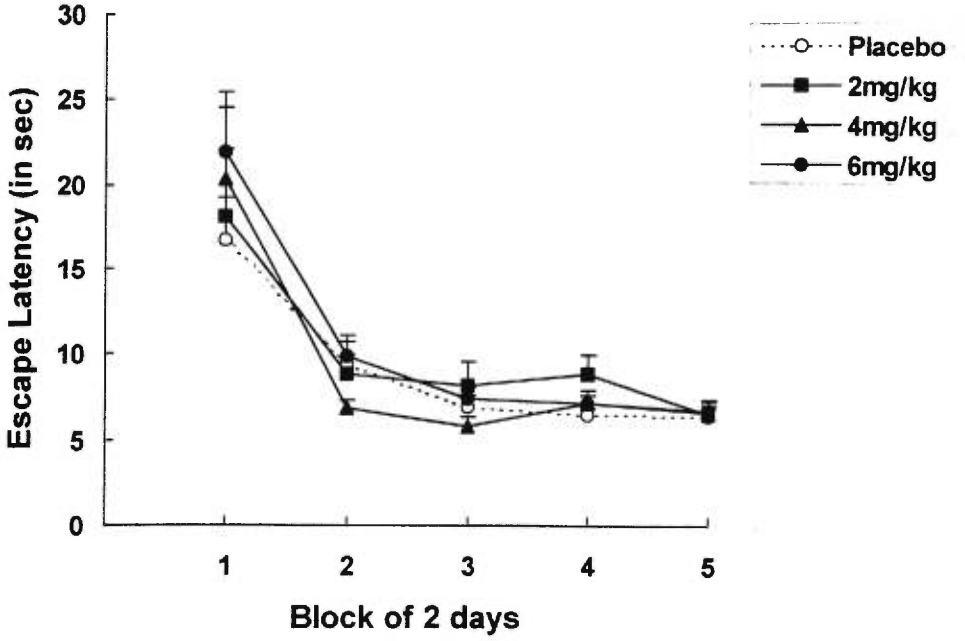

A
Body Weight (in g +SEM)
300
290
280
270
260
250
240
230
220
210
200
1
3
5
7
9
11
Day
Placebo
2 mg/kg
4 mg/kg
6 mg/kg

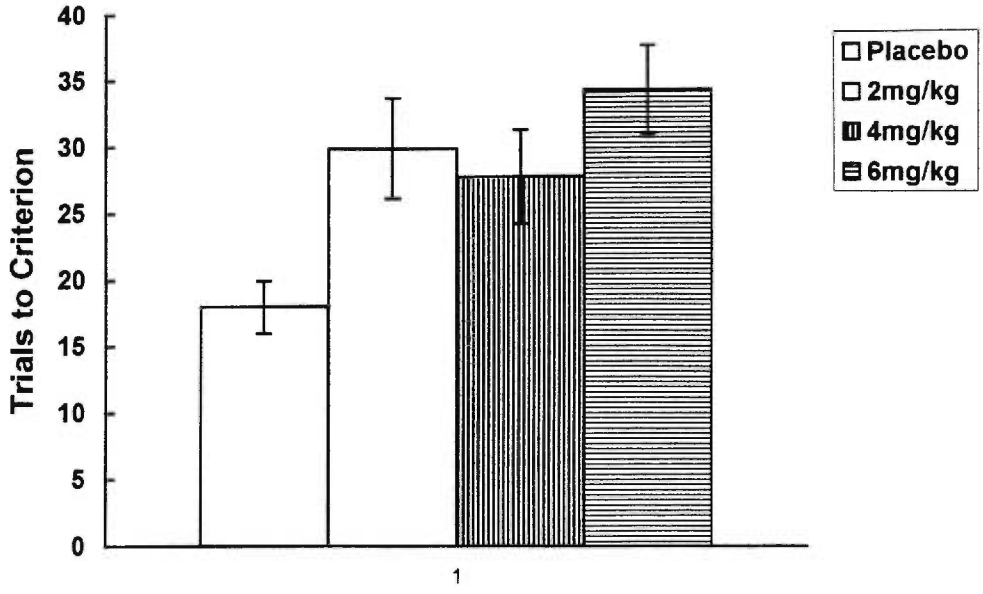

B
Body Weight (in g + SEM)
380
360
340
320
300
280
260
240
220
200
16
21
26
31
36
41
46
51
Day
Placebo
2 mg/kg
4 mg/kg
6 mg/kg

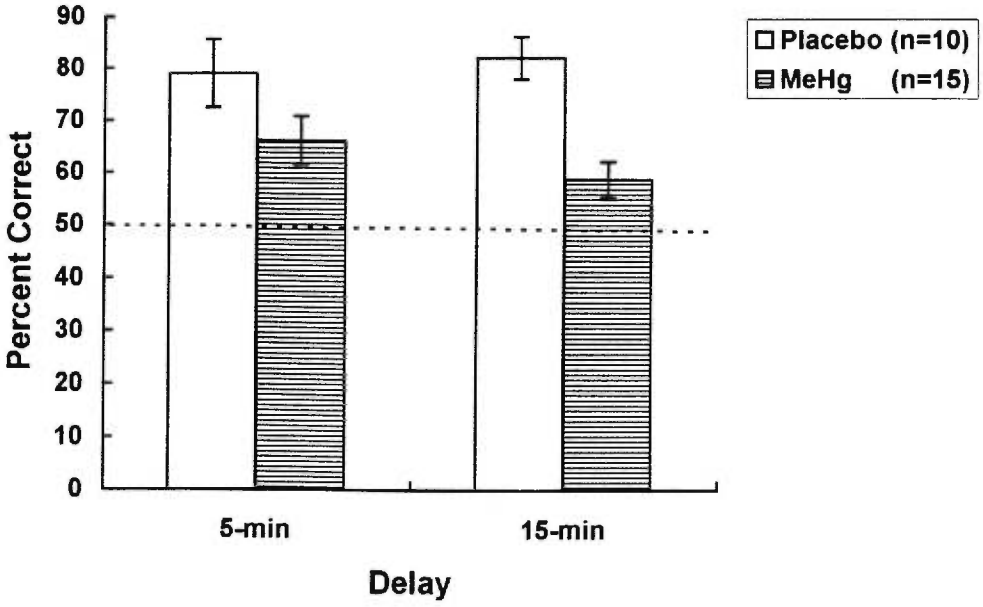

Fall Latency (in sec)
100
90
80
70
60
50
40
30
20
10
0
4
5
6
7
8
9
10
Day
Placebo
2mg/kg
4mg/kg
6mg/kg


A
Escape Latency (in sec)
60
50
40
30
20
10
0
4 5 6 7 8 9 10 11 12 13 14 15
Day
Placebo
2mg/kg
4mg/kg
6mg/kg


B
Number of Quadrant Entries
14
12
10
8
6
4
2
0
4 5 6 7 8 9 10 11 12 13 14 15
Day
Placebo
2mg/kg
4mg/kg
6mg/kg


Escape Latency (in sec)
30
25
20
15
10
5
0
1
2
3
4
5
Block of 2 days
Placebo
2mg/kg
4mg/kg
6mg/kg


Trials to Criterion
40
35
30
25
20
15
10
5
0
1
Placebo
2mg/kg
4mg/kg
6mg/kg


90
80
70
60
50
40
30
20
10
0
Percent Correct
5-min
15-min
Delay
Placebo (n=10)
MeHg (n=15)

2.3 Effets protecteurs de l'anti-oxidant glutathion et du bloqueur de canaux calciques nifédipine contre les déficits d'apprentissage et de mémoire induits par le mercure méthylique chez des rats juvéniles

Soumis à *NeuroToxicology*

sous le tire de

Protective effects of the anti-oxidant glutathione and of the Ca^{++} channel blocker nifedipine against methylmercury-induced learning and memory impairments in juvenile rats

Protective Effects of the Anti-oxidant Glutathione and the Ca^{++} Channel Blocker Nifedipine Against Methylmercury-Induced Learning and Memory Impairments in Juvenile Rats

Stéphane Beaudin[1], Francois Y. Doré[2], Saroj K. Chakrabarti[3] and Maurice Ptito[1]

[1]Groupe de recherche en neuropsychologie expérimentale, Département de psychologie, Université de Montréal, Montréal, Qué., Canada H3C 3J7

[2]École de psychologie, Université Laval, Québec, Qué., Canada G1K 7P4

[3]Département de médecine du travail et d'hygiène du milieu, Faculté de médecine, Université de Montréal, Montréal, Qué., Canada H3C 3J7

Correspondence: Stéphane Beaudin
Department of Nutritional Sciences and Psychology
Savage Hall Building
Cornell University
Ithaca, NY

Abstract

Anti-oxidants such as glutathione and Ca^{++} channel blockers such as nifedipine have been found *in vitro* to protect cells against toxic effects of methylmercury. However, the beneficial effects of these compounds have not been assessed at the behavioral level. The present study was therefore aimed to examine in juvenile rats the potential protective role of 0.5 g/kg/day of glutathione and of 5 mg/kg/day of nifedipine against the effects on learning and memory due to postnatal subacute exposure to MeHg at a dose of 6 mg/kg/day for 10 days. The behavioral task chosen, water maze delayed matching-to-position (DMTP), has already proven to be impaired by exposure to low and moderate doses of MeHg. Glutathione and nifedipine were both found to antagonize the detrimental effect of MeHg on performance in water maze DMTP. Thus, the present results have shown that these two compounds possess the potential protective effects *in vivo* against learning and memory deficits induced by subacute exposure to MeHg in rats.

Running Head: Protective effects against MeHg intoxication

Key Words: Glutathione - Nifedipine - MeHg - Water maze DMTP

Introduction

The ubiquity of the highly toxic environmental pollutant methylmercury (MeHg) represents a serious threat to the well-being of populations of all ages everywhere around the world (WHO, 1989). In humans as well as in animals, the main target of MeHg is the nervous system, the developing organism being especially vulnerable. Consumption of fish, which is well known as a major vehicle of intoxication, is extensive worldwide. In some populations, fishing is not only the primary source of proteins, it is also a central component of the social organization, culture and tradition (Wheatley, 1996). For the forseeable future, the cardinal principle of management for any toxic substance, minimizing exposure (Marsh, 1994), may be extremely difficult to apply or take generations to implement. Therefore, there is an urgent need to detect early signs of MeHg-induced toxicity, to stop the progression of neural degeneration in identified cases, and to prevent irreversible damage to the nervous system.

In the 1960s and 1970s, it was found that selenium and vitamin E antagonized to some degree the toxicity of MeHg (Ganther et al., 1972; Kasuya, 1975; Parizek and Ostadalova, 1967; Welsh, 1979). Recently, the hypothesis that oxydative injury may be involved in the pathogenesis of neurotoxic effects of MeHg has gained increased recognition and emphasized the possible protective role of anti-oxydants against the toxicity of MeHg (Yee and Choi, 1994). Several mechanisms might limit the MeHg "oxydative stress", including scavenging compounds such as glutathione which catalyzes the reduction of superoxyde radicals. Because oxydative stress is part of any

metabolic activity, the brain possesses its own anti-oxydant mechanisms through superoxyde dismutase (SOD) and glutathione. However, in the presence of MeHg, these endogeneous mechanisms are suppressed and the oxydative state of the brain increases through the rise of free radicals (i.e. superoxyde and hydrogen peroxide). Therefore, elevating the level of glutathione exogeneously could reduce or suppress MeHg-induced oxydative effects. Moreover, glutathione is appealing as a specific therapeutic because, unlike selenium, it also accelerates the excretion of MeHg from the brain by complexing directly with MeHg (Kromidas et al., 1990). The effectiveness of oxygen radical scavengers such as glutathione has been shown recently to block MeHg-induced neurotoxicity in vitro (Park et al., 1996).

Together with increased reactive oxygen species, MeHg has also been found to disturb Ca^{++} homeostasis by elevating intracellular Ca^{++} concentration, a common mechanism by which many neurotoxic substances promote neuronal cell death (Komulainen and Bondy, 1987). Support for the protective role of Ca^{++} channel blockers arose from in vitro neurochemical studies (Pauwesl et al., 1991; Yoshimura et al., 1993). These studies showed that MeHg-induced increased intracellular Ca^{++} level was delayed by high concentrations of nifedipine, which in turn delayed the access of MeHg to critical intracellular sites (e.g. mitochondria). Interestingly, the dihydropyridine compounds, including nifedipine and nimodipine which are both L-type Ca^{++} channel antagonists, have been found to improve learning and memory in old and lesioned animals (Finger et al., 1990; Izquierdo, 1990; Sandin et al., 1990).

Taken together, the above results indicate that MeHg-induced elevation in oxygen free radicals and disruption of cation homeostasis can be manipulated to increase the resistance to the neurotoxic action of MeHg. In spite of the beneficial effects of anti-oxidant and Ca^{++} channel blockers on cellular activity *in vitro*, the potential functional improvement offered by these therapeutics have not, to the best of our knowledge, been addressed at the behavioral level.

In animal models, the most salient behavioral effect of MeHg intoxication is impairment of sensory and motor functions (Rice, 1996). In rodents, crossing of hind legs on being held by the tail (Magos et al., 1981; 1985) and rotating movements of the tail (Ohi et al., 1978), which have been reported after exposure to high doses of MeHg (8 to 10 mg/kg), are considered typical signs of intoxication. Decreased spontaneous motor activity (Kobayashi et al., 1981), impaired performance on the rotarod task (Berthoud et al., 1976; Gilbert and Maurissen, 1982; Kobayashi et al., 1981), and increased hindlimb foot-spread (Gilbert & Maurissen, 1982) have also been reported.

The effects of prenatal or postnatal exposure to MeHg on learning and memory in rodents are not as well investigated as motor deficits and negative results are preponderant. However, several lines of evidence from morphological (Berthoud et al., 1976; Burbacher et al., 1990; Diamond and Sleight, 1972; Magos et al., 1981) and neurochemical studies (Abd-Elfattah and Shamoo, 1981; Castoldi et al., 1996; Cavanagh and Chen, 1971; Chakrabarti et al., submitted; Dwidedi et al., 1980; Eldefrawi et al., 1977; Loua et al., submitted Okuda et al., 1978; Omata et al., 1980;

Von Burg et al., 1980; Yoshino et al., 1966) suggest that exposure to MeHg might interfere with learning and memory processes. Indeed, recent experiments in our laboratory have confirmed that motor coordination as well as learning and memory deficits are early manifestations of intoxication and appear without overt motor signs, after postnatal subacute exposure to low or moderate doses of MeHg (Beaudin et al., submitted).

The purpose of the present investigation was to assess the potential protective effects of the anti-oxidant glutathione and the Ca^{++} channel blocker nifedipine on learning and memory impairment resulting from MeHg intoxication. To this end, the water maze Delayed Matching-to-Position (DMTP), which was previously shown to be impaired in postnatal subacute exposure to low and moderate doses of MeHg, was selected as the testing procedure.

The water maze DMTP has been developed by Means (1988; Comer and Means, 1989) to test working memory. A circular pool surrounded by extramaze cues is divided into radial thirds. A position at the center of the arc forming the perimeter of one section served as the starting point for all trials. The other two sections (goal) are separated from the starting section by doors. In the center of one of the goal sections, a platform is submerged. Each trial is divided into a sample run and a test run. The sample run is a forced choice: the door of the section containing the hidden platform is opened whereas the door of the other goal section is closed. The rat is placed into the water and allowed to swim until it reaches the platform. Then, the rat is removed from

the pool and after a delay, the test run (free choice) is presented. The hidden platform is in the same section as in the sample run (DMTP) but this time the doors of the two goal sections are opened. Again the rat is placed in the pool and allowed to reach and climb onto the platform.

According to Means (1995), in the water maze DMTP, rodents have a strong unlearned bias to return to the location where they escape on their last trial. Forced-choice run (information or sample trial), a barrier between sections to prevent short cuts and detention following incorrect test run choices are all necessary to counteract this bias. Although counterintuitive and inconsistent with findings in appetitive working memory procedures, performance on test runs given either immediately or after a delay longer than 1 min is better than on test runs with a delay of 1 min (Means, 1995). The enhancing effect of longer retention intervals is explained by nonmemory hypotheses: longer escape from the water provides a more effective reward as well as the needed time to recover from the emotional effects of the sample run.

In addition to the water maze DMTP task, the effects of the different treatments were assessed by measuring body weight gain, mortality, and the appearance of overt motor signs.

Material and Methods

Subjects

Sprague-Dawley male rats (Charles River Canada Inc., St-Constant, Québec), approximately 60-day old and weighing 240-250 g at the start of the experiment, served as subjects. They were housed individually in a standard Plexiglass cage and placed in a room maintained at a constant temperature (21±3°C) and on a standard 12:12 light/dark cycle. Purina laboratory chow and demineralized water were available *ad libitum* throughout the experiment.

Neurotoxic Exposure and Treatment

Methyl mercury chloride (MeHgCl) (purity over 99%), was obtained from Johnson Matthey Company (Ward Hill, MA, USA). Glutahione and Nifedipine were obtained from Sigma Chemical Compagny (St-Louis, MO, USA). Naive rats were randomly assigned to four groups: Group MeHg (n=12), Group GSH (n=12), Group NFD (n=10), and Group Placebo (n=10). Group MeHg, GSH, and NFD all received by gastric gavage a dose of 6 mg/kg/day of MeHgCl for 10 consecutive days, for a total cumulative dose of 60 mg/kg. The compound was dissolved in a 0.9% saline solution and administered in a volume of 4 ml/kg. Group Placebo received an equal volume of vehicle only. Seventy-two hours after the first exposure to MeHg, Group GSH and Group NFD were treated with glutathione and nifedipine, respectively, throughout the end of the experiment.

Group GSH received 0.5g/dg/day po of gluthatione whereas Group NFD was injected with 5mg/kg/day i.p. Glutathione was administered approximately 20 hours before each exposure to MeHg and nifedipine was injected 30 min before behavioral testing.

Body weights were monitored at 2-day intervals from the beginning of MeHg exposure to 13 days after the end of behavioral testing. Rats were observed periodically for any crossing of hind legs.

Testing in Water Maze DMTP

Behavioral testing started on the 4^{th} day of MeHg treatment (Day 4 to Day 13) because steadily accumulation of methymercury in the brain is known to occur after 72 hours. The swimming pool was a circular plastic tank (200 cm in diameter with 60 cm high walls) and was filled to a height of 32 cm with 23^{o} C water. It occupied the center of the experimental room. A T-shaped opaque plastic insert (height: 51 cm; stem: 105 cm long; bar: 72 cm) divided the swimming pool into a starting section (maximal width: 35 cm) and two equal goal sections (maximal width: 17.5 cm) on the left and on the right of the starting section. The doors to the goal sections were 20-cm wide. A black circular platform (diameter: 9 cm) was placed in the correct goal section and submerged 2 cm beneath the surface of water. The absence of contrast between the black tank and the black platform made the submerged platform invisible. The extramaze cues were provided by four dim spotlights (two 75 and two 40 W bulbs) placed on the walls of the room at 150 cm above the swimming pool and by the

experimenter who was standing between the SE and SW quadrants. The spotlights in the NE, SE, and SW quadrants were at a distance of 100, 200, and 200 cm from the edge of the swimming pool respectively, whereas the spotlight located between the SW and NW quadrants was at 100 cm from the pool. Because water maze DMTP was expected to be especially difficult, the cues were made especially salient.

Rats received 2 trials/day for 10 consecutive days, each trial consisting of a sample run and a test run. During the sample run, the rat was allowed 60 sec to enter the correct goal section and to escape onto the hidden platform where it was left for 15 sec. If the platform was not found within 60 sec, the rat was gently guided to it and allowed to remain on it for 15 sec. During the test run, the rat had also 60 sec to choose the correct goal section and escape. If it chose the wrong goal section, the door of this section was closed and re-opened after a 30-sec delay to allow a correct choice (correction procedure). At the beginning sample and test runs, the rat was placed in the pool facing the outside wall of the starting section. The sample run and the test run were separated by a 2-min retention interval during which the rat was returned to its home cage. The correct goal section was varied semirandomly so that it was never the same for more than two consecutive trials and overall, it was on the right and the left of the starting section an equal number of times. The two daily trials were separated by 3 hours during which the rat was returned to its home cage and room. On sample runs, escape latency was calculated from release in the water to climbing onto the hidden platform. First choice was recorded on test runs.

Statistics

ANOVAs (Treatment x Session or Block of trials) with repeated measures on the second factor were used for the weights and the water maze DMTP data. Simple main effects were analyzed when the interaction was significant (Satterthwhaite's Mean Square of Error and df) and post hoc comparisons were made with the Newman-Keuls' test. One-way Anovas were used when appropriate.

Results

General Health Parameters

One rat from Group NFD died at the end of the toxic exposure stage of the experiment, probably as a result of bad intubation during gastric gavage. Crossing of hind legs did not appear in any rat from the treatment groups during exposure to MeHg or in the following days of testing, thus confirming that the dose level used did not result in overt motor deficits.

Results for weight gain were divided into two stages: from the first day of toxic exposure to the day after the last gastric gavage (Day 1 to 11), and from the third day after the last gavage to the end of the experiment (Day 13 to 25). During the period of toxic exposure (Figure 2a), the weights of Group MeHg, GSH, and NFD were depressed and even slightly decreased. The ANOVA showed that the factor Group, $F(3, 40) = 10.69$ $p < .0001$, the factor Day, $F(5, 200) = 103.03$ $p < .0001$, and the interaction, $F(15, 200) = 24.03$ $p < .0001$, were significant. The analysis of simple main effects revealed that at the beginning of exposure, that is, on Day 1, $F(3, 61) =$

.12, and on Day 3, $F(3, 61) = 1.19$, the groups did not differ. The effect of MeHg intoxication on body weight began to appear on Day 5, $F(3, 61) = 15.79$ $p < .001$, and this difference between the groups was also significant on the following days (Day 7: $F(3, 61) = 10.47$ $p < .001$; Day 9: $F(3, 61) = 22.13$ $p < .001$; Day 11, $F(3, 61) = 40.80$ $p < .001$). As it can be seen in Figure 2a, Group Placebo was different from the three treated groups.

Although the weights still differed three days after the last gastric gavage (Figure 2b), all groups gained weight in the following days (Day: $F(6, 240) = 430.25$ $p < .0001$). The factor Group, $F(3, 40) = 25.36$ $p < .0001$, and the interaction Group x Day: $F(18, 240) = 4.85$ $p < .0001$) were significant. Again, the Group Placebo was heavier than the three treated groups but at the end of testing, Group GSH was also significantly heavier than Group NFD and MeHg, as confirmed by post hoc comparisons on Day 25. Results from the period of toxic exposure and from the following days suggest that depressed weight gain in treated rats is caused by gastric irritation and anorexia rather than by a general deterioration of physiological functions.

Water Maze DMTP

On sample runs, escape latencies (Fig. 3) were recorded as an indication of visuo-motor abilities. The results confirmed that throughout the whole task, the groups did not differ on this basic behavioral measurement. The ANOVA showed that escape latencies during sample runs decreased across blocks of 2 days, $F(4, 156)= 120,95$ $p <$

.0001), but the factor Group, F(3, 39)= 1,58, and the interaction, F(12, 156)= 0,52) were not significant.

As to the performance on the water maze DMTP task (Fig. 4), it was superior in all groups to the level expected by chance (Placebo: $t(9)=9.6$ $p < .0001$; Group GSH: $t(11)=5.6$ $p < .0005$; Group NFD: $t(8)=4.9$ $p < .005$; Group MeHg: $t(11)=6.2$ $p < .0001$). However, the success rate was clearly affected by toxic exposure at least in some groups, $F(3. 39)=4.12$ $p < .05$). Post hoc comparisons ($p < .05$) revealed that Group MeHg was significantly impaired compared to Group Placebo. Performance in Group GSH and Group NFD did not differ from Group Placebo and was superior to performance in Group MeHg.

Discussion

The present results have confirmed that learning and memory impairments can be detected after postnatal subacute exposure to a dose of 6 mg/kg of MeHg. It also shows that the anti-oxidant glutathione and the Ca^{++} channel blocker nifedipine have a protective effect on learning and memory impairments resulting from MeHg intoxication.

None of our exposed animals displayed crossing of hind legs, which is a typical motor indication of severe toxicity. Weight gain was depressed during toxic exposure but this effect was short-lived and was probably related to gastric irritation and anorexia as suggested by increased weight in all groups in the following days.

Recovery from depressed weight gain was better in Group GSH than in Group NFD or Group MeHg. The superior action of glutathione might be related to three mechanisms (Sarafian et al., 1996): reduced toxic activity of MeHg by the formation of a MeHg-GSH complex; facilitation of excretion of MeHg from the cell by this complex; and anti-oxidant effects. Visuo-motor abilities did not seem to be impaired in the treated groups as shown by performance during sample runs of training to water maze DMTP. Therefore, learning and memory impairments can serve as early signs of intoxication when the general health of the animal and basic sensory and motor functions are not overtly affected.

The learning and memory deficits observed in the present study replicate those reported by Beaudin et al. (submitted) with a dose of 6 mg/kg. They are consistent with recent studies on the crucial role of cholinergic-dopaminergic interaction on spatial working memory (Kim and Levin, 1996; Levin et al., 1990; McGurk et al., 1988), as well as with recent neurochemical analyses of the effect of MeHg on cholinergic and monoaminergic systems (Chakrabarti et al., submitted; Loua et al., submitted). However, the most striking result from the present investigation is the protective effect of glutathione and nifedipine. Thus, it confirms further the potential protective roles of these compounds against learning and memory deficits induced by repeatitive exposure to MeHg in juvenile rats.

The direct relationship between the neural systems most affected by exposure to MeHg and learning and memory deficits however remains to be established with

precision. Similarly, the protective effects of anti-oxidants and of Ca^{++} channel blockers on early signs of MeHg intoxication will have to be investigated in more details, using different dose levels and other exposure conditions of MeHg. However, neurobehavioral parameters can contribute to assess the therapeutic effects of such compounds that could stop the progression of neural degeneration in identified cases of MeHg intoxication and hence, could prevent irreversible damage to the nervous system due to MeHg.

Acknowledgments

This article includes portions of a doctoral dissertation submitted by Stéphane Beaudin in partial fulfillment of the requirements for the doctoral degree. The research was supported in part, by Grants from the Medical Research Council of Canada (MRC), Hydro-Québec, Bay James Committee on MeHg and Canadian Electricity Association. Stéphane Beaudin was supported by Fonds pour la Formation des Chercheurs et l'Aide à la Recherche of Gouvernement du Québec (FCAR). The present research protocol was approved by the Comité de protection des animaux de laboratoire de l'Université de Montréal, in accordance with the rules of the Canadian Council on Animal Care. We thank Mr. Chengiang Bai for his assistance in gastric gavage.

References

Abd-Elfattah A-SA., Shamoo AE. Regeneration of a functionally active rat brain muscarinic receptor by d-penicillamine after inhibition with methylmercury and mercuric chloride. Mol Pharmacol 1981; 20: 492-497.

Beaudin S, Doré FY, Chakrabarti SK, Ptito M (submitted). Subacute postnatal exposure to methylmercury impairs motor coordination and water maze learning tasks in juvenile rats.

Berthoud HR, Garman RH, Weiss B. Food intake, body weight, and brain histopathology in mice following chronic methylmercury treatment. Toxicol Appl Pharmacol 1976; 36: 19-30.

Burbacher TM, Rodier PM, Weiss B. Methylmercury developmental neurotoxicity: A comparison of the effects in humans and animals. Neurotoxicol Teratol 1990; 12: 191-202.

Castoldi AF, Candura SM, Costa P, Manzo L, Costa LG. Interaction of mercury compounds with muscarinic receptor subtypes in the rat brain. NeuroToxicology 1996; 17: 735-742.

Cavanagh JB, Chen FCK. Amino acid incorporation in protein during the silent phase before organo-mercury and P-bromophenylacetylurea neuropathy in the rat. Act Neuropathol 1971; 19: 216-224.

Comer TR, Means LW. Overcoming unlearned response biases: Delayed escape following errors facilitates acquisition of a win-stay and win-shift water-escape tasks in rats. Behav Neural Biol 1989; 52: 239-250.

Diamond SS, Sleight SD. Acute and subchronic methylmercury toxicosis in the rat. Toxicol Appl Pharmacol 1972; 23: 197-207.

Durham, H., Loua, K. M. Chakrabarti, S. Modulation of neurotransmitter amines and their metabolites and monoamines oxidase activity in different brain regions following repeated exposure of rats to methylmercury. Toxicologist, 952, 1998.

Dwidedi C, Raghunathan R, Joshi, BC, Foster HW. Effect of mercury compounds on cholinacetyl transferase. Res Comm Chem Pathol Pharmacol 1980; 30, 381-384.

Eldefrawi ME, Mansour NA, Eldefrawi AT. Interactions of acetylcholine receptors with organic mercury compounds. Adv Exp Med Biol 1977; 84: 449-463.

Finger S, Green L, Tarnoff ME, Mortman KD, Andersen A. Nimodipine enhances new learning after hippocampal damage. Exp Neurol 1990; 109: 279-285.

Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P, Oh S, Hoekstra WG. Selenium: Relation to decreased toxicity of methyl mercury added to diets containing tuna. Science 1972; 175: 1122-1124.

Gilbert SG, Maurissen JPJ. Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice. J Toxicol Env Health 1982; 10: 31-41.

Izquierdo I. Nimodipine and the recovery of memory. TINS 1990; 11: 309-310.

Kasuya M. The effect of vitamin E on the toxicity of alkyl mercurials on nervous tissue in culture. Toxicol Appl Pharmacol 1975; 32: 347-354.

Kim JS, Levin ED. Nicotinic, muscarinic and dopaminergic actions in the ventral hippocampus and the nucleus accumbens: effects on spatial working memory in rats. Brain Res 1996; 725: 231-240.

Kobayashi H, Yuyama A, Matsuaka, N, Takeno, K, Yanagiva I. Neuropharmacological effect of methylmercury in mice with special reference to the central cholinergic system. Japan J Pharmacol 1981; 31: 711-718.

Komulainen H, Bondy SC. Increased free intrasynaptosomal Ca^{2+} by neurotoxic organometals: Distinctive mechanisms. Toxicol Appl Pharmacol 1987; 88: 77-86.

Kromidas L, Trombetta LD, Jamall IS. The protective effects of glutathione against methylmercury cytotoxicity. Toxicol Lett 1990; 51: 67-80.

Levin ED, McGurk SR, Rose JE, Butcher LL. Cholinergic-dopaminergic interactions in cognitive performance. Behav Neural Biol 1990; 54: 271-299.

Loua KM, Chakrabarti SK, Durham H (submitted). Alteration of the cholinergic system in brain and blood lymphocytes, following repeated exposure to methylmercury in the adult rat.

Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. The comparative toxicity of ethyl- and methylmercury. Arch Toxico 1985; 57: 260-267.

Magos L, Peristianis GC, Clarkson TW, Brown A, Preston S, Snowden RT. Comparative study of the sensitivity of male and female rats to methylmercury. Arch Toxicol 1981; 48: 11-20.

Marsh DO. Organic mercury: clinical and neurotoxicological aspects. In Handbook of Clinical Neurology, Vol 20 (64): Intoxications of the nervous system, de Wolff, ed., Amsdterdam, Elsevier Science, pp. 413-429.

McGurk SR, Levin ED, Butcher, LL. Cholinergic-dopaminergic interactions in radial-arm maze performance. Behav Neural Biol 1988, 49: 234-239.

Means LW. Rats acquire win-stay more readily than win-shift in a water-escape situation. Anim Learn Behav 1988; 16: 303-311.

Means LW. Working memory for water-escape loci in rodents. In Neurobehavioral plasticity: learning, development, and response to brain insults, Spear NE. Spear LP Woodruff ML, eds., Hillsdale, Lawrence Erlbaum, 1995. pp. 67-89.

Ohi G, Nishigaki S, Seki H, Tamura Y, Mizoguchi I, Yagyu H, Nagashima K. Tail rotation, an early neurological sign of methylmercury poisoning in the rat. Envir Res 1978; 16: 353-359.

Okuda J, Tsuzuki Y, Yamada T. Some neurochemical changes in the brain of rats with acute intoxication of methylmercury chloride. Japanese J Legal Med 1978; 32: 51-58.

Omata S, Hirakawa E, Daimon Y, Uchiyama M, Nakashita H, Horigome T, Sugano I, Sugano H. Methylmercury-induced changes in the activities of neurotransmitter enzymes in nervous tissues of the rat. Arch Toxicol 1982; 51: 285-294.

Parizek J, Ostadalova I. The protective effect of small amount of selenite in sublimate intoxication. Experientia 1967; 23: 142-143.

Park ST, Lim KT, Chung YT, Kim SU. Methylmercury-induced neurotoxicity in cerebral neuron culture is blocked by antioxydants and NMDA receptor antagonists. NeuroToxicology 1996; 17: 37-46.

Pauwels PJ, Leysen JE, Janssen PAJ. Ca^{++} and Na^{+} channels in neuronal cell death. Protection by flumarizine. Life Sci 1991; 48: 1881-1893.

Rice DC. Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents. NeuroToxicology 1996; 17: 139-154.

Sandin M, Jasmin S, Levere TE. Aging and cognition: Facilitation of recent memory in aged non-human primates by nimodipine. Neurobiol Aging 1990; 11: 573-575.

Sarafian TA, Bredesen DE, Verity MA. Cellular resistance to methylmercury. NeuroToxicol 1996; 17: 27-36.

Von Burg R, Northington FK, Shamoo A. Methylmercury inhibition of rat brain muscarinic receptors. Toxicol Appl Pharmacol 1980; 53: 283-292.

Welsh SO. The protective effect of vitamine E and N.N'-diphenyl-p-phenylenediamine (DPPD) against methyl mercury toxicity in rat. J Nutr 1979; 109: 1673-1681.

Wheatley MA. The importance of social and cultural effects of mercury on arboriginal peoples. NeuroToxicology 1996; 17: 251-256.

WHO. Environmental health criteria 86: mercury-environmental aspects. Geneva, World Health Organization, 150 pp.

Yee S, Choi BH. Methylmercury poisoning induces oxidative stress in the mouse brain. Exp Mol Pathol 1994; 60: 188-196.

Yoshimura Y, Watanabe Y, Shibuya T. Inhibitory effects of calcium channel antagonists on motor dysfunction induces by intracerebroventricular administration of paraquat. Pharmacol Toxicol 1993; 72: 229-235.

Yoshino Y, Mozai T, Nakao K. Distribution of mercury in the rat brain and its subcellular units in experimental organic mercury poisoning. J Neurochem 1966; 13: 397-406.

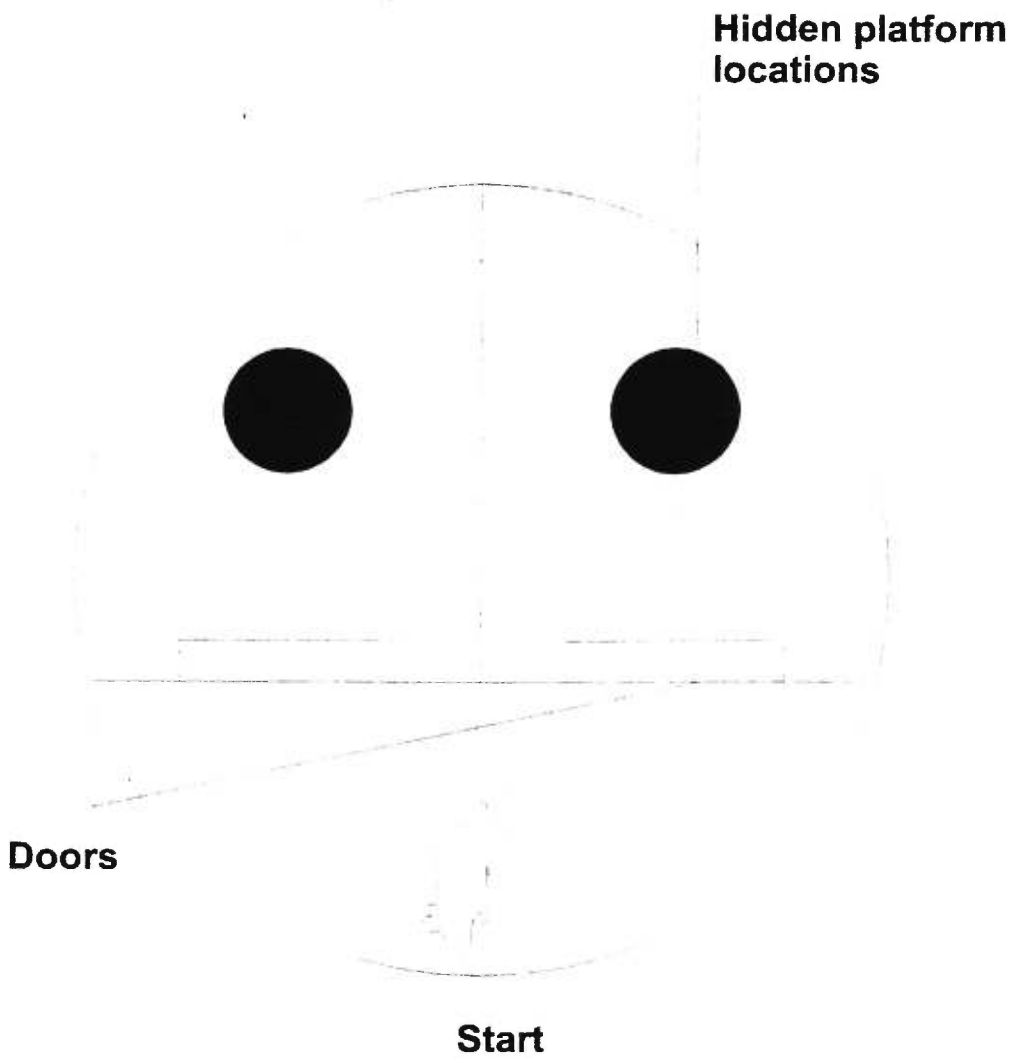
Figure Legends

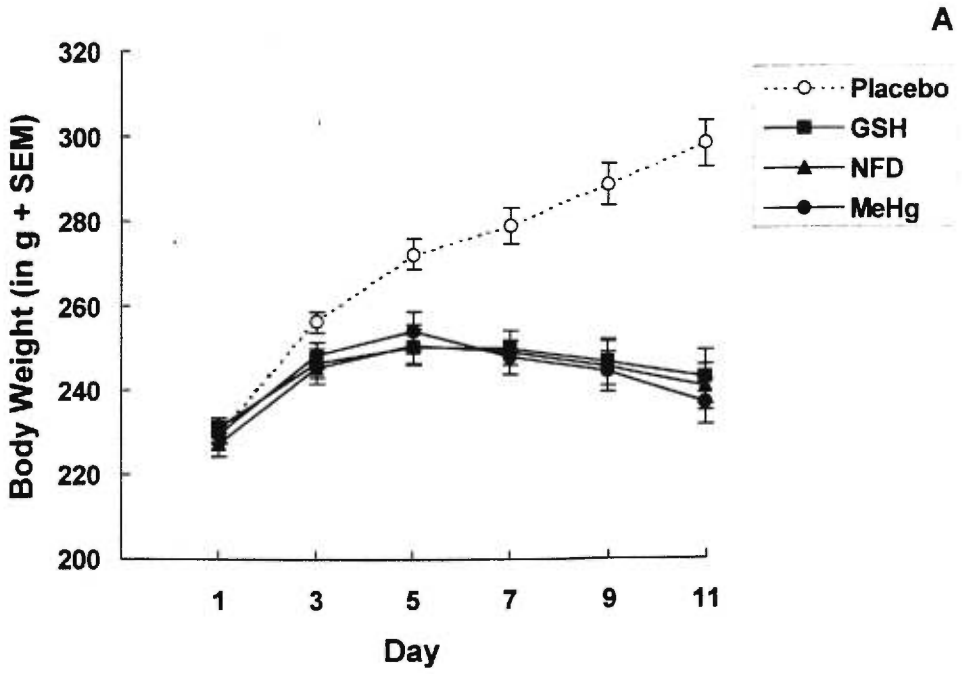
Figure 1. Schematic representation of the swimming pool used in the water maze DMTP.

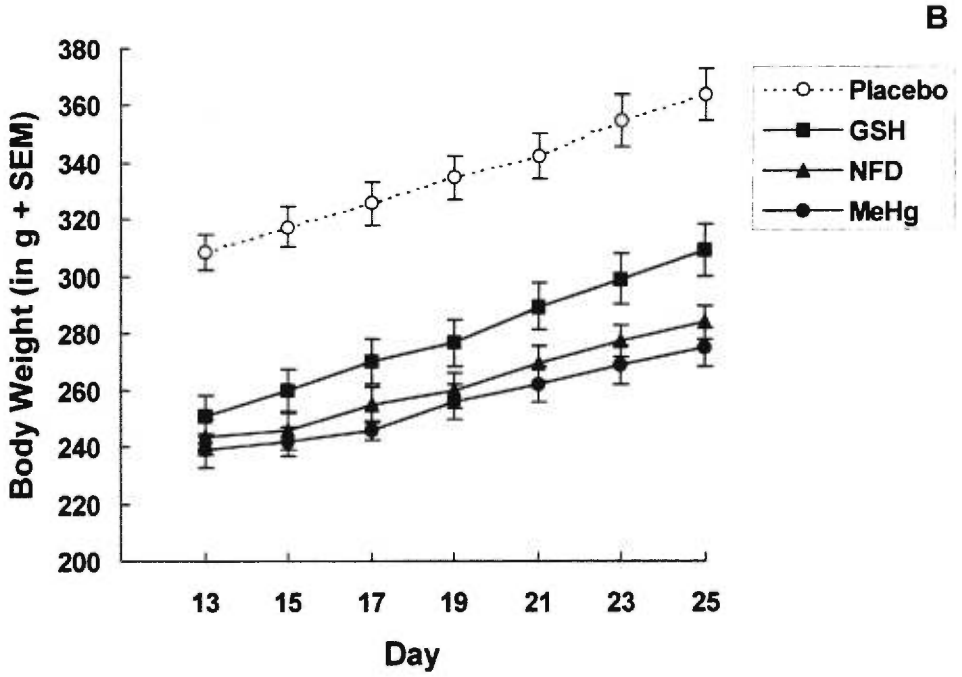
Figure 2. Body weight of the four groups during toxic exposure (A) and in the rest of the experiment (B).

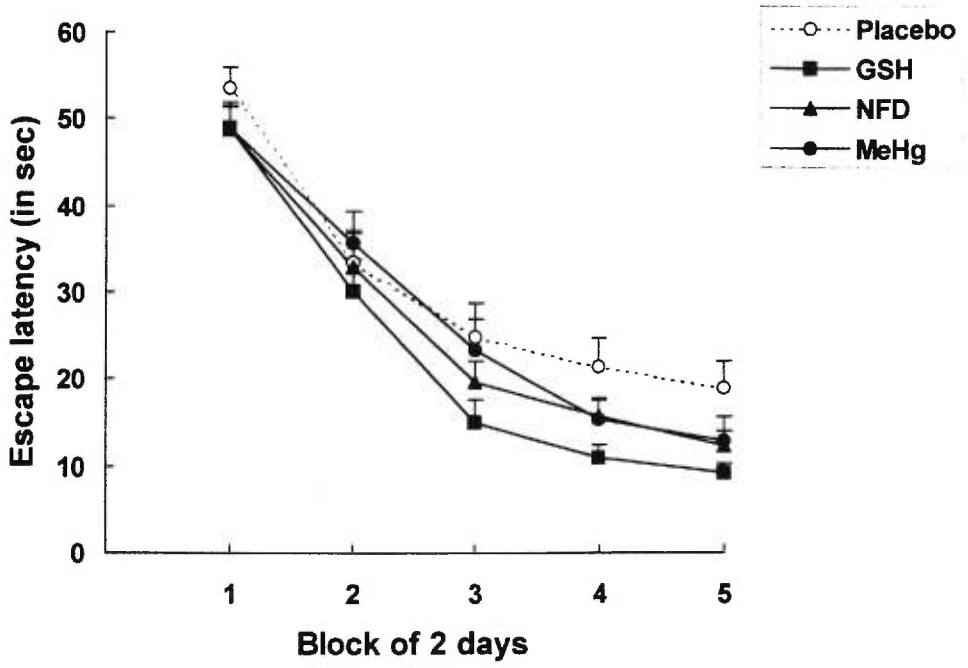
Figure 3. Escape latencies during sample runs in the water maze DMTP.

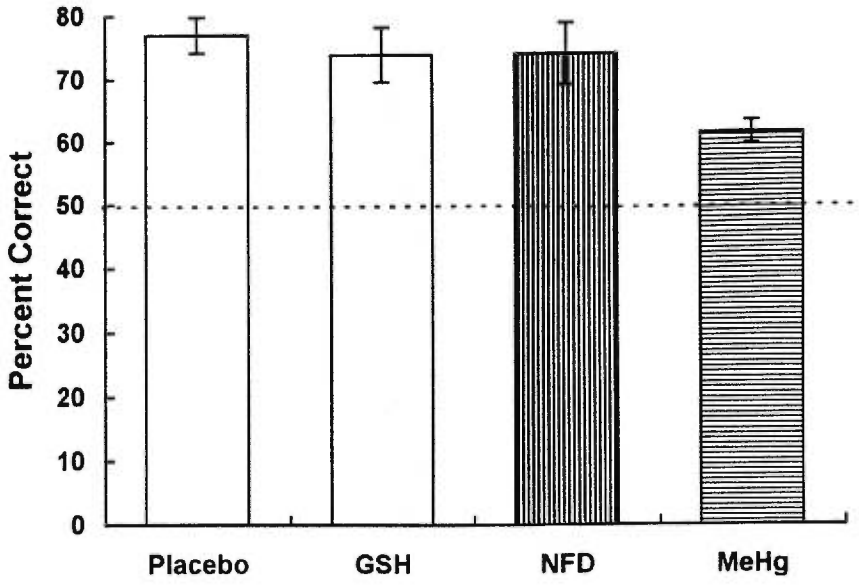
Figure 4. Success rate during test runs in the water maze DMTP.


Hidden platform locations
Doors
Start


A
Body Weight (in g + SEM)
320
300
280
260
240
220
200
1
3
5
7
9
11
Day
Placebo
GSH
NFD
MeHg


B
Body Weight (in g + SEM)
380
360
340
320
300
280
260
240
220
200
13
15
17
19
21
23
25
Day
Placebo
GSH
NFD
MeHg


Placebo
GSH
NFD
MeHg
Escape latency (in sec)
60
50
40
30
20
10
0
1
2
3
4
5
Block of 2 days


Percent Correct
80
70
60
50
40
30
20
10
0
Placebo
GSH
NFD
MeHg

CHAPITRE 3

DISCUSSION GÉNÉRALE

Les expériences de la présente thèse présentent quatre caractéristiques particulières, comparativement à la majorité des recherches expérimentales sur les effets comportementaux et cognitifs de l'exposition au le mercure méthylique chez les rongeurs. Premièrement, l'exposition au MeHg est faite durant la période postnatale plutôt que durant la période intra-utérine et de plus, elle a lieu dans une phase tardive du développement, à savoir un peu avant la maturité sexuelle. Deuxièmement, les doses de MeHg utilisées sont relativement faibles ou modérées par rapport à celles qui sont couramment employées en période postnatale. En effet, les trois expériences de cette thèse ont recours à des doses variant de 1 à 6 mg/kg alors que généralement, le dosage se situe entre 12 et 20 mg/kg. Troisièmement, l'exposition à laquelle ont été soumis les rats juvéniles peut être considérée comme subaiguë (10 jours) tandis que la plupart des études en période postnatale emploie des expositions aiguës (1 jour) ou chroniques (allant jusqu'à 60 jours). Quatrièmement, les doses et la durée d'exposition choisies n'induisent pas les signes moteurs typiques (croisement des pattes postérieures) observés dans les expériences avec un régime d'exposition plus soutenu.

Même avec un régime d'exposition postnatale modéré et malgré l'absence de signes moteurs indicatifs d'un dommage cérébral irréversible, les expériences de la présente thèse montrent de façon répétée et convergente que l'intoxication par le mercure méthylique peut rapidement produire des déficits comportementaux et cognitifs, plus subtils et difficiles à observer certes, mais non moins nuisibles au fonctionnement normal de l'organisme. Ces expériences démontrent également que les déficits sont dépendants du dosage (dose-dependent) et que la détection de ces signes

précoces d'exposition permet l'élaboration de thérapeutiques potentiellement efficaces, avant que les fonctions nerveuses ne soient complètement et définitivement compromises.

Dans toutes les expériences effectuées dans cette thèse, les rats n'ont pas subi de perte nette de poids contrairement aux recherches avec des doses plus fortes. Ils ont seulement souffert d'un ralentissement du gain pondéral durant la période d'exposition au MeHg. Dans les jours ou les semaines qui suivaient, les rats exposés aux doses les plus élevées (2, 4 et 6 mg/kg) ne réussissaient pas à récupérer entièrement le retard pris par rapport aux rats du groupe Placebo, mais leur poids augmentait de façon régulière et continue. Ces résultats suggèrent que le ralentissement de gain pondéral consécutif à une exposition au MeHg est davantage relié à l'irritation gastrique produite par la procédure d'exposition (gavage) qu'à un dérèglement permanent du métabolisme et des fonctions physiologiques de base.

Le régime d'exposition utilisé dans cette thèse n'affecte la coordination motrice, telle que mesurée dans le test du rotarod, que si la dose est maximale (6 mg/kg) et l'effet se manifeste surtout dans les deux derniers jours de mesure, même si l'amélioration quotidienne de la performance n'est pas aussi rapide que chez le groupe Placebo ou des groupes exposés à des doses de 2 et 4 mg/kg. La performance inférieure dans les deux derniers jours coïncide avec les deux dernières expositions au MeHg. Il est donc possible que ce résultat soit dû à l'accumulation de mercure méthylique dans le système nerveux et plus particulièrement dans le cervelet. En effet,

alors que le dosage cumulatif chez les autres groupes ne dépasse pas 20 ou 40 mg/kg, il pourrait atteindre 60 mg/kg chez le groupe ayant reçu une dose de 6 mg/kg. En somme, il semble que la coordination motrice n'est affectée que si une quantité significative de MeHg s'est déjà accumulée dans le système nerveux. Pour le vérifier de façon plus probante, il faudrait mettre en corrélation le déficit de coordination motrice et les quantités accumulées de MeHg. Toutefois, cette hypothèse est compatible avec les données de Sakamato et al. (1993) qui ont obtenu un déficit postnatal au test de rotarod avec une dose de 7.14 mg/kg pendant 10 jours, de même qu'avec les densités de mercure observées dans différentes régions cérébrales par Loua, Chakrabarti, Durham, (1998).

Dans l'open field, aucun effet n'est observé quant à l'exploration horizontale ou verticale de cet espace, peu importe la dose à laquelle les rats ont été exposés. Par contre, l'exploration d'objets disposés dans cet espace, elle, est affectée par l'exposition à une dose de 4 mg/kg. Les rats soumis à cette condition ne manifestent aucune réaction observable au changement de position des objets à l'intérieur d'une configuration stable et familière, contrairement aux sujets du groupe Placebo ou à ceux ayant subi une exposition à des doses plus faibles (1 et 2 mg/kg). Cette absence de réaction peut s'expliquer soit par un déficit attentionnel, les rats ne remarquant pas le changement de position, soit par un déficit mnémonique, les rats ne se souvenant pas de la disposition particulière des objets. Les données telles que recueillies ne permettent pas de départager l'une ou l'autre de ces interprétations et des travaux ultérieurs devront tester ces hypothèses de façon systématique.

Le labyrinthe aquatique de Morris est l'une des tâches standards pour mesurer la mémoire spatiale. Contrairement au labyrinthe radial qui, dans sa version usuelle, inclut une composante de mémoire de travail (évitement d'un lieu déjà visité à l'intérieur d'un essai) et une composante de mémoire de référence (relations spatiales stables d'un essai à l'autre; règles de contingence du renforcement), le labyrinthe aquatique est une tâche spatiale qui ne met en jeu que la mémoire de référence. En effet, bien que le point de départ de l'animal varie d'un essai à l'autre, la plate-forme invisible est toujours localisée au même endroit et maintient des relations invariantes avec les indices spatiaux de l'environnement.

Dans la tâche de Morris, la performance de rats exposés à des doses de 1, 2 et 4 mg/kg de MeHg est normale en termes de latences d'échappement et de nombre de quadrants traversés pour atteindre la plate-forme invisible. Par contre, des rats exposés à une dose de 6 mg/kg traversent plus de quadrants par unité de temps que des rats Placebo ou des rats exposés aux doses inférieures. Ce résultat ne peut être attribué à des difficultés visuomotrices puisque la performance dans la tâche avec plate-forme visible est normale. Il suggère par ailleurs que les rats avec le dosage le plus élevé sont incapables d'adopter la stratégie spatiale optimale et qu'ils réussissent à s'échapper aussi vite que les autres groupes en parcourant plus rapidement la surface du labyrinthe. Une performance inférieure dans le labyrinthe aquatique est habituellement associée à une dysfonction du système hippocampique. Il faut noter, cependant, que les rats exposés à 6 mg/kg ne sont pas complètement désorientés dans cette tâche. Ils n'ont simplement pas tendance à adopter la meilleure stratégie. Il semble donc que le

fonctionnement du système hippocampique, tout en commençant à être perturbé, ne soit pas définitivement compromis. Il faut noter aussi que dans les deux expériences où cette tâche était utilisée, les sujets étaient mesurés principalement durant la période d'exposition. S'ils avaient été mesurés après la fin de l'exposition, il est possible que l'accumulation de mercure dans le système hippocampique aurait perturbé davantage son fonctionnement.

Dans toutes les expériences de cette thèse, le DMTP aquatique est la tâche qui s'est avérée la plus sensible aux effets de l'exposition juvénile au MeHg. De plus, les résultats sont très cohérents d'une expérience à l'autre et même en variant la procédure. Le déficit de mémoire de travail, mis en évidence par cette tâche, apparaît avec des doses aussi faibles que 2 mg/kg. Il se manifeste autant dans l'acquisition de la tâche (chapitres 2, 3 et 4) que dans un test de performance (chapitre 3) où des intervalles de rétention plus longs sont testés. Cette robustesse et cette cohérence des résultats peuvent s'expliquer par le fait que les sujets étaient soumis à cette tâche après la période d'exposition. Elles peuvent aussi s'expliquer par les exigences plus grandes de la tâche comparativement à un test de mémoire de référence (Means, 1995): les conditions de test requièrent une mise à jour continue de l'information en mémoire, le maintien temporaire d'une information unique et la résistance à l'interférence rétroactive générée par l'essai précédent. D'autres tâches du même type, mais qui mettent en jeu autant la mémoire des objets que la mémoire spatiale, pourraient servir à corroborer la spécificité du déficit en mémoire de travail consécutif à une exposition au MeHg.

Le substrat neuroanatomique du DMTP aquatique n'a pas été exploré jusqu'à maintenant et demeure donc ambigu. Comme la version de cette tâche utilisée dans la thèse fait appel à la stratégie *win-stay*, la procédure présente des similitudes avec la tâche de réponse différée qui est généralement associée au cortex préfrontal; l'animal doit en effet retrouver, après un délai variable, un lieu ou un objet avec lequel il a été préalablement en contact. Elle présente aussi des similitudes avec différentes tâches d'appariement différé de l'échantillon, soit en relation avec des lieux, soit en relation avec des objets, ces tâches étant associées au fonctionnement du système hippocampique, plus particulièrement du cortex entorhinal, et au fonctionnement du cortex préfrontal ventral. La comparaison des versions *win-stay* et *win-shift* du DMTP aquatique pourraient contribuer à élucider et à préciser les systèmes mnémoniques impliqués. De plus, comme la présente thèse a maintenant démontré une absence de déficit moteur sévère à des doses faibles et modérées d'intoxication par le MeHg, les versions sèches des labyrinthes qui sont plus faciles d'usage pourraient servir à cette fin. Néanmoins, il reste que le DMTP est une tâche particulièrement sensible à l'intoxication par le mercure, spécifique et fiable comme le démontrent nos résultats.

Les résultats rapportés dans le troisième article suggèrent qu'un anti-oxydant, le glutathion, et un bloqueur des canaux calciques, la nifédipine, pourraient avoir un rôle protecteur quand l'intoxication par le MeHg est détectée rapidement grâce aux signes précoces fournis par les déficits comportementaux et cognitifs. Le potentiel thérapeutique de ces composés, qui n'avait pas été évalué jusqu'à maintenant sur les plans comportemental et cognitif, est prometteur. Le glutathion est particulièrement

intéressant. Non seulement il protège les systèmes mnémoniques et prévient un déficit d'apprentissage comme la nifédipine, mais il limite aussi le ralentissement du gain pondéral. De plus, le glutathion accélère l'excrétion du mercure par la formation d'un complexe MeHg-glutathion et réduit le niveau oxydatif du système nerveux. Il semble que la sous-production endogène du glutathion puisse être compensée par une intervention exogène.

La transposition des procédures expérimentales et des tests comportementaux, employés dans cette thèse, à l'évaluation des déficits chez des sujets humains est relativement facile. D'une part, il existe déjà des tests psychométriques mesurant les déficits de mémoire à court terme et à long terme ainsi que de mémoire sémantique, épisodique et de travail chez des adultes et des enfants d'âge scolaire. D'autre part, des tâches d'apprentissage et de mémoire, équivalentes à celles employées avec les animaux, ont été mises au point pour des enfants en bas âge (Overman, Bachevalier, Miller et Moore, 1996; Overman, Bachevalier, Schuhmann et Ryan, 1996; Overman, Bachevalier, Sewell et Drew, 1993; Overman, Bachevalier, Turner et Peuster, 1992; Overman, Pate, Moore et Peuster, 1996).

CHAPITRE 4

CONCLUSION GÉNÉRALE

Dans la présente thèse de doctorat, il est démontré que certains tests comportementaux et cognitifs peuvent être perturbés suite à une exposition subaiguë au mercure méthylique chez des rats juvéniles et ce en l'absence de perte nette de poids corporel et de signes typiques d'intoxication. Le test de mémoire de travail dans le DMTP aquatique est parmi les tests comportementaux et cognitifs le plus sensible à ce type d'exposition. La sélection de tests spécifiques et sensibles pour l'étude des effets neurotoxiques d'une substance d'intérêt peut ainsi servir potentiellement de signes comportementaux d'exposition. A l'aide d'autres marqueurs biologiques d'effet ou d'exposition, ces changement précoces dans le comportement peuvent, ultimement, prévenir le développement de dommages cérébraux irréversibles et permanents et ainsi empêcher l'intoxication.

La démonstration, par ailleurs, des effets protecteurs de l'anti-oxydant glutathion et du bloqueur des canaux calciques nifédipine contre le déficit mnésique induit par l'exposition au mercure méthylique est parmi l'un des résultats les plus importants de cette étude. Il indique que l'action neurotoxique du MeHg et ses effets néfastes sur le comportement et l'apprentissage peuvent être contrecarrés par l'administration exogène de drogues spécifiques. Par ailleurs, ce résultat montre que l'action amnésique du MeHg peut être corrigée par deux mécanismes distincts; l'un agissant sur l'oxydation induite par le MeHg, l'autre agissant sur l'équilibre ionique du calcium intra-cellulaire.

décrire en termes fonctionnels clairs et spécifiques l'action de ces composés sur le fonctionnement du système nerveux. La compréhension de la neurotoxicologie comportementale des nombreuses toxines naturelles et synthétiques de l'environnement ne peut que profiter de l'union éminente entre l'approche comportementale et l'approche biochimique et morphologique. Seules ces conditions de rapprochement scientifique permettront de prendre des actions fermes quant aux seuils ou aux doses minimales acceptables chez l'humain Entre temps, retenons que le mercure méthylique est une neurotoxine et qu'elle est là pour y rester.

CHAPITRE 5

BIBLIOGRAPHIE

Anger, W.K. (1984). Neurobehavioral testing of chemicals: Impact on recommended standards. Neurobehavioral Toxicology and Teratology, 6, 147-153.

Anger, W.K. (1986). Workplace exposures. Dans Z. Annau (Ed.), Neurobehavioral toxicology (pp. 331-347). Baltimore: Johns Hopkins University Press.

Anger, W.K. et Johnson, B.L. (1985). Chemicals affecting behavior. Dans J.L. O'Donoghue (Ed.), Neurotoxicity of industrial and commercial chemicals (pp. 51-148). Boca Raton: CRC Press.

Bornhausen, M., Müsch, H.R. et Greim, H. (1980). Operant behavior performance changes in rats after prenatal methylmercury exposure. Toxicology and Applied Pharmacology, 56, 305-310.

Buelke-Sam, J., Kimmel, C.A., Adams, J., Nelson, C.J., Vorhees, C.V., Wright, D.C., St Omer, V., Korol, B.A., Butcher, R.E., Geyer, M.A., Holson, J.F., Kutscher, C.L., et Wayner, M.J. (1985). Collaborative behavioral teratology study: Results. Neurobehavioral Toxicology and Teratology, 7, 591-624.

Burbacher, T.M., Rodier, P.M. & Weiss, B. (1990). Methylmercury developmental neurotoxicity: A comparison of the effects in humans and animals. Neurotoxicology and Teratology, 12, 191-202.

Calme, D.B., Eisen, A., McGeer, E. et Spencer, P. (1986). Alzheimer's disease, Parkinson's disease, and motoneurone disease: A biotrophic interaction between ageing and environment? Lancet, II (8565), 1067-1070.

Cohen, N.J. et Eichenbaum, H. (1993). Memory, amnesia, and the hippocampal system. Cambridge, Mass.: MIT Press.

Durham, H., Loua, K. M. Chakrabarti, S. Modulation of neurotransmitter amines and their metabolites and monoamines oxidase activity in different brain regions following repeated exposure of rats to methylmercury. Toxicologist, 952, 1998.

Eccles, C.U. et Annau, Z. (1982). Prenatal methylmercury exposure: II: Alterations in learning psychotropic drug sensitivity in adult offspring. Neurobehavioral Toxicology and Teratology, 4, 377-382.

Eichenbaum, H. (1996). Is the rodent hippocampus just for "place"? Current Opinions in Neurobiology, 6, 187-195.

Fagan, J.F. et McGrath, S.K. (1981). Infant recognition memory and later intelligence. Intelligence, 5, 121-130.

Finger, S., Green, L., Tarnoff, M.E., Mortman, K.D., et Andersen, A. (1990). Nimodipine enhances new learning after hippocampal damage. Experimental Neurology, 109, 279-285.

Ganther, H.E., Goudie, C., Sunde, M.L., Kopecky, M.J., Wagner, P., Oh, S., et Hoekstra, W.G. (1972). Selenium: Relation to decreased toxicity of methyl mercury added to diets containing tuna. Science, 175, 1122-1124.

Geyer, M.A., Butcher, R.E. et Fite, K. (1985). A study of startle and locomotor activity in rats exposed prenatally to methylmercury. Neurobehavioral Toxicology and Teratology, 7, 759-765.

Gunderson, V.M., Grant, K.S., Burbacher, T.M., Fagan, J.F. et Mottet, N.K. (1986). The effect of low-level prenatal methylmercury exposure on visual recognition memory in infant crab-eating macaques. Child Development, 57, 1076-1083.

Gunderson, V.M., Grant-Webster, K.S., Burbacher, T.M., et Mottet, N.K. (1988). Visual recognition memory deficits in methylmercury-exposed Macaca fascicularis infants. Neurotoxicology and Teratology, 10, 373-379.

Harada, M. (1995). Minamata disease: Methylmercury poisoning in Japan caused by environmental pollution. Critical Reviews in Toxicology, 25, 1-24.

Hughes, J.A. et Annau, Z. (1976). Postnatal behavior effects in mice after prenatal exposure to methyl mercury. Pharmacology, Biochemistry and Behavior, 4, 385-391.

Hughes, J.A. et Sparber, S.B. (1978) d-Amphetamine unmasks postnatal consequences of exposure to methyl mercury in utero: Methods for studying behavioral teratogenesis. Pharmacology, Biochemistry and Behavior, 8, 365-375.

Hunter, D., Bomford, R.R. et Russell, D.S. (1940). Poisoning by methylmercury compounds. Quarterly Journal of Medecine, 9, 193-219.

Inouye, M., Murao, K., et Kajiwara, Y. (1985). Behavioral and neuropathological effects of prenatal methylmercury exposure in mice. Neurobehavioral Toxicology and Teratology, 7, 227-232.

Inskip, M.J. et Piotrowski, J.K. (1985). Review of the health effects of methylmercury. Journal of Applied Toxicology, 5, 113-133.

Izquierdo, I. (1990). Nimodipine and the recovery of memory. Trends in Neurosciences, 11, 309-310.

Kasuya, M. (1975). The effect of vitamin E on the toxicity of alkyl mercurials on nervous tissue in culture. Toxicology and Applied Pharmacology, 32, 347-354.

Kimura, M. (1995). Role of basal ganglia in behavioral learning. Neuroscience Research, 22, 353-358.

Klaassen, C.D. (1986). Distribution, excretion, and absorption of toxicants. Dans C.D. Klaassen, M.O. Amdur et J. Doull (Eds.), Casarett and Doull's toxicology: The basic science of poisons (3e édition) (pp. 33-63). New York: Plenum Press.

Kobayashi, H., Yuyama, A., Matsuaka, N., Takeno, K. & Yanagiva, I. (1981). Neuropharmacological effect of methylmercury in mice with special reference to the central cholinergic system. Japan Journal of Pharmacology, 31, 711-718.

Komulainen, H., et Bondy, S.C. (1987). Increased free intrasynaptosomal Ca^{2+} by neurotoxic organometals: Distinctive mechanisms. Toxicology and Applied Pharmacology, 88, 77-86.

Kromidas, L., Trombetta, L.D., et Jamall, I.S. (1990). The protective effects of glutathione against methylmercury cytotoxicity. Toxicology Letters, 51, 67-80.

Lindberg, S., Stokes, P., Goldberg, E. et Wren, C. (1987). Group report: Mercury. Dans T.W. Hutchison et K.M. Meena (Eds.), Lead, mercury, cadmium and arsenic in the environment (pp. 17-34) . New York: John Wiley.

Loua, K.M., Chakrabarti, S.K. & Durham, H. Modulation of cholinergic system in different brain regions following repeated exposure of rats to methylmercury. Toxicologist, 953, 1998.

Magos, L., Brown, A.W., Sparrow, S., Bailey, E., Snowden, R.T. & Skipp, W.R. (1985). The comparative toxicity of ethyl- and methylmercury. Archives of Toxicology, 57, 260-267.

Magos, L., Peristianis, G.C., Clarkson, T.W., Brown, A., Preston, S., & Snowden, R.T. (1981). Comparative study of the sensitivity of male and female rats to methylmercury. Archives of Toxicology, 48, 11-20.

Marsh, D.O. (1994). Organic mercury: clinical and neurotoxicological aspects. Dans F.A. de Wolff (Ed.), Handbook of Clinical Neurology, Vol 20 (64): Intoxications of the nervous system (pp. 413-429), Amsdterdam: Elsevier Science.

Means, L.W. (1995). Working memory for water-escape loci in rodents. Dans N.E. Spear, L.P. Spear et M.L. Woodruff (Eds.), Neurobehavioral plasticity: learning, development, and response to brain insults (pp. 67-89). Hillsdale, Lawrence Erlbaum.

Müsch, H.R., Bornhausen, M., Kriegel, H. et Greim, H. (1978). Methylmercury chloride induces learning deficits in prenatally treated rats. Archives of Toxicology, 40, 103-108.

Ohi, G., Nishigaki, S., Seki, H., Tamura, Y., Mizoguchi, I., Yagyu, H. & Nagashima, K. (1978). Tail rotation, an early neurological sign of methylmercury poisoning in the rat. Environmental Research, 16, 353-359.

Ohi, G., Seki, H., Maeda, H. et Yagyu, H. (1975). Protective effect of selenite against methylmercury toxicity: Observations concerning time, dose and route factors in the development of selenium attenuation. Industrial Health, 13, 93-99.

Overman, W.H., Bachevalier, J., Miller, M. et Moore, K. (1996). Children's performance on "animal tests" of oddity: Implications for cognitive processes required for tests of oddity and delayed non-match to sample. Journal of Experimental Child Psychology, 62, 223-242.

Overman, W.H., Bachevalier, J., Schuhmann, E. et Ryan, P. (1996). Cognitive gender differences in very young children parallel biologically based cognitive gender differences in monkeys. Behavioral Neuroscience, 110, 673-684.

Overman, W.H., Bachevalier, J., Sewell, F. et Drew, J. (1993). A comparison of children's performance on two recognition memory tasks: Delayed nonmatch-to-sample versus visual paired comparison. Developmental Psychobiology, 26, 345-357.

Overman, W.H., Bachevalier, J., Turner, A., et Peuster, A. (1992). Object recognition versus object discrimination: Comparison between human infants and infant monkeys. Behavioral Neuroscience, 106, 15-29.

Overman, W.H., Pate, B.J., Moore, K. et Peuster, A. (1996). Ontogeny of place learning in children as measured in the radial arm maze, Morris search task, and open field task. Behavioral Neuroscience, 110, 1205-1228.

Parizek, J., et Ostadalova, I. (1967). The protective effect of small amount of selenite in sublimate intoxication. Experientia, 23, 142-143.

Park, S.T., Lim, K.T., Chung, Y.T. et Kim, S.U. (1996). Methylmercury-induced neurotoxicity in cerebral neuron culture is blocked by antioxydants and NMDA receptor antagonists. NeuroToxicology, 17, 37-46.

Pauwels, P.J, Leysen, J.E, et Janssen, P.A.J. (1991). Ca^{++} and Na^{+} channels in neuronal cell death. Protection by flumarizine. Life Sciences, 48, 1881-1893.

Post, E.M., Yang, M.G., King, J.A., et Sanger, V.L. (1973). Behavioral changes of young rats force-fed methyl mercury chloride. Proceedings of the Society of Experimental and Biological Medicine, 143, 1113-1116.

Rice, D.C. (1992). Effects of pre- plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance. NeuroToxicology, 13, 443-452.

Rice, D.C. (1996). Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents. NeuroToxicology, 17, 139-154.

Rice, D.C. et Gilbert, S.G. (1990). Effects of developmental exposure to methylmercury on spatial and temporal visual function in monkeys. Toxicology and Applied Pharmacology, 102, 151-163.

Rice, D.C. et Gilbert, S.G. (1992). Exposure to methylmercury from birth to adulthood impairs high-frequency hearing in monkeys. Toxicology and Applied Pharmacology, 115, 6-10.

Rice, D.C. et Gilbert, S.G. (1995). Effects of developmental methylmercury exposure or lifetime lead exposure on vibration sensitivity function in monkeys. Toxicology and Applied Pharmacology, 134, 161-169.

Rice, D., Hodgson, T.A. et Kopstein, A.N. (1985). The economic cost of illness: A replication and update. Health Care Financing Review, 7, 68-69.

Sakamato, M., Nakano, A., Kajiwara, Y., Naruse, I. & Fujisaki, T. (1993). Effects of methyl mercury in postnatal developing rats. Environmental Research, 61, 43-50.

Sandin, M., Jasmin, S., et Levere, T.E. (1990). Aging and cognition: Facilitation of recent memory in aged non-human primates by nimodipine. Neurobiology of Aging, 11, 573-575.

Shalock, R.L., Brown, W.J., Kark, R.A. et Menon, N.K. (1981). Perinatal methylmercury intoxication: Behavioral effects in rats. Developmental Psychobiology, 14, 213-219.

Spyker, J.M., Spaber, S.B. et Goldberg, A.M. (1972). Subtle consequences of methylmercury exposure: Behavioral deviation in offspring of treated mothers. Science, 18, 621-623.

Su, M. et Okita, G.T. (1976). Behavioral effects on the progeny of mice treated with methylmercury. Toxicology and Applied Pharmacology, 38, 195-205.

Thompson, R.F., et Kim, J.J. (1996). Memory systems in the brain and localization of a memory. Proceedings of the National Academy of Sciences USA, 93, 13438-13444.

Thompson, R.F., et Krupa, D.J. (1994). Organization of memory traces in the mammalian brain. Annual Review of Neuroscience, 17, 519-549.

Vorhees, C.V. (1985). Behavioral effects of prenatal methylmercury in rats: A parallel trial to the Collaborative Behavioral Teratology study. Neurobehavioral Toxicology and Teratology, 7, 717-725.

Welsh, S.O. (1979). The protective effect of vitamine E and N.N'-diphenyl-p-phenylenediamine (DPPD) against methyl mercury toxicity in rat. Journal of Nutrition, 109, 1673-1681.

Wheatley, M.A. (1996). The importance of social and cultural effects of mercury on arboriginal peoples. NeuroToxicology; 17, 251-256.

White, N.M. (1997). Menmonic functions of the basal ganglia. Current Opinions in Neurobiology, 7, 164-169.

WHO (World Health Organization) (1990). Methylmercury. Vammala: World Heath Organization.

Wise, S. P. (1996). The role of the basal ganglia in procedural memory. Neuroscience, 8, 39-46.

Yoshimura, Y., Watanabe, Y., et Shibuya, T. (1993). Inhibitory effects of calcium channel antagonists on motor dysfunction induces by intracerebroventricular administration of paraquat. Pharmacology and Toxicology, 72, 229-235.

Zenick, H. (1974). Behavioral and biochemical consequences of methylmercury chloride toxicity. Pharmacology, Biochemistry and Behavior, 2, 709-713.