

## Effect of Perinatal Lead Exposure on the Social Behaviour of Laboratory Mice Offspring at Adolescent Age

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### Abstract

Lead (Pb) was given to Swiss-Webster female mice at the concentrations of 0.1 and 0.2% (w/v), containing 550 and 1100ppm of lead respectively, in their drinking water. Treatment started from day 1 of pregnancy until day 15 postnatally. Thereafter, the dams were switched to plain tap water. After the weaning period (21 days), all male offspring were isolated (one animal per cage) for 14 days, and the isolated male offspring were subjected to "Standard Opponent" test at the age of 36 days. The results of this test showed a significant and dose-dependent increase in the non-social behaviour, whereas such results showed a significant decline in the social behaviour including naso-genital and naso-nasal contact, number of fights, rear, wall rear and displacement activities of the Pb exposed young adult male offspring. The present perinatal Pb effects in the male offspring are possibly via in utero exposure and/or via mother's milk.

**Key Words:** lead; perinatal exposure; mice offspring; standard opponent test; social behaviour.

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### Introduction

Lead (Pb) exposure/toxicity is a leading environmental health issue for children and women of childbearing age (Mushak, 1992). In both humans and experimental animals, Pb readily crosses the placental-fetal barrier (Donald *et al.*, 1986; Goyer, 1990; Lataillade *et al.*, 1995), causing a direct relation between the Pb-exposed mother and the possibility for irreversible developmental damage to her offspring (Oliver, 1911; Paul, 1960; Rom, 1976).

Many recent reports showed that Pb exposure continues to be a major public health problem worldwide (Dykeman *et al.*, 2002; Lidsky and Schneider, 2003; Huang and Schneider, 2004; Shalan *et al.*, 2005; Bellinger, 2006). Moreover, animal studies have confirmed that exposure to low levels of Pb during the perinatal period can produce long-lasting changes in the brain (Kuhlmann *et al.*, 1977). The nervous system is the primary target for the low levels of Pb exposure and the developing brain is extremely sensitive to the toxic effects of Pb during the perinatal (gestational and lactational) period (Zawia *et al.*, 1998; Chetty *et al.*,

2001; Basha *et al.*, 2003; Huang and Schneider, 2004; De Marco *et al.*, 2005). Thus, chronic exposure to Pb during the perinatal period produces central nervous system impairments as indicated by behavioural, physiological and biochemical measures (Ernhart and Greene, 1990; Lanpear *et al.*, 2000, 2001; Antonio *et al.*, 2003; Ajarem *et al.*, 2003).

Many investigators have studied the influence of lead on behavioural activities both in humans (Bellinger *et al.*, 1984; Ernhart *et al.*, 1985; Singh and Ashraf, 1989; Rothenberg *et al.*, 1989; McMichael *et al.*, 1992) and in animals (Levin and Bowman, 1983, 1986; Laughlin *et al.*, 1983; Donald *et al.*, 1987; Draski *et al.*, 1989; Davis *et al.*, 1990; Ajarem, 1999). However, experimental studies concerning the effects of lead exposure perinatally on social behavioural of the offspring during age are very limited. Thus, the aim of the present work was to study the effects of perinatal Pb exposure on the social behaviour of the offspring at adolescent age.

## Materials and Methods

### Experimental animals

Adult male and female Swiss-Webster mice were housed in opaque plastic cages (three females with one male in each cage) in the animal facility of Zoology Department, King Saud University, Riyadh, Saudi Arabia. Animals were kept under reversed lighting conditions with white light on from 22.30 to 10.30 hours local time. The ambient temperature was regulated between 18 and 22° C. On day 1 of pregnancy (appearance of vaginal plug was considered as day one of pregnancy), the males were removed from the cages and the females were exposed to experimental treatments. Food (Pilsbury's Diet) and water were available *ad libitum*, unless otherwise indicated.

### Lead administration

Lead acetate (analytical grade, Riedel de Haen, Germany) was dissolved in deionized distilled water in the concentrations of 0.1 and 0.2% (w / v), containing 550 and 1100 ppm of lead respectively. These lead concentrations formed the sole drinking fluid source for the experimental group of dams during the perinatal period of the experiment. The drinking fluid containing lead concentrations were changed with fresh preparations every five days. The control group received deionized distilled water only . In order to preclude the precipitation of insoluble lead salts, 1-2 drops of acetic acid (vehicle of lead) were added to all bottles (including controls). No "vehicle control" group was included in this study. All pregnant mice were housed individually. Treatment of mothers was started from day 1 of pregnancy and it continued until the postnatal day15 (PD15) after delivery and thereafter the mothers were

switched to plain deionized distilled water.

### "Standard Opponent" Test

After weaning at the age of 22 days, 10 male offspring from each treatment category and control group were individually housed in new cages for 14 days. After this isolation period, these male offspring were subjected to "standard opponent" tests under dim red lighting (ca. 8 lux) as described by Brain *et al.*, (1989). The docile and age-matched male "standard opponents" were rendered anosmic by applying 25µl of 4% zinc sulphate solution to the nasal tract under ether anesthesia for three days prior to encounters (Brain *et al.*,1981). The anosmic 'standard opponent' intruders were introduced in the home cages of 'test animals' and the "standard opponent" test of each 'test animal' was observed visually for 500 seconds. The opponents were used only once and the selected "elements" of behaviour were studied ( Brain *et al.*,1987 ; Ajarem and Ahmed, 1991).

### Statistical Analysis

Data of "Standard opponent" tests were compared within the experimental groups by the analysis of variance ( ANOVA ) and subsequently were analysed using Mann-Whitney U test (Sokal and Rohlf, 1981).

## Results

The behavioural data (median with ranges) in the "Standard Opponent" test of male offspring are given in (Tables 1 and 2). Almost all of the elements of behaviour were affected by perinatal Pb exposure and these effects were statistically significant in a dose-dependent manner. The non-social and social behaviour of the exposed offspring were significantly

**Table 1.** Effect of the perinatal lead exposure on the social behaviour of male laboratory mice offspring , at adolescent age, in a 'standard opponent test'.

Treatment group	Median number (with ranges) Number of seconds allocated to behaviours like:					
	Nonsocial investigation	Social investigation	Defense	Threat	Attack	Displacement
Control	104.7 (163.9 - 39.1)	231.3 (290.3 - 200.0)	4.4 (18.5 - 1.9)	7.7 (20.8 - 4.0)	120.4 (159.8 - 98.2)	24.9 (53.3 - 3.2)
w/v % 0.1	* 300.1 (340.4 - 262.2)	** 125.9 (182.8 - 95.2)	5.9 (9.8 - 0)	15.8 (38.1 - 9.1)	* 25.8 (59.2 - 9.1)	17.6 (38.2 - 5.5)
w/v % 0.2	*** 364.1 (399.1 - 279.1)	*** 110.4 (190.6 - 82.7)	3.5 (12.4 - 0)	13.4 (39.4 - 2.2)	*** 4.0 (11.2 - 0)	* 6.5 (17.8 - 3.6)

\*, \*\* and \*\*\* statistically significant at  $P < 0.05$  ,  $P < 0.01$  and  $P < 0.001$  respectively from the control by Mann-Whitney U-test .

**Table 2.** Effect of the perinatal lead exposure on the number of various acts and postures of social behaviour of male laboratory mouse offspring at adolescent age, in a 'standard opponen test'.

Treatment group	Median number (with ranges) of acts and postures						
	Latency to (Threat (sec	Latency to Attack (sec)	Number of fights	Number of Naso-Nasal contact	Number of Naso-Genital contact	Wall rears	Rears
Control	3 (60 - 2)	66 (210 - 9)	25 (37 - 13)	35 (46 - 23)	31 (43 - 27)	19 (35 - 15)	12 10 (31 -
w/v % 0.1	* 130 (155 - 90)	150 (170 - 115)	9 (15 - 3)	* 19 (28 - 12)	* 17 (20 - 12)	* 11 (14 - 8)	* 8 (9 - 7)
w/v % 0.2	*** 220 (350 - 180)	** 305 (430 - 0)	*** 2 (4 - 0)	*** 7 (19 - 5)	*** 9 (12 - 6)	*** 5 (9 - 3)	*** 2 (5 - 0)

\*, \*\* and \*\*\* statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from the control by Mann-Whitney U-test.

and dose-dependently affected showing an increase in the former and a decrease in the later, respectively. The elements of social behaviour including attack, numbers of fights, naso-nasal and naso-genital contacts, wall rears and rears were decreased significantly in a dose-dependent manner. The latencies to threat and attack were also increased significantly and dose-dependently. Overall, the results indicate that the social behaviour is significantly decreased due to the perinatal Pb exposure in a dose-dependent manner. Conversely, the nonsocial behaviour and its elements like wall rears and rears, in young male adult offspring were increased significantly and dose-dependently.

### Discussion

The present results clearly suggest that the dams exposure to lead perinatally (prenatally and postnatally), caused lead exposure to the developing offspring in utero during the gestational development (prenatal period) as well as during lactational period through milk (postnatal period). After the weaning period, various behavioural indices in young male offspring were affected in the "Standard Opponent" test. Thus, these results clearly emphasize that prenatal lead exposure is extremely dangerous and a strong correlation exists between maternal and umbilical cord blood Pb levels indicating prenatal transfer of lead from mother to developing fetus in utero (Gardella, 2001). It may further be ascertained that the in utero developing central nervous system in the fetuses, is also a critical point of development,

and the prenatal period is extremely vulnerable to the toxic insult of Pb exposure. Such toxic insult of Pb exposure has been reportedly expressed in disturbed form of morphological development, sensory motor reflexes, biochemical and behavioural outcomes in rodents (Grant *et al.*, 1980; Draski *et al.*, 1989; Ferguson *et al.*, 1998; Antonio *et al.*, 2003; Ajarem *et al.*, 2003; DeMarco *et al.*, 2005).

It is now well documented that significant quantities of compounds that are given to mothers during late pregnancies and during postnatal period, may be transmitted to the offspring *in utero* and/or via mother's milk during lactation (Draski *et al.*, 1989; Ajarem and Ahmad, 1991; 1998a and b; Ajarem and Brain, 1993; Ajarem, 1999; Ajarem *et al.*, 2003). It is known that a major portions of brain cells (70%) of the closely related rats are formed after birth (Patel, 1983). Furthermore it has been established that the cerebellum in the brain may be most vulnerable to the neurotoxicity of lead in the very rapid growth period during the first 20 postnatal days (Gietzen and Wooley, 1984). Thus, lead could have produced cumulative developmental abnormalities in the brain perinatally, which is expressed in the young adult male offspring in disturbed form of long lasting social behavioural outcomes.

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## تأثير التعرض للرصاص قبل الولادة وبعدها على السلوك الاجتماعي لنسل الفئران المعملية في مرحلة البلوغ

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قسم علم الحيوان – كلية العلوم – جامعة الملك سعود . ص . ب . ٢٤٥٥ . الرياض – ١١٤٥١ . المملكة العربية السعودية .

### الملخص

أعطيت إناث الفئران السويسرية الرصاص بالتركيزين ( ٠,١ و ٠,٢ وزن / حجم ) اللذين يحتويان على ٥٠٠ و ١١٠٠ جزء لكل مليون من الرصاص على التوالي عن طريق ماء الشرب ، بالإضافة إلى المجموعة الضابطة. بدأت المعاملة من بداية الحمل وحتى اليوم الخامس عشر بعد الولادة حولت بعدها الأمهات إلى ماء الشرب العادي. عزلت المواليد الذكور عن أمهاتها في اليوم الحادي والعشرين من عمرها ، بحيث يعزل كل ذكر على حدة في قفص لمدة أربعة عشر ( ١٤ ) يوماً، ثم أخضعت تلك المواليد بما فيها مواليد العينة الضابطة لاختبار المواجهة القياسية . أظهرت نتائج الاختبارات زيادة معنوية معتمدة على الجرعة في الحركات غير الاجتماعية قبالها نقص معنوي في الحركات الاجتماعية، حيث نقصت عدد مرات الاتصال عن طريق الأنف أو المؤخرة ، عدد مرات العراك، الوقوف في الهواء أو على الجدار و سلوك التنحي. وقد أوضحت النتائج عموماً أن التعرض للرصاص خلال فترة النمو المبكرة أدت إلى تغيرات في سلوك الفئران الاجتماعي ، وأن هذه التغيرات أشارت إلى سهولة وصول الرصاص إلى الصغار المعاملة أمهاتها بالرصاص عن طريق الرحم أو عن طريق حليب الأم أو بكليهما، مما يوحى بخطورة التعرض للرصاص خلال فترة النمو المبكرة من حياة الحيوان .

**الكلمات المفتاحية :** الرصاص ، التعرض قبل الولادة وبعدها ، ذرية الفئران ، اختبار المواجهة القياسية ، السلوك الاجتماعي.