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ORIGINAL PAPER

EFFECT OF PERINATAL LEAD EXPOSURE ON INTESTINAL MICROBIOTA OF RATS: PRELIMINARY RESULTS*

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Abstract

Lead is a heavy metal which is widespread in the environment, and the exposure to Pb during the body's development can alter the gut microbiota. The aim of this study was to assess the effects of prenatal and neonatal exposure to lead (Pb) on the number of commensal intestinal bacteria in rats. Pregnant female rats received 0.1% lead acetate (PbAc) in drinking water supplied *ad libitum*. Pregnant females from the control group received distilled water. During the feeding of offspring, mothers from the experimental group were still receiving PbAc. The offspring were weaned at postnatal day 21. The number of enterococci was significantly lower in all the studied parts of the intestine of the rats pre- and neonatally exposed to PbAc than in the control group. The total number of coliforms in all the examined sections of the intestine was lower in the Pb-exposed group. In contrast, streptococci were found only in all the analysed parts of the intestine in the Pb-exposed animals. In the control group, yeasts were not found, contrary to the Pb-exposed group. The amount of *Candida* observed in the colon was approximately ten times higher than their amounts in every other examined section of the intestine. Prenatal and neonatal exposure to lead may decrease the number of coliforms and enterococci bacteria, and may promote colonization of the intestine by streptococci (especially in

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the duodenum) and yeasts (particularly in the colon). Streptococci and yeasts are more alkaline microorganisms than coliforms and enterococci. Exposure to Pb can effect changes in pH of the environment in the gastrointestinal tract and this way it can lead to the selection of specific species of bacteria.

Keywords: bacteria, intestine, lead, offspring.

INTRODUCTION

The microflora of the human gastrointestinal tract includes thousands of different bacterial species, which provide many kinds of antigens constantly stimulating the gut-associated lymphoid tissue (Eckburg et al. 2005). Similar to other mammals, these are aerobic and anaerobic species belonging to *Escherichia*, *Bacteroides*, *Bifidobacterium* and *Lactobacillus* genera, etc. (Sadykov et al. 2009), and majority of them are found in the terminal ileum and colon. Involved in the metabolism of otherwise unassimilable carbohydrates, including pectin, cellulose, hemicellulose and arabinose, they are an important factor preventing or inhibiting the colonization of pathogens.

Although data on the composition of indigenous intestinal flora are still incomplete, the available information suggests that colonization in the mammalian intestine occurs in the first year of life (Berg 1996, Palmer et al. 2007) and the eventual composition of intestinal microbiota in an adult is the result of various endogenous and exogenous factors (Mackie et al. 1999). For a long time it was held that the intestinal flora of a newborn is similar to the flora of the mother (Mackie et al. 1999). However, recent studies based on molecular biology techniques have shown that the composition of intestinal bacteria in the stool samples of a child resembles similar samples collected from other adults in a given population (Palmer et al. 2007). The stability of the gut flora's composition may result from the immune system recognising bacteria acquired in early childhood, identifying them as its own and developing tolerance (Ouwehand et al. 2002).

The amounts and types of bacteria in the intestinal tract are also dependent on changes in the microenvironment, including pH and the availability of oxygen and food in the subsequent parts of the tract. Changes in microbe-host relationships in the gastrointestinal tract can cause abnormal expansion of intestinal microbiota resulting in acute pancreatitis, bacterial gastroenteritis and irritable bowel syndrome (Schmidt, Stallmach 2005). Changes in gut flora may play an important role in the pathogenesis of infantile colic in the first months of life (Savino et al. 2004).

Factors associated with environmental pollution are among suggested causes of inflammatory and proliferative diseases of the large intestine. It is likely that gut microbiota are an important mediator of the bioavailability and toxicity of environmental pollutants, including heavy metals, such as lead (Pb), which is the most common and dangerous toxic metal (Breton et al. 2013).

The amount of Pb absorbed from the environment into the gastrointestinal system depends on its form, route of absorption, exposure period, and the gender, age and physiological state of an organism (Sharma, Barber 2014). Lead is absorbed mainly through the respiratory and digestive systems and is transported by the blood to all tissues; it is excreted from the body in faeces and urine. The toxic effects of Pb are manifested in the impairment of enzymatic activity associated with heme synthesis. Chronic Pb exposure leads to anaemia and damage to the nervous system and kidneys (Jurkiewicz et al. 2004, Gerhardsson et al. 2005, Wiechuła et al. 2008). Moreover, Pb causes gastrointestinal dysfunction (Sharma and Barber 2014).

There is much research on the impact of Pb on the kidney and liver, considered as primary organs, with the bones, blood cells and the nervous system also often examined. However, there is much less research focusing on the effects of this element on the digestive system, particularly on the small intestine and colon. For example, it is not known whether exposure to Pb affects the bacterial flora of the large intestine and stimulates the proinflammatory activity of Pb, increasing the risk of large intestine cancer in individuals chronically exposed to lead. The aim of this study was to evaluate the effects of prenatal and neonatal exposure to 0.1% lead acetate on potential changes in intestinal microbiota composition in offspring of Wistar rats.

MATERIALS AND METHODS

The research was carried out on 18 offspring of albino Wistar rats. The study was approved by the Local Ethics Committee for Scientific Experiments on Animals in Szczecin, Poland (No. 5/2014, dated 23 April 2014).

Three-month-old female (250 ± 20 g) rats (n = 6) were bred with sexually mature males (2:1). The animals were kept in controlled conventional conditions (temp. 22 ± 1°C, relative humidity approximately 50% and 12/12 h light-dark cycle).

Standard food – Labofeed H diet, Label Food "Morawski", Kcynia (Pastuszewska et al. 2000) and tap water were freely available. Once females tested positive for copulatory plugs, males were removed from the home cage and the females were transferred to individual cages. Pregnant females were divided into two groups: control and Pb-exposed. Females from Pb-exposed group (n = 3) received 0.1% lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$; PbAc, ultra-pure grade, Merck, Poland) in drinking water *ad libitum*, starting from the first day of gestation (Kosik-Bogacka et al. 2011). The PbAc solution was prepared daily in disposable plastic bags (hydropac, Anilab, Poland) directly from a solid reagent to obtain the desired concentration; the solution was not acidified. Pregnant females from the control group (n = 3) received untreated

distilled water until the weaning of the offspring. The amount of liquid drunk did not differ significantly between Pb-exposed and control groups. The offspring (males and females) stayed with their mothers and were fed by them; meanwhile the mothers from Pb-exposed group still received PbAc in drinking water *ad libitum*. The offspring were weaned on the 21st post-natal day (PND 21) and placed in separate cages. After that, the young rats of the Pb-exposed group (n = 11) still received PbAc in the drinking water and the control group (n = 7) received untreated distilled water *ad libitum* until PND 28. Afterwards, the young rats of both groups received only distilled water *ad libitum* until PND 60, when the animals were sacrificed and the alimentary tracts from both groups of young rats were collected.

We chose oral administration of 0.1% PbAc, as it imitates environmental exposure and is widely used as a model for Pb poisoning in rats (Kang et al. 2004, Xu et al. 2005). Moreover, owing to our preliminary study and previous experiments (Baranowska-Bosiacka et al. 2012), we know that this treatment protocol results in a Pb concentration in the whole blood of the rat offspring which is similar to that found in children from contaminated urban areas (Barton 2011) – above the threshold of 10 µg dL^{-1} considered safe for humans (CDC 2007).

The animals were sacrificed by decapitation. We used the following sections of the alimentary tract in the analysis: a 1-cm segment of the upper part of the small intestine 5 cm from the pylorus; a 1-cm segment of the lower part of the small intestine 5 cm from the ileocecal junction; the cecum; and a 1-cm fragment of the colon 5 cm from the cecum junction. The intestinal sections were transferred into 50 ml of sterile saline. After 30 min, the materials were shaken and decimal dilutions performed. From each dilution 1 ml of suspension was collected and placed on the following media: on MacConkey Agar (Oxoid) – for the counting of coliform bacteria, on Mannitol Salt Agar (Oxoid) – for the isolation of staphylococci, on Edwards Medium (Oxoid) – for the isolation of *streptococci*, on Salnetz and Bartley Medium (Oxoid) – for the isolation of enterococci, and on Sabouraud Dextrose Agar (Oxoid) – for the detection and enumeration of the *Candida* genus. Cultures were incubated under aerobic conditions at 37°C for 24-48 hours.

Bacterial colonies were counted and expressed as colony forming units per ml (cfu ml^{-1}), 1 cfu ml^{-1} detection limit. The results were expressed in $\log_{10}$ cfu ml^{-1}. Basic biochemical reactions for identification were performed. Each test was performed in duplicate, and each result was an arithmetic mean.

The results were analysed statistically using Statistica 6.1 software. Arithmetic mean (AM) and standard deviation (SD) were calculated for each of the studied parameters. A non-parametric Mann-Whitney *U*-test was used to check the significance of differences between the experimental and control groups. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

Table 1 shows the composition of aerobic microbial flora in the different sections of the digestive tracts in the control group and in the prenatally and neonatally Pb-exposed rats. In the duodenum of the control rats, the presence of coliforms and enterococci was observed. In the Pb-exposed rats, coliforms, enterococci, streptococci and yeast were found. In the Pb-exposed rats, the number of detected streptococci in the duodenum was higher than in other parts of the intestine. Similarly, an analysis of the ileum of the control rats revealed the presence of coliforms and enterococci. In the Pb-exposed group we detected the presence of coliforms, enterococci and yeasts at levels similar to the ones in the duodenum, although the number of streptococci was significantly lower than in the duodenum. In the caecum of the control rats, we observed the presence of coliforms and enterococci. In the Pb-exposed rats we detected coliforms at a level similar to that in the duodenum and ileum, enterococci in significantly higher amounts than in the duodenum and

Table 1

Composition of aerobic microbial flora of different compartments of the digestive tracts of control and exposed led rats

Specification	Group (n)	Coliform	Enterococci	Streptococci	*Candida*
Duodenum	C-6 days (5)	0.44 ± 0.40	0.47 ± 0.13	0.28 ± 0.15	ND
	C-22 days (5)	0.66 ± 0.08	0.41 ± 0.13	0.42 ± 0.20	ND
	control (7)	0.78 ± 0.04	0.63 ± 0.06	ND	ND
	Pb (11)	0.62 ± 0.11	0.39 ± 0.12	2.33 ± 2.0	0.38 ± 0.14
Ileum	C-6 days (5)	0.66 ± 0.08	0.41 ± 0.13	0.42 ± 0.20	ND
	C-22 days (5)	0.66 ± 0.08	0.41 ± 0.13	0.42 ± 0.20	ND
	control (7)	0.71 ± 0.07	0.57 ± 0.20	ND	ND
	Pb (11)	0.62 ± 0.10	0.34 ± 0.34	0.34 ± 0.21#	0.40 ± 0.11
Caecum	C-6 days (5)	0.66 ± 0.08	0.42 ± 0.13	0.42 ± 0.2	ND
	C-22 days (5)	0.66 ± 0.08	0.41 ± 0.12	0.42 ± 0.2	ND
	control (7)	0.71 ± 0.07	0.57 ± 0.1	ND	ND
	Pb (11)	0.62 ± 0.10	0.48 ± 0.13*	0.57 ± 0.15#	0.34 ± 0.10
Colon	C-6 days (5)	0.66 ± 0.08	0.41 ± 0.13	0.42 ± 0.2	ND
	C-22 days (5)	0.66 ± 0.8	0.41 ± 0.13	0.42 ± 0.2	ND
	control (7)	0.71 ± 0.07	0.57 ± 0.09	ND	ND
	Pb (11)	0.62 ± 0.1	0.50 ± 0.16*	0.57 ± 0.15#	3.9 ± 0.1

Data are expressed as $\log_{10}$ mean ± SD of bacterial population density (cfu ml^{-1}), C-6 days – 6-day-old control rats, C-22 day – 22-day-old control rats, control – three-month-old control rats, Pb – three--month-old exposed lead rats, ND – not detected;

* ($p < 0.05$) difference statistically significant in comparison with exposed lead rats in duodenum related to Enterococci (Mann-Whitney test);

($p < 0.05$) difference statistically significant in comparison with exposed lead rats in duodenum related to Streptococci (Mann-Whitney test).

ileum, and streptococci at a level comparable to those in the ileum, but significantly lower than in the duodenum. The number of *Candida* in this group was comparable to the ones in the duodenum and ileum. In the colon of the control rats group we found coliforms and enterococci. In the Pb-exposed group we detected coliforms at levels similar to those in the duodenum, ileum and caecum, and enterococci levels were similar to the ones in the caecum but significantly higher than in the duodenum and ileum. The number of streptococci identified in this group was comparable to that in the caecum, but was significantly higher than in the ileum and significantly lower than in the duodenum. The number of yeasts in the colon samples of the Pb-exposed group was approximately ten times higher than the amounts in the other sections of the intestine.

In 6-day-, 22-day- and three-month-old control rats we observed coliforms and enterococci in all parts of the digestive tract. Streptococci were noted only in 6-day- and 22-day-old control rats.

DISCUSSION

Studies on bacterial cultures show that the lower part of the gastrointestinal tract is colonized by a higher number of predominantly anaerobic microorganisms than the upper part, which is colonized by aerobic microorganisms (Berg 1996, Rolf 1984). The final part of the ileum serves as a kind of transition zone between aerobic flora above and the large intestine with its anaerobic microflora below (Berg 1996). The development of microbiota in the body starts from the moment of birth. First, the sterile gut is colonized by aerobic bacteria: *Staphylococcus*, *Streptococcus* and *Enterobacteriaceae*. As the amount of oxygen in the intestine decreases, obligatory anaerobes appear: *Bacterioides* and *Clostridium* (Collado et al. 2012, Mackie et al. 1999). Initial differences in the composition of the intestinal flora disappear after going on a steady diet.

Our study showed that only Gram-negative rods (coliform bacteria) and Gram-positive bacteria (*Enterococcus*) were present in the intestine of the control group. In the rats which were prenatally and neonatally exposed to PbAc in drinking water, there were significantly fewer enterococci in all the examined sections of the intestine compared with the control group. In all the tested sections, the number of coliforms was lower in the Pb-exposed group than in the control group. In contrast, streptococci were found in all the parts of the intestine of the Pb-exposed group, but were not detected in the control group. The largest number of streptococci was found in the duodenum of the rats exposed to PbAc. There were no yeasts in the control group, but they were present in the Pb-exposed group. The number of *Candida* in the duodenum was approximately ten times higher than the amounts in the other examined sections.

The prenatal and neonatal exposure to lead may decrease the number of commensal coliforms and enterococci species and promote colonization of pathogenic streptococci (especially in the duodenum) and yeasts (especially in the colon). Exposure to Pb may affect the bacterial flora of the large intestine, stimulating the pro-inflammatory activity of Pb, as well as increasing the risk of a large intestine cancer in individuals chronically exposed to lead.

Lead is absorbed through the digestive and respiratory systems, and its transport in the small intestine has been studied in the mouse intestine perfused *in situ*, and in segments of the rat intestine isolated *in vitro* (Diamond 1998). It crosses the apical membrane of the small intestine possibly through Ca^{2+} channels or by the endocytosis of a lead-binding protein. Pb may displace Ca^{2+} in the epithelial cells from calcium-binding proteins such as calbindins, or bind with other proteins, including metallothionein (Pb-MT). Lead may exit the cell through the basolateral membrane by displacing Ca^{2+} from the Ca^{2+}-ATPase or Ca^{2+}/Na^{+}- exchanger (Diamond 1998). Colic is an early symptom of Pb poisoning, indicating the potential onset of more acute effects which may appear with prolonged exposure, usually due to industrial poisoning. The symptoms of colic include abdominal pain, constipation and diarrhoea (Hackett et al. 1982).

The impact of heavy metals on the gastrointestinal tract depends *inter alia* on the integrity of the intestinal barrier which serves as the first line of defence and is determined *inter alia* by host-microbial interactions. A change in the composition of gut microbiota may have been caused by decreased intestinal pH. In our examination, bacteria grew at higher pH, when staphylococci were not found. This decrease provided evidence for the influence on changes in the number of acidic preferring bacteria such as coliforms.

Our previous studies showed that chronic Pb exposure in rats caused inhibition of both spontaneous and stimulated transport of ions in the colon (Kosik-Bogacka et al. 2011). It is reported that mucin glycoproteins play an important role in protecting the intestine against chemical or physical damage. Their protective function depends on the amount of mucin released and the viscosity of the mucus blanket. They are also a barrier through which immunological, allergic and inflammatory reactions are triggered as a result of infection or intoxication, for example by Pb (Field, Semrad 1993). Moreover, microorganisms such as bacteria and fungi can absorb metal ions (Morozzi et al. 1986, Halttunen et al. 2008). Enteric microflora and its metabolites can also affect environmental parameters such as pH, oxidative balance, detoxification enzymes, and host proteins metabolizing and carrying xenobiotics (Claus et al. 2008), which may indirectly affect the bioavailability of metals in the intestine.

Streptococci, which were undetected in the control group, were found in all the studied sections of the Pb-exposed rat intestine, with the highest number found in the duodenum. In this study, we also observed that the

number of *Candida* in the colon of the rats exposed to lead was approximately ten times higher than in any other examined section of the intestine. The presence of fungi in stool samples is common, as yeasts as normal flora can colonize the gastrointestinal tract. Diarrhoea as the result of infection caused by yeasts can occur in immuno-compromised patients. The occurrence of diarrhoea in all the animals of the study group confirms that exposure to lead can increase the probability of ailments in the gastrointestinal tract.

CONCLUSIONS

Prenatal and neonatal exposure to lead decreases the number of coliforms and enterococci bacteria, and may promote colonization of the intestine by streptococci (especially in the duodenum) and yeasts (particularly in the colon). Streptococci and yeasts are more alkaline microorganisms than coliforms and enterococci. Exposure to Pb can effect changes in the pH of the environment in the gastrointestinal tract and this way it can lead to the selection of specific species of bacteria.

REFERENCES

Baranowska-Bosiacka I., Struzynska L., Gutowska I., Machalinska A., Kolasa A., Klos P., Czapski G.A., Kurzawski M., Prokopowicz A., Marchlewicz M., Safranow K., Machalinski B., Wiszniewska B., Chlubek D. 2012. *Perinatal exposure to lead induces morphological, ultrastructural and molecular alterations in the hippocampus*. Toxicology, 303:187-200.

Barton H.J. 2011. *Advantages of the use of deciduous teeth, hair, and blood analysis for lead and cadmium bio-monitoring in children. A study of 6-year-old children from Krakow (Poland)*. Biol. Trace Elem. Res., 143: 637-658.

Berg R.D. 1996. *The indigenous gastrointestinal microflora*. Trends Microbiol., 4: 430-435.

Breton J., le Cclere K., Daniel C., Sauty M., Nakab L., Chassat T., Dewulf J., Penet S., Carnoy C., Thomas P., Pot B., Nesslany F., Foligne B. 2013. *Chronic ingestion of cadmium and lead alters the bioavailability of essential and heavy metals, gene expression pathways and genotoxicity in mouse intestine*. Arch. Toxicol., 87: 1787-1795.

CDC (Centers for Disease Control and Prevention). 2007. *Interpreting and managing blood lead levels <10 μg/dl in children and reducing childhood exposures to lead: recommendations of CDC's Advisory Committee on Childhood Lead Poisoning Prevention*. MMWR Recomm. Rep., 56: 1-16.

Claus S.P., Tsang T.M., Wang Y., Cloarec O., Skordi E., Martin F.P., Rezzi S., Ross A., Kochhar S., Holmes E., Nicholson J.K. 2008. *Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes*. Mol. Syst. Biol., 4: 219.

Collado M.C., Cernada M., Bauerl C., Vento M., Perez-Martinez G. 2012. *Microbial ecology and host-microbiota interactions during early life stages*. Gut Microbes, 3: 352-365.

Diamond G.L., Goodrum P.E., Felter S.P., Ruoff W.L. 1998. *Gastrointestinal absorption of metals*. Drug Chem Toxicol., 21: 223-251.

Eckburg P.B., Bik E.M., Bernstein C.N., Purdom E., Dethlefsen L., Sargent M., Gill S.R., Nelson K.E., Relman D.A. 2005. *Diversity of the human intestinal microbial flora*. Science, 308: 1635-1638.

Field M., Semrad C.E. 1993. *Toxigenic diarrheas, congenital diarrheas, and cystic fibrosis: disorders of intestinal ion transport*. Annu. Rev. Physiol., 55: 631-655.

Gerhardsson L., Akantis A., Lundstrom N.G., Nordberg G.F., Schutz A., Skerfving S. 2005. *Lead concentrations in cortical and trabecular bones in deceased smelter workers*. J. Trace Elem. Med. Biol., 19: 209-215.

Hackett P.L., Hess J.O., Sikov M.R. 1982. *Effect of dose level and pregnancy on the distribution and toxicity of intravenous lead in rats*. J. Toxicol. Environ. Health, 9: 1007-1020.

Halttunen T., Salminen S., Meriluoto J., Tahvonen R., Lertola K. 2008. *Reversible surface binding of cadmium and lead by lactic acid and bifidobacteria*. Int. J. Food. Microbiol., 125: 170-175.

Jurkiewicz A., Wiechula D., Nowak R., Gazdzik T., Loska K. 2004. *Metal content infemoral head spongious bone of people living in regions of different degrees of environmental pollution in Southern and Middle Poland*. Ecotoxicol. Environ. Saf., 59: 95-101.

Kang J.K., Sul D., Kang J.K., Nam S.Y., Kim H.J., Lee E. 2004. *Effects of lead exposure of phospholipid hydroperoxidase glutathione peroxidase mRNA in rat brain*. Toxicol. Sci., 82: 228-236.

Kosik-Bogacka D.I., Baranowska-Bosiacka I., Marchlewicz M., Kolasa A., Jakubowska K., Olszewska M., Lanocha N., Wiernicki I., Millo B., Wiszniewska B., Chlubek D. 2011. *The effect of L-ascorbic acid and/or tocopherol supplementation on electrophysiological parameters of the colon of rats chronically exposed to lead*. Med. Sci. Monit., 17 BR16-BR26.

Mackie R.I., Sghir A., Gaskins H.R. 1999. *Developmental microbial ecology of the neonatal gastrointestinal tract*. Am. J. Clin. Nutr., 69: 1035S-1045S.

Morozzi G., Cenci G., Scardazza F., Pitzurra M. 1986. *Cadmium uptake by growing cells of gram--positive and gram-negative bacteria*. Microbios, 48: 27-35.

Ouwehand A., Isolauri E., Salminen S. 2002. *The role of the intestinal microflora for the development of the immune system in early childhood*. Eur. J. Nutr., 41: 132-137.

Palmer C., Bik E.M., Digiulio D.B., Relman D.A., Brown P.O. 2007. *Development of the human infant intestinal microbiota*. PLOS Biol., 5: e177.

Pastuszewska B., Ochtabińska A., Morawski A. 2000. *A note on the nutritional adequacy of stock diets for laboratory rats and mice*. J. Anim. Feed *Sci.,* 9: 533-542.

Rolf R. 1984. *Interactions among microorganisms of the indigenous intestinal flora and their influence on the host*. Rev. Infect. Dis., 6: S73-S79.

Sadykov R., Digel I., Artmann A.T., Porst D., Linder P., Kayser P., Artmann G., Savitskaya I., Zhubanova A. 2009. *Oral lead exposure induces dysbacteriosis in rats*. J. Occup Health, 51: 64-73.

Savino F., Cresi F., Pautasso S., Palumeri E., Tullio V., Roana J., Silvestro L., Oggero R. 2004. *Intestinal microflora in breastfed colicky and non-colicky infants*. Acta Paediatr., 93: 825-829.

Schmidt C., Stallmach A. 2005. *Etiology and pathogenesis of inflammatory bowel disease*. Minerva Gastroenterol. Dietol., 51: 127-145.

Sharma R., Barber I. 2014. *Lead toxicity and postnatal development of gastrointestinal tract*. Univers. J. Environ. Res. Technol., 4: 121-133

Wiechuła D., Jurkiewicz A., Loska K. 2008. *An assessment of natural concentrations of selected metals in the bone tissues of the femur head*. Sci. Total Environ., 406: 161-167.

Xu S.Z., Bullock L., Shan C.J., Cornelius K., Rajanna B. 2005. *PKC isoforms were reduced by lead in the developing rat brain*. Int. J. Dev. Neurosci., 23: 53-64.