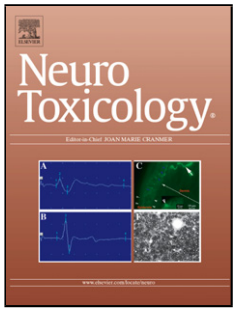

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comparLow arsenic concentrations impair memory in rat offpring exposed during pregnancy and lactation: Role of α7 nicotinic receptor, glutamate and oxidative stress

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Highlights

- **Exposure to arsenic during development impairs memory in female offspring.**
- **Exposure to arsenic decreases the expression of α7-AChR in hippocampus.**
- **Low arsenic levels cause oxidative damage in whole brain and hippocampus.**
- **Exposure to arsenic increases glutamate levels that may produce excitotoxicity.**

Abstract

Inorganic arsenic (iAs) is an important natural pollutant. Millions of individuals worldwide drink water with high levels of iAs. Arsenic exposure has been associated to cognitive deficits. However, the underlying mechanisms remain unknown. In the present work we investigated in female adult offspring the effect of the exposure to low arsenite sodium levels through drinking water during pregnancy and lactation on short- and long-term memory. We also considered a possible underlying neurotoxic mechanism. Pregnant rats were exposed during pregnancy and lactation to environmentally relevant iAs concentrations (0.05 and 0.10 mg/L). In 90-day-old female offspring, short-term memory (STM) and long-term memory (LTM) were evaluated using a step-down inhibitory avoidance task. Otherwise, we evaluated the α7 nicotinic receptor (α7-nAChR) expression, the transaminases and the oxidative stress levels in hippocampus. The results showed that the exposure to 0.10 mg/L iAs in this critical period produced a significant impairment in the LTM retention. This behavioral alteration might be associated with several events that occur in the hippocampus: decrease in α7-nAChR expression, an increase of glutamate levels that may produce excitotoxicity, and a decrease in the antioxidant enzyme catalase (CAT) activity.

Keywords: Arsenic; α7-nicotinic receptor; Oxidative stress; Glutamate; female rats

1. Introduction

Inorganic Arsenic (iAs) is a metalloid present in the earth's crust. Moreover, due to its ubiquitous presence in rocks and soil, high levels of arsenic are present in drinking water as a contaminant in Indo-Gangetic plains, regions of South East Asia and many parts of South America (Argos et al., 2010; Smith and Steinmaus, 2011; Steinmaus et al., 2010). iAs in drinking water is a global threat to health, and it affects about 200 million people in at least 70 countries worldwide (WHO, 2013). Arsenic causes a wide range of adverse health effects. The acute As (III) toxicity is more noxious than As (V) toxicity, but considering that As (V) is reduced to As (III) in the body, the two species are being considered equally toxic.

Chronic exposure to iAs through contaminated drinking water can damage tissue throughout the body and it is therefore associated to a wide range of human diseases, such as hyperpigmentation and keratosis, different cancer types (bladder, lung, kidney, liver, skin) and, vascular pathological conditions, such as Blackfoot disease, atherosclerosis, hypertension, and diabetes (Brown and Ziese, 2004; Jovanovic et al., 2012; Kapaj et al., 2006). Taking into account that the deleterious health effects are associated with chronic arsenic exposure, the

maximum limit of iAs in drinking water has been reduced to 0.01 mg/L by the World Health Organization and US Environmental Protection Agency (2001; WHO, 2013).

It has been found that arsenic affects both the central and peripheral nervous systems causing subclinical and clinical effects (Kim et al., 2012; Navas-Acien et al., 2006). Studies about the iAs effects in the development and function of the central nervous system (CNS) received less attention than the researches on genotoxicity and carcinogenicity. There are few reports about the iAs effects on the CNS and development and behavior toxicity evaluations. Epidemiological studies have revealed an association between iAs in drinking water and the risk of cognitive impairment including disturbed visual perception and visuomotor integration, psychomotor speed, attention, speech and memory (Calderon et al., 2001; Tsai et al., 2003). Decrease in the IQ of children exposed to ground water arsenic has frequently been reported (Wang et al., 2007)). In recent years, there has been heightening concern about the potential of occupational or environmental iAs exposure that affects neurological function in children and adolescents (Tsai et al., 2003; Tseng, 2009). Studies on rats and mice have been carried out to assess the impact of iAs on neurobehavioral performance (Bardullas et al., 2009; Gumilar et al., 2015; Luo et al., 2009; Rodriguez et al., 2010; Yadav et al., 2011). Some studies suggest that arsenic can adversely affect brain development and neural function even when it is present at very low concentrations in drinking water of 0.05 mg/L (Chaudhuri et al., 1999).

Multiple mechanisms in arsenic neurotoxicity have been suggested in experimental studies. Different animal studies demonstrate that neurons may be the major

targets of arsenic neurotoxicity and show myelin damage, disappearance of axons, vacuolar degeneration, and loss of cell-cell junction (Ma et al., 2010; Piao et al., 2011; Zarazua et al., 2010). Arsenic has been found to cause cholinergic dysfunctions associated with decreased activity of brain acetylcholinesterase and impaired learning and memory in experimental studies (Flora et al., 2009; Kopf et al., 2001; Nagaraja and Desiraju, 1994; Rodriguez et al., 2001; Wang et al., 2007). The key role of the hippocampus in the formation of many forms of memory, including inhibitory avoidance and maze tasks, has been broadly documented (Izquierdo et al., 2002). The hippocampus plays a central role in the memory formation and it could be critical for memory processes because the septohippocampal cholinergic input to the hippocampus is crucial (Drever et al., 2011; Dutar et al., 1995; Hasselmo, 2006).Acetylcholine release within hippocampal circuits results in the activation of both muscarinic (mAChRs) and nicotinic acetylcholine receptors (nAChRs), causing the subsequent modulation of cellular excitability and synaptic transmission. These two types of receptors are differentially expressed across the hippocampus and have different functions. nAChRs are ionotropic pentameric receptors made up of heteromeric or homomeric assemblies of α2-α10 and β2-β4 subunits. In the hippocampus, the nAChR subtypes predominantly expressed are α7, α4β2, and α3β4 (Albuquerque et al., 2009; Dani and Bertrand, 2007; Drever et al., 2011). α7-AChRs are widely expressed in dentate granule cells, pyramidal cells and interneurons both pre- and postsynaptically (Corradi and Bouzat, 2016; Fabian-Fine et al., 2001; Zoli et al., 2017). Also, ionotropic glutamate receptors are required and well-studied in the context of learning and memory. It was pointed out that AMPA subunit knockout

animals show deficient spatial working memory capabilities (Sanderson et al., 2008) and AMPA activation is necessary for the consolidation/retention processes (Yoshihara and Ichitani, 2004).

The underlying molecular mechanisms of iAs toxicity are not fully understood, but generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during metabolism is postulated as the possible mechanism (Liu et al., 2011). (Shi et al., 2004). Oxidative stress and apoptosis in the hippocampus have been associated with neurotoxicity induced by arsenic (Ahmed et al., 2012; Chandravanshi et al., 2014). The role of oxidative stress in iAs toxicity is further confirmed by *in vivo* and *in vitro* experiments indicating that iAs leads to reduction in superoxide dismutase activity, glutathione pools, and to increases of lipid peroxidation shown that *in utero* exposure may occur (Hood et al., 1987; Jin et al., 2006; Lindgren et al., 1984). Similar findings have been described in humans. Strong positive correlations have been found between cord and maternal blood arsenic levels in arsenic-exposed pregnant women (Hall et al., 2007), and it has been demonstrated that arsenic levels in cord and maternal blood were nearly identical among pregnant women living in an arsenic contaminated area, suggesting virtually free passage of arsenic across the placenta from the mother to the fetus (Concha et al., 1998). These findings suggest that the developing fetus is at risk for iAs exposure via placental transfer. In view of increasing risk of exposure to arsenic and associated vulnerabilities of the developing brain, studies have been carried out to assess the impact of arsenic exposure during prenatal and early postnatal periods (Gumilar et al., 2015; Herrera et al., 2013; Lu et al., 2014; Xi et

al., 2010a). While arsenic can be found in breast milk at very low concentrations (Xi et al., 2010b), it contributes to the exposure during gestation. In addition, there are results that show that iAs decreases the breast milk nutrients, which can indirectly contribute to the growth deficits in the offspring (Kozul-Horvath et al., 2012).

Although developmental neurotoxicity of arsenic in offspring rats by transplacental and early life exposure to high iAs concentration in drinking water has been studied (Xi et al., 2009), there is little information on the teratogenic effects of exposure to low levels of iAs during gestation and lactation. Recently, we have shown that rat offspring exposed to very low iAs concentrations, through drinking water during gestational and lactation period, evidence a delay in the development of sensory-motor reflexes and a decrease in locomotor activity. Our results suggest that exposure to low iAs concentrations produces dysfunction in the CNS mechanisms whose role is to regulate motor and sensory development and locomotor activity (Gumilar et al., 2015).

The present study has been carried out to assess the impact of low iAs exposure from gestational day 1 (GD1) to post-natal day 22 (PD22) about selected behavioral and neurochemical end-points on adult rats. Pregnant rats were therefore exposed to environmentally relevant 0.05 and 0.10 mg/L of iAs concentrations (~ 0.0065 mg/kg/day and 0.013 mg/kg/day in rats, respectively, based on our own measurements) during pregnancy and lactation. In 90-day-old female offspring, short-term memory (STM) and long-term memory (LTM) were evaluated using a step-down inhibitory avoidance task. In addition, we determine

the α7-nAChR expression, the transaminases and the oxidative stress levels in the hippocampus to elucidate a possible mechanism of the memory performance.

2. Materials and Methods

2.1 Animals and experimental design

Parent animals were male and nulliparous female Wistar rats (90-120 days old) obtained from the animal colony at the *Bioterio* of the *Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del* Sur, Bahía Blanca, Argentina. They were housed in polycarbonate cages and maintained at a constant condition of temperature (22º ± 1º C) and humidity (50% - 60%) in a 12L:12D cycle (lights on at 7:00 a.m) and with food (Ganave®, Alimentos Pilar S.A., Argentina) and water *ad libitum*. There was no environmental enrichment in the cages. Proestrus phase in female rats was determined according to the cell types observed in vaginal smear. The evening of the females' proestrus day, male and female rats were housed overnight in couples. The presence of spermatozoa in the vaginal smears was registered as an index of pregnancy and was referred to as gestational day 0 (GD 0). Pregnant females were weighed and housed individually in cages (~860 cm^2 and 18 cm height) and were randomly assigned to one of the three following groups: control group, a treated group with 0.05 mg/L of iAs in drinking water and other group treated with 0.10 mg/L of iAs in drinking water. Sodium arsenite (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in tap water and was administered to pregnant rats at concentrations levels of 0.05 and 0.10 mg/L iAs during the GD0 to PD21 throughout pregnancy and lactation, until weaned. Sodium arsenite is one of the most common trivalent compounds used in toxicological

studies and resembles the presence of this form in wells of contaminated areas. Drinking water was changed daily to minimize oxidation of arsenite to arsenate. The control group was supplied with tap water. Maternal weight gain and food intake were recorded at different GDs and drink consumption was recorded daily as described before (Gumilar et al., 2015).

The number of dams who delivered live litters indeed was n = 12 for each group. Within 24 h after birth, all pups were weighed and the litter size was determined. On postnatal day (PND) 3, litter size was randomly maintained at five males and five females, and the following data were analyzed: length of gestation, litter size and body weight of pups at different PNDs as described in (Gumilar et al., 2015). Pups were weaned and housed in groups of six rats according to treatment until PND 90. One female from each litter was used for the behavioral test, other female for the α7-AChR expression and other for the rest of neurochemical measure. For memory test we used a n = 10 per group, and for neurochemicals measures we used a n = 5 – 6 per group.

2.2 Step-down inhibitory avoidance

Rats were trained in a trial, step-down inhibitory avoidance paradigm, the hippocampal-dependent learning task in which stepping down from a platform present in a given context is associated with a footshock resulting in an increase in step-down latency (Bevilaqua et al., 2003). The inhibitory avoidance apparatus was a box with a floor consisting of parallel nonrusting steel bars. A 2.5 cm high platform was placed on the left end of the box. Latency of the rats to step down placing the four paws on the grid was measured. 24 hours prior to training, we

conducted a habituation of rats to the new environment, which consisted of placing the rat on the platform and let them explore for 180 seconds. In the training session, the animals were gently placed on the platform and received a 0.6 mA, 2 sec scrambled footshock immediately after they stepped down placing their four paws on the grid. Test sessions were carried out 1.5 and 24 h after training for short-term memory (STM) and long-term memory (LTM) evaluations, respectively. They were exactly like the training session, except that the footshock was omitted. A 180 sec ceiling was imposed on test session latency measurements. In the test sessions, step-down latency was used as measure of retention of memory (Canto-de-Souza and Mattioli, 2016; Izquierdo and Medina, 1997).

2.3 RNA extraction and cDNA synthesis

Ninety-day-old female offspring were sacrificed by decapitation. The brain rinsed in ice cold isotonic saline solution and hippocampus was immediately isolated, rinsed briefly in ice cold isotonic saline and immediately separated into hippocampus. Hippocampus region was extracted in accordance with coordinates taken from the atlas of Paxinos and Watson (A −4.2, L ±3.0, V 1.4) (Paxinos and Watson, 1986). The hipoccampus of control and exposed female offspring were individually collected in 500 µL of ice-cold TRIzol (SIGMA-Aldrich, Buenos Aires, Argentina) and homogenized. After chloroform extraction and isopropyl alcohol precipitation, RNA was dissolved in RNAse free water. To check RNA integrity, sample of RNA was loaded onto a 1% agarose gel, subjected to electrophoresis, stained with ethidium bromide, and visualized by UV transillumination. The RNA concentration

and purity were measured in a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA).

2.4 Reverse transcription and real time PCR (RT-qPCR) analysis

For each sample, 15 µg of total mRNA was converted into cDNA with Molony Murine Leukaemia virus reverse transcriptase (MLV-RT; Promega, USA) and random primers (Promega, USA) according to the protocol recommended by the manufacturer. The gene-specific primers are listed in Table 1. Relative quantification of mRNA by qPCR was performed using a 2X Syber Green-mastermix (Biodynamics, Argentina) and a Rotor-Gene 6000 (Corbett Reasearch, Australia). The comparative CT method was used to determine the relative gene expression (Schmittgen and Livak, 2008). The α7-nAChR transcript levels were estimated by using the formula $2^{-\Delta Ct}$ where ΔCt represents the difference in Ct values between α7-nAChR and GAPDH, the endogenous control gene (Dionisio et al., 2013).

Table 1
Primers used for RT-qPCR analysis

Target gene	Forward primer (5'-3')	Reverse primer (5'-3')	Product (bp)
α7	GCTGTACAAGGAGCTGGTCA	TGGATGTGGATGAGAAGAAC	116
GADPH	TTCACCACCATGGAGAAGGC	AGTGATGGCATGGACTGTGGTC	221

2.5 Preparation of brain homogenates

Ninety-day-old female offspring were sacrificed by decapitation. The whole brain or the hippocampus were collected and homogenized 1:40 (w/v) in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA and then were centrifuged at 4500 rpm for 10 min. The supernatant was kept cold for further determination of transaminases, enzymes activities and lipid peroxidation level.

2.6 Evaluation of the metabolism of glutamate

In the metabolism of glutamate, aspartate transaminase (AST) and alanine aminotransferase (ALT) take part. Therefore, the levels of transaminases in the hippocampus were determined. Biochemical determinations were made using specific kits (Wiener Lab, Argentina) by measurement of the optical density of reaction products at the corresponding wavelength with a spectrophotometer (Shimadzu UV-1203, UV–VIS spectrophotometer).

2.7 Assessment of oxidative stress:

2.7.1 Lipid peroxidation assay

As an indicator of the level of lipid peroxidation products presented in the whole brain, malondialdehyde (MDA) was assayed as thiobarbituric acid-reactive substance (TBARS) (Sepand et al., 2016). Finally, the level of MDA was quantified by a spectrophotometer at wavelength of 532 nm. MDA concentration was expressed as nmol/mg protein.

2.7.2 Catalase (CAT) assay

Catalase activity of each brain samples (whole brain or hippocampus region) were assayed following the procedure of Aebi (Aebi, 1984), with a slight modification. The catalase activity was measured by calculating the rate of degradation of hydrogen peroxide, the substrate of the enzyme. The enzyme activity was expressed as the rate constant of a first-order reaction (k) related to the protein content.

2.7.3 Glutathione peroxidasa (GPx) assay

In whole brain and hippocampus region, glutathione peroxidase activity was measured by the method used by Lawrence and Burk (Lawrence and Burk, 1976). The absorbance was recorded at 340 nm. The enzyme activity was expressed as μmoles of NADPH oxidized per min per mg protein.

2.7.4 Protein assay

Protein content was determined by the micromethod of Bradford (Bradford, 1976) with bovine serum albumin (BSA) as the standard protein.

2.8 Statistics

The step-down inhibitory avoidance task was analyzed by non-parametric statistics Kruskal-Wallis ANOVA followed by Mann Whitney test. Data of quantitative expression of α7-nAChR, level of transaminases, level of antioxidant enzymes and TBARS were detected by one-way analysis of variance (ANOVA) to analyze the between-group differences. If the results of ANOVA were significant ($p < 0.05$),

LSD test was then applied to compare the treated groups with the control ones. All statistical analyses were carried out using the software SPSS 21.0 for Windows.

2.9 Ethics

Animal care and handling were in accordance with the internationally accepted standard Guide for the Care and Use of Laboratory Animals (2011) as adopted and promulgated by the National Institute of Health. Experimental designs were also approved by the local standard for protecting animal's welfare, Institutional Committee for the Care and Use of Experimental Animals (Protocols Number 002/2014 and 077/2016).

3. Results

3.1. Data about the dams and their litters

There were no statistical differences in body weight between the groups of dams on GD 0, in maternal weight gain, food intake and drink comsumption during the different periods registered, and in gestational length or litter size (Fig. 1 and Table 1), as observed in our previous study (Gumilar et al., 2015). Also, iAs treatment during pregnancy and lactation did not affect the body weight of pups at PND 1, 4, 7, 14 and 21 (Table 2). No visible teratogenic malformation was observed in any pup of the different groups.

Table 2. Data of mothers and their litters

	Control group n = 12	**0.05 mg/L** n = 12	**0.10 mg/L** n = 12
Body weight of dams (g)			
GD0	283.5 ± 18.9	285.6 ± 7.4	285.8 ± 8.5
Body weight gain of dams (g)			
GD 0-3	11.9 ± 1.8	12.2 ± 2.0	10.2 ± 1.7
GD 3-6	10.1 ± 2.0	6.9 ± 2.0	7.1 ± 1.7
GD 6-9	11.0 ± 2.0	8.8 ±1.5	8.7 ± 0.5
GD 9-12	17.3 ± 2.3	16.4 ± 1.9	15.2 ± 1.7
GD 12-15	15.2 ± 2.6	11.6 ± 1.5	15.0 ± 1.5
GD 15-18	32.4 ± 3.4	27.8 ± 3.7	35.4 ± 3.4
GD 18-20	23.2 ± 2.9	17.9 ± 3.5	20.3 ± 3.8
GD 0-20	122.8 ± 6.1	100.8 ± 9.9	114.4 ± 9.0
Length of gestation (days)	22.0 ± 0	22.0 ± 0	22.0 ± 0
Litter size			
Female	5.2 ± 1.4	5.8 ± 1.2	5.8 ± 1.0
Male	6.2 ± 1.3	4.6 ± 1.0	5.1 ± 0.6
Total	10.8 ± 1.4	8.5 ± 1.7	10.9 ± 1.5
Body weight gain of Offspring			
PND1	6.3 ± 0.2	6.4 ± 0.1	6.5 ± 0.2
PND4	9.1 ± 0.4	8.7 ± 0.3	10.1 ± 0.4
PND7	14.0 ± 0.8	12.1 ± 0.5	13.8 ± 0.4
PND10	18.9 ± 0.9	17.3 ± 0.6	18.3 ± 0.7
PND13	23.7 ± 0.9	22.3 ± 0.8	23.5 ± 0.7
PND16	28.9 ± 1.2	28.0 ± 1.3	29.7 ± 1.8
PND19	30.0 ± 2.6	32.4 ± 1.2	34.6 ± 2.6
PND21	39.8 ± 2.4	39.5 ± 2.3	39.6 ± 2.9
Body Weight PND 90 (g)			
Female	195 ± 9.5	191 ± 10.7	197 ± 9.0

Values are mean ± SEM. GD: Gestational Day. PND: Post-Natal Day.

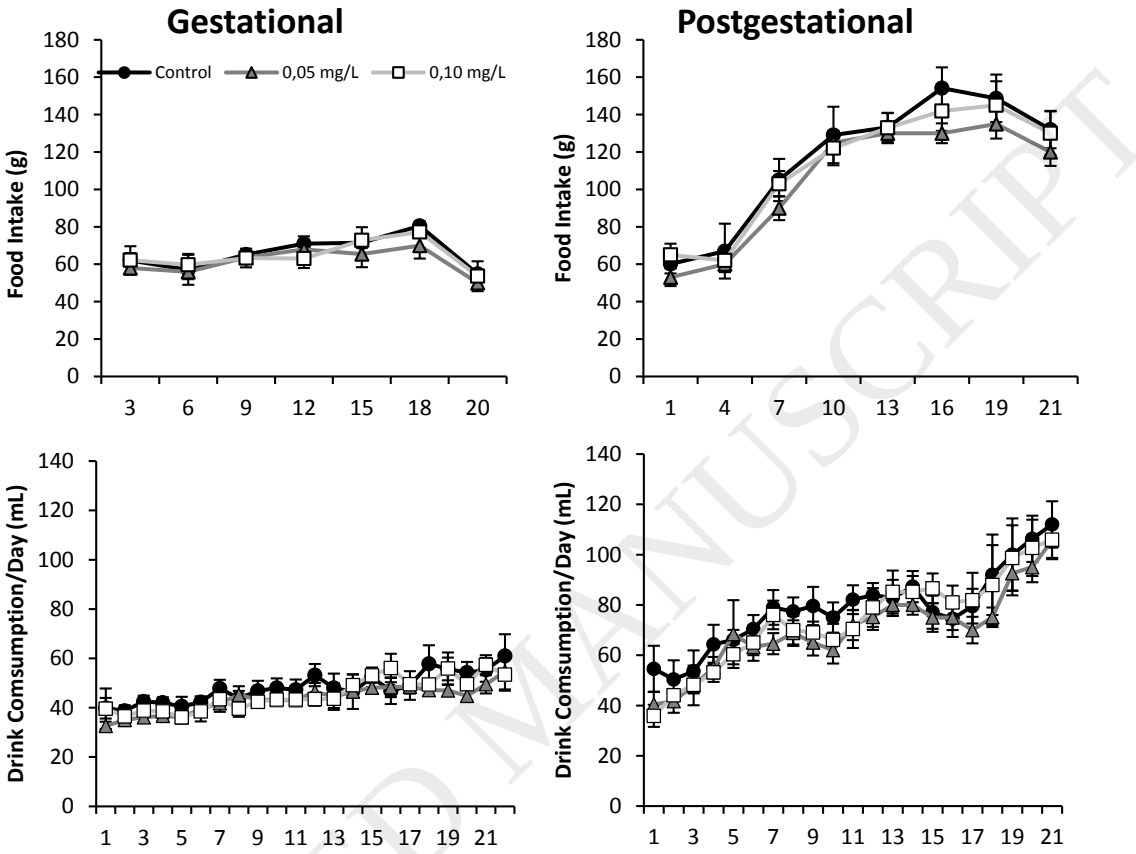


Fig. 1. Food intake of dams was recorded every 3 day during gestation development and lactation whereas drink comsumption was recorded daily. All results are expressed as Mean ± SEM of 12 animals per group.

3.2 Step-Down Inhibitory Avoidance

To evaluate the Short- and Long-Term Memory (STM and LTM), we performed the inhibitory avoidance test. The data represented in Fig. 2 show the results of the step-down inhibitory avoidance test. In female 90-day-old offspring, the exposure to 0.10 mg/L iAs produced a significant impairment in the retention of LTM. The latencies of these rats to step down on the grid were lower than those of the control group ($p < 0.01$) 24 h after training. This exposure did not affect memory retrieval

during the first retention test session (STM) carried out 90 min after shock. The latencies measured in the training session were similar for all groups. No differences in step-down latencies were detected between the animals exposed to the lower dose of iAs (0,05 mg/L) and the control group.

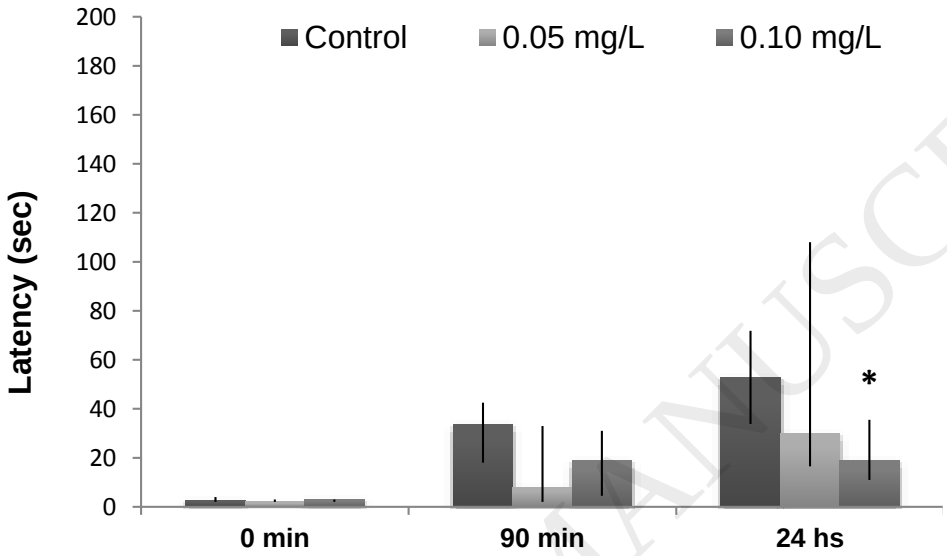


Fig. 2. Latencies measured in the step-down inhibitory avoidance test during the training session (0 min) and the test sessions for the evaluation of STM (90 min after training) and LTM (24 h after training) in the adult female offspring exposed to 0.05 and 0.10 mg/L iAs during gestation development and lactation. Bars represent median (±interquartile range) of step-down latencies in seconds of 10 animals per group. *$p < 0.01$, compared to control group.

Thus, 0.10 mg/L iAs supplied to dams during pregnancy and lactation impairs long-term memory of the offspring on PD90. Short-term memory is not affected at any of the tested iAs concentrations.

3.3 α7nAChR mRNA Expression in the Hippocampus

To investigate possible mechanisms by which iAs affect the long-term memory, we first explored α7-nAChR mRNA expression in the hippocampus region of 90 day-old rats using relative quantitative RT-qPCR. One-way ANOVA showed significant differences between groups ($F_{(2,15)}$ = 12.49, p < 0.001) in the expression of α7-nAChR in the hippocampus. As shown in Fig.3, post hoc comparisons showed that the female rats exposed to both iAs concentration exhibited a significant decrease in the α7-nAChR mRNA levels in the hippocampus compared to control group (p < 0.001).

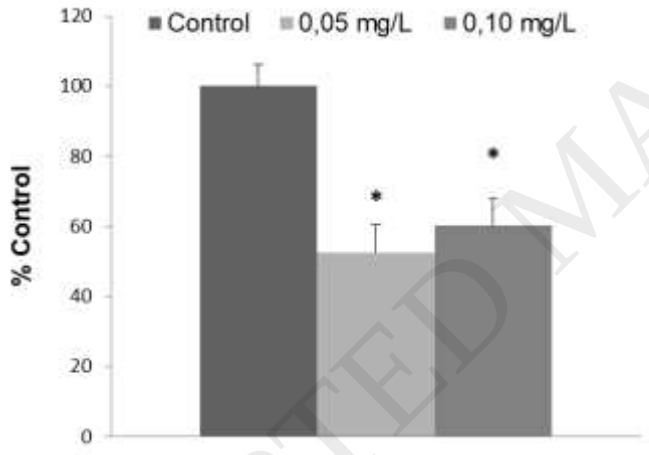
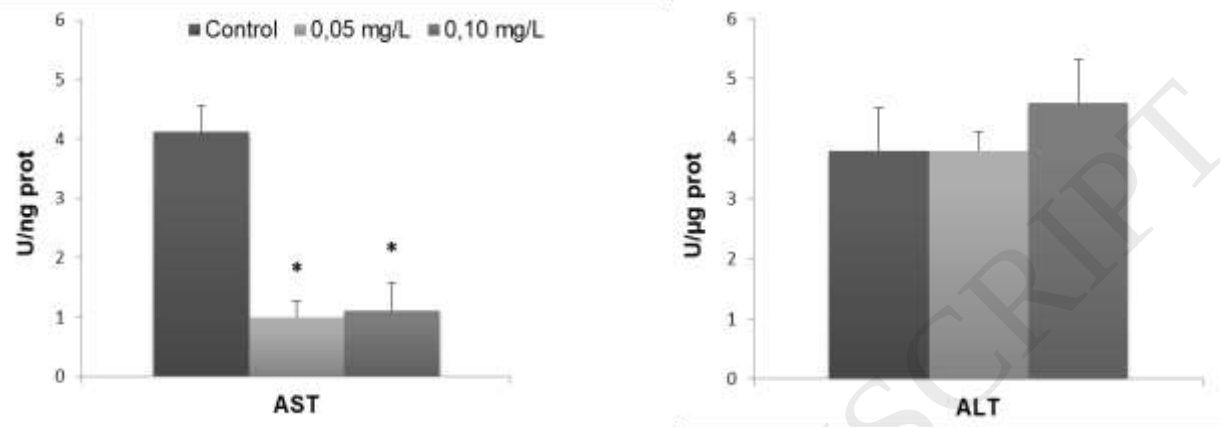


Fig. 3. Expression of α7-nAChR subunit at mRNA level in hippocampus from 90-day-old female offspring exposed during gestation development and lactation to 0.05 and 0.10 mg/L iAs. Quantitative real-time PCR was performed to determine mRNA level. The values are shown as the means ± SD. n = 5-6. *p < 0.001, compared to control group.

3.4. Evaluation of the metabolism of glutamate

To evaluate the glutamatergic pathway, we indirectly measured transaminase levels in 90-day-old rats. As show in Fig. 4, transaminases concentrations in supernatant of hippocampus of control and treated groups were assessed. One-

way ANOVA showed significant differences between groups ($F_{(2,12)}$ = 19.44, p < 0.001) in the levels of AST in hippocampus area. Post hoc comparisons showed a



significantly decrease in both treated groups compared to control group (p < 0.001, for both iAs concentrations). No significant differences were observed in ALT levels among groups.

Fig. 4. Transaminase levels in hippocampus from 90-day-old female offspring exposed during gestation development and lactation to 0.05 and 0.10 mg/L iAs. The values are shown as the means ± SD of n = 5. *p < 0.001, compared to control group.

Therefore, this significant decrease of AST in the hippocampus can be translated as an increase of glutamate that could produce excitotoxicity in this brain area.

3.5 Oxidative stress in whole brain and the hippocampus area.

In order to identify whether the decreased in the long-term memory is due to oxidative stress stimulated by low iAs concentrations during the gestational development and lactation, we studied the anti-oxidative enzyme activity and lipid peroxidation in brains of 90-day old rat offspring. Figure 5 illustrates the activities of antioxidant enzymes, namely CAT and GPx, and levels of lipid peroxidation as

MDA content, in the whole brain of the control and experimental groups of adult female offspring exposed to 0.05 and 0.10 mg/L iAs during development.

One-way ANOVA showed a significant differences between groups ($F_{(2,12)}$ = 3.98, p < 0.05) in the levels of CAT in whole brain. Post hoc comparisons showed a significant decrease in CAT activity in 0.10 mg/L iAs treated group compared to control group (p < 0.05). No significant differences were observed in one-way ANOVA in GPx levels. With respect to MDA content in whole brain, one-way ANOVA showed a significant differences between groups ($F_{(2,12)}$ = 40.63, p < 0.001). Post hoc comparisons showed a significant decrease in MDA in brains of offspring exposed to both concentrations of iAs (p < 0.001 for both concentrations).

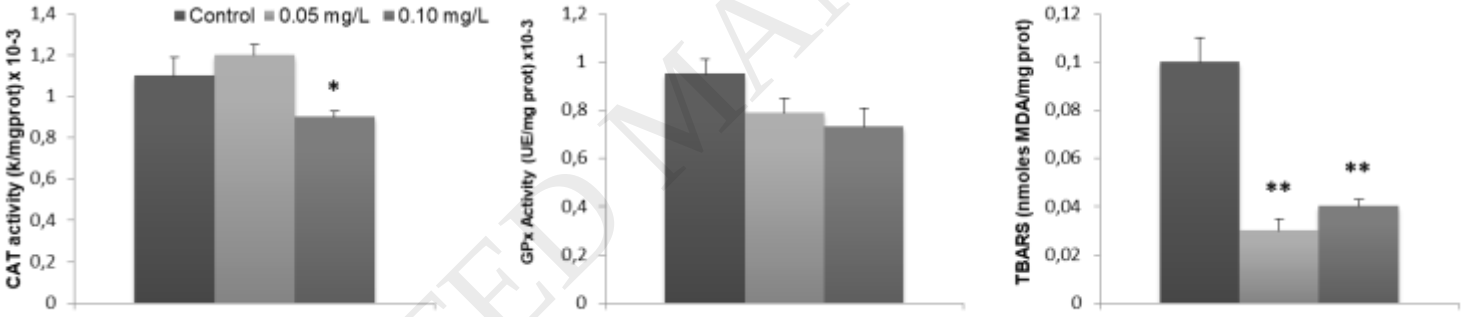


Fig. 5. Activities of CAT (A) and GPx (B), and MDA content (C) in the whole brain homogenates of adult female offspring exposed to 0.05 and 0.10 mg/L iAs during the gestation development and lactation. The values are shown as the means ± SD. n = 5. * p < 0.05; ** p < 0.001 compared to the control group.

We subsequently analyzed CAT activity as another parameter of oxidative stress in the hippocampus. One-way ANOVA revealed significant differences in CAT activity between groups, ($F_{(2,12)}$ = 51.19, p < 0.001). When offspring were exposed to 0.05 and 0.10 mg/L iAs during the pregnancy and lactation we observed a decreased CAT activity in hippocampus (p < 0.001) compared to control group (Fig. 6). No

statistically significant changes were observed in the GPx activity in this brain area when offspring were exposed to both iAs concentrations.

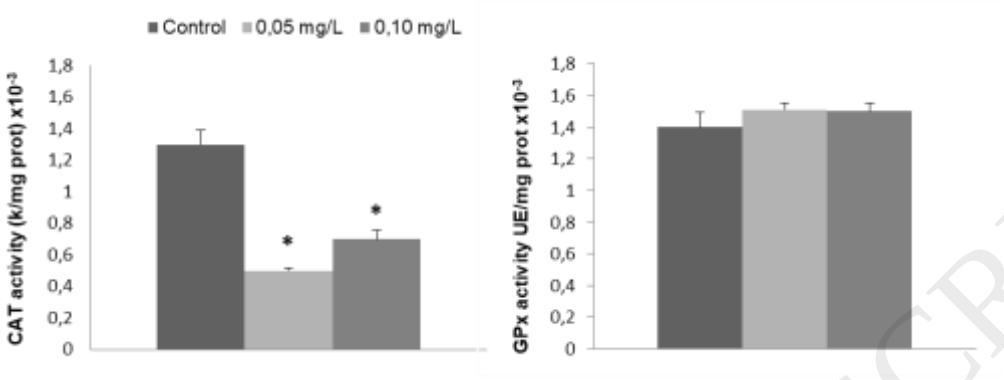


Fig. 6. Activities of CAT (A) and GPx (B) in the hippocampus of 90-day-old female offspring exposed to 0.05 and 0.10 mg/L iAs during the gestation development and lactation. The values are shown as the means ± SD. n = 5. * $p < 0.001$, compared to control group.

Discussion

The present study evaluates the potential effect of iAs on the developing nervous system of offspring rats whose dams were exposed to iAs in drinking water during pregnancy and lactation. Few investigations focused on long-lasting behavioral and neurochemical alterations in offspring prenatally exposed to low iAs concentrations. Previously, we demonstrated that early exposure to low iAs concentrations through contaminated maternal drinking water does not produce alterations to progeny development as shown in Fig.1 and Table 2, but it produces dysfunction in CNS mechanisms involved in the regulation of motor and sensory development and locomotor activity (Gumilar et al., 2015).

We here demonstrated that the early exposure to low levels of iAs impairs the retention of memory evaluated in the step down inhibitory avoidance task. The

arsenic concentrations here used are present in different water courses worldwide. We chose to work with female rats because we have previously shown that the two iAs concentrations here tested delayed the maturation of some reflexes and produced hypoactivity in female rats. In contrast, in male rats, delayed maturation of reflexes and hypoactivity were observed only in the animals exposed to the highest iAs concentrations (Gumilar et al., 2015). In the inhibitory avoidance tasks, we observed that the 90-day-old female offspring of dams exposed to 0.10 mg/L iAs during pregnancy and lactation evidenced deficits in the retention of long-term memory. In step-down inhibitory avoidance, during the training session, rodents associate stepping down from a platform with an unconditioned stimulus (foot-shock). The conditioned response is to avoid stepping down (Cammarota et al., 2005; Gold, 1986; Vianna et al., 2001). Learned fear may be elicited by exposure to environmental cues or contexts that were previously associated with an aversive stimulus (Gross and Canteras, 2012). Fear conditioning to aversive stimuli requires not only the amygdala but also the hippocampus (Burwell et al., 2004; Johansen et al., 2010), and the medial prefrontal cortex (Cenquizca and Swanson, 2007; Corcoran and Quirk, 2007). While the hippocampus, amygdala and medial septum plays an important role in memory consolidation, the entorhinal cortex is apparently critical for memory storage, subsequent to the previous processing of learned information by the hippocampus, amygdala and medial septum (Farioli-Vecchioli et al., 2008; Izquierdo et al., 1992; McGaugh, 2002; Olton and Papas, 1979).

The full biological significance of the changes in neurotransmitter levels induced by arsenic is not yet clear, but previous studies suggested that these alterations may precede the appearance of cognitive effects. Several studies suggest that

cholinergic and glutamatergic systems are involved in regulating learning and memory; the stimulation of these pathways facilitates learning and memory (Dennis et al., 2016; Jerusalinsky et al., 1997; Morris, 1989; Myhrer, 2003). Acetylcholine (ACh) has been extensively studied due to its prominent role in attention, learning, memory and synaptic plasticity (Everitt and Robbins, 1997; Hasselmo, 2006; Micheau and Marighetto, 2011). NMDA receptors are important for triggering learning-related plasticity. It has been suggested that the activation of the NMDA receptor is required for long-term potentiation in the hippocampus (Izquierdo, 1994).

In order to elucidate a possible mechanism by which iAs impairs the LTM, we evaluated the expression of α7-nAChR mRNA level in the hippocampus of adult female offspring. In female 90-day-old offspring exposed to both iAs concentrations above mentioned, we found a significantly decrease in the α7-nAChR subunit expression at mRNA level in hippocampus. The homomeric α7 receptor is one of the most abundant nAChRs in the nervous system. It plays an important role in cognition, memory, pain, neuroprotection, and inflammation. Potentiation of the α7 receptor emerges as a novel therapeutic strategy for several neurological diseases that impairs memory, such as Alzheimer (Bouzat et al., 2017).

Several studies show lesions to the septohippocampal pathway, and the consequent loss of cholinergic neurons related to deficits in memory functions usually associated with aging related disabilities or neurodegenerative diseases (Davis et al., 1999; Fibiger, 1991; McGaughy et al., 2000; Micheau and Marighetto, 2011; Muir, 1997; Schliebs and Arendt, 2011). Similarly, pharmacological or genetic inhibition of ACh receptors cause memory deficits but it is often unclear

which receptor subtypes are involved and in which part of the brain they are located (Anagnostaras et al., 2003; Atri et al., 2004; De Rosa and Hasselmo, 2000; Hasselmo et al., 2000; Warburton et al., 2003; Wess, 2004). Therefore, the cholinergic system and/or the α7-nAChR in hippocampus may participate in part of the loss of long-term memory.

As regards the glutamate system, the enzymes glutamine synthetase, aspartate aminotransferase and alanine aminotransferase are involved in glutamate metabolism. Our results showed that the activity of aspartate aminotransferase was inhibited by both concentrations of iAs in rat hippocampus. The reduced activity may impact on glutamate concentrations. Astrocytes play a key role when it comes to remove glutamate from the synaptic cleft and metabolizing it to glutamine. Glutamine is released from these cells and then taken up by neurons to serve as a glutamate precursor (Danbolt, 2001). On the other hand, Torres et al. (2013) have demonstrated that once in astrocytes, glutamate is preferentially used as a fuel in the tricarboxylic acid (TCA) cycle instead of being converted into glutamine. The authors suggested that oxidation of glutamate could buffer excitotoxic conditions due to high glutamate concentration. Our results show that the exposure to low iAs concentrations reduced glutamate degradation in glial cells due to a reduction in the AST involved in glutamate metabolism. Therefore, the excessive extracellular glutamate levels could be responsible for excitotoxic condition in the rat hippocampus and the deficits in the retention of long-term memory.

The neurotoxicity of iAs also could be due to the formation of reactive oxygen species (ROS) in the brain, which may cause a cell damage through lipid

peroxidation. ROS have been demonstrated to be present in the rat brain exposed to iAs (Flora et al., 2005). In addition, iAs exposure decrease the concentration of brain glutathione (GSH) in rats (Kannan and Flora, 2004) and depress antioxidant enzyme activities (Shila et al., 2005), which may account for the behavioral alterations in offspring rats. TBARS is an indicator of lipid peroxidation whose end product is malondialdehyde (MDA). It is known that many metals/metalloid affect cell membranes by altering their integrity via lipid peroxidation causing deterioration of their components (Gurer and Ercal, 2000). This is why we studied the levels of CAT, GPx and TBARS in whole brain and CAT and GPx in hippocampus. In whole brain we observed a significant decrease in CAT levels only in offspring exposed early to 0.10 mg/L iAs and a significant decrease in TBARS levels in the whole brain of offspring exposed to both concentrations of iAs, compared to control group. The decrease in TBARS levels reflects the lower degree of lipid peroxidation. We did not observe significant changes in the GPx levels. When we determined CAT activity in the hippocampus, we observed a remarkable decrease in offspring exposed to both iAs concentrations. We did not observe significant changes in the GPx levels in hippocampus. Therefore, we can infer that the decrease in CAT levels in the offspring exposed to 0.10 mg / L iAs, both in the whole brain and in the hippocampus, could be responsible in part, of impairs in the LTM. In contrast, in offspring exposed to 0.05 mg/L iAs, the antioxidant effect observed in whole brain (CAT activity is not affected and TBARS levels decrease) could produce a protective effect on the LTM, although it is not ruled out that there may be other compensatory mechanisms and brain areas involved in memory.

Conclusion

The results of this study demonstrated that the exposition to low iAs concentrations during pregnancy and lactation impair the retention of long term memory in female adult offspring. The possible mechanism by which impair the long-term memory is due to several events that may occur in the hippocampus: decrease in α7-nAChR expression, increase of glutamate levels that may produce excitotoxicity, and the decrease in CAT levels in offspring exposed to 0.10 mg/L iAs although other mechanisms may be involved.

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References

2001. US EPA Federal Register, 66.,6975-7066.
2011. Guide for the Care and Use of Laboratory Animals. 2011. The National Institute of Health, Eighth Edition.
Aebi, H., 1984. Catalase in vitro. Methods Enzymol 105, 121-126.
Ahmed, S., Ahsan, K.B., Kippler, M., Mily, A., Wagatsuma, Y., Hoque, A.M., Ngom, P.T., El Arifeen, S., Raqib, R., Vahter, M., 2012. In utero arsenic exposure is associated with impaired thymic function in newborns possibly via oxidative stress and apoptosis. Toxicol Sci 129(2), 305-314.
Albuquerque, E.X., Pereira, E.F., Alkondon, M., Rogers, S.W., 2009. Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev 89(1), 73-120.
Anagnostaras, S.G., Murphy, G.G., Hamilton, S.E., Mitchell, S.L., Rahnama, N.P., Nathanson, N.M., Silva, A.J., 2003. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci 6(1), 51-58.
Argos, M., Kalra, T., Rathouz, P.J., Chen, Y., Pierce, B., Parvez, F., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Slavkovich, V., van Geen, A., Graziano, J., Ahsan, H., 2010. Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. Lancet 376(9737), 252-258.
Atri, A., Sherman, S., Norman, K.A., Kirchhoff, B.A., Nicolas, M.M., Greicius, M.D., Cramer, S.C., Breiter, H.C., Hasselmo, M.E., Stern, C.E., 2004. Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word paired-associate memory task. Behav Neurosci 118(1), 223-236.
Bardullas, U., Limon-Pacheco, J.H., Giordano, M., Carrizales, L., Mendoza-Trejo, M.S., Rodriguez, V.M., 2009. Chronic low-level arsenic exposure causes gender-specific alterations in locomotor activity, dopaminergic systems, and thioredoxin expression in mice. Toxicol Appl Pharmacol 239(2), 169-177.
Bevilaqua, L.R., Kerr, D.S., Medina, J.H., Izquierdo, I., Cammarota, M., 2003. Inhibition of hippocampal Jun N-terminal kinase enhances short-term memory but blocks long-term memory formation and retrieval of an inhibitory avoidance task. Eur J Neurosci 17(4), 897-902.
Bouzat, C., Lasala, M., Nielsen, B.E., Corradi, J., Esandi, M.D.C., 2017. Molecular function of alpha7 nicotinic receptors as drug targets. J Physiol.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72, 248-254.
Brown, J., Ziese, L., 2004. Public health goal for arsenic in drinking water. Drinking Water Public Health Goals. Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.
Burwell, R.D., Saddoris, M.P., Bucci, D.J., Wiig, K.A., 2004. Corticohippocampal contributions to spatial and contextual learning. J Neurosci 24(15), 3826-3836.
Calderon, J., Navarro, M.E., Jimenez-Capdeville, M.E., Santos-Diaz, M.A., Golden, A., Rodriguez-Leyva, I., Borja-Aburto, V., Diaz-Barriga, F., 2001. Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 85(2), 69-76.
Cammarota, M., Bevilaqua, L.R., Barros, D.M., Vianna, M.R., Izquierdo, L.A., Medina, J.H., Izquierdo, I., 2005. Retrieval and the extinction of memory. Cell Mol Neurobiol 25(3-4), 465-474.
Canto-de-Souza, L., Mattioli, R., 2016. The consolidation of inhibitory avoidance memory in mice depends on the intensity of the aversive stimulus: The involvement of the amygdala, dorsal hippocampus and medial prefrontal cortex. Neurobiol Learn Mem 130, 44-51.
Cenquizca, L.A., Swanson, L.W., 2007. Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res Rev 56(1), 1-26.

Concha, G., Vogler, G., Lezcano, D., Nermell, B., Vahter, M., 1998. Exposure to inorganic arsenic metabolites during early human development. Toxicol Sci 44(2), 185-190.
Corcoran, K.A., Quirk, G.J., 2007. Recalling safety: cooperative functions of the ventromedial prefrontal cortex and the hippocampus in extinction. CNS Spectr 12(3), 200-206.
Corradi, J., Bouzat, C., 2016. Understanding the Bases of Function and Modulation of alpha7 Nicotinic Receptors: Implications for Drug Discovery. Mol Pharmacol 90(3), 288-299.
Chandravanshi, L.P., Yadav, R.S., Shukla, R.K., Singh, A., Sultana, S., Pant, A.B., Parmar, D., Khanna, V.K., 2014. Reversibility of changes in brain cholinergic receptors and acetylcholinesterase activity in rats following early life arsenic exposure. Int J Dev Neurosci 34, 60-75.
Chaudhuri, A.N., Basu, S., Chattopadhyay, S., Das Gupta, S., 1999. Effect of high arsenic content in drinking water on rat brain. Indian J Biochem Biophys 36(1), 51-54.
Danbolt, N.C., 2001. Glutamate uptake. Prog Neurobiol 65(1), 1-105.
Dani, J.A., Bertrand, D., 2007. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47, 699-729.
Davis, K.L., Mohs, R.C., Marin, D., Purohit, D.P., Perl, D.P., Lantz, M., Austin, G., Haroutunian, V., 1999. Cholinergic markers in elderly patients with early signs of Alzheimer disease. JAMA 281(15), 1401-1406.
De Rosa, E., Hasselmo, M.E., 2000. Muscarinic cholinergic neuromodulation reduces proactive interference between stored odor memories during associative learning in rats. Behav Neurosci 114(1), 32-41.
Dennis, S.H., Pasqui, F., Colvin, E.M., Sanger, H., Mogg, A.J., Felder, C.C., Broad, L.M., Fitzjohn, S.M., Isaac, J.T., Mellor, J.R., 2016. Activation of Muscarinic M1 Acetylcholine Receptors Induces Long-Term Potentiation in the Hippocampus. Cereb Cortex 26(1), 414-426.
Dionisio, L., Arias, V., Bouzat, C., Esandi Mdel, C., 2013. GABAA receptor plasticity in Jurkat T cells. Biochimie 95(12), 2376-2384.
Drever, B.D., Riedel, G., Platt, B., 2011. The cholinergic system and hippocampal plasticity. Behav Brain Res 221(2), 505-514.
Dutar, P., Bassant, M.H., Senut, M.C., Lamour, Y., 1995. The septohippocampal pathway: structure and function of a central cholinergic system. Physiol Rev 75(2), 393-427.
Everitt, B.J., Robbins, T.W., 1997. Central cholinergic systems and cognition. Annu Rev Psychol 48, 649-684.
Fabian-Fine, R., Skehel, P., Errington, M.L., Davies, H.A., Sher, E., Stewart, M.G., Fine, A., 2001. Ultrastructural distribution of the alpha7 nicotinic acetylcholine receptor subunit in rat hippocampus. J Neurosci 21(20), 7993-8003.
Farioli-Vecchioli, S., Saraulli, D., Costanzi, M., Pacioni, S., Cina, I., Aceti, M., Micheli, L., Bacci, A., Cestari, V., Tirone, F., 2008. The timing of differentiation of adult hippocampal neurons is crucial for spatial memory. PLoS Biol 6(10), e246.
Fibiger, H.C., 1991. Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. Trends Neurosci 14(6), 220-223.
Flora, S.J., Bhadauria, S., Pant, S.C., Dhaked, R.K., 2005. Arsenic induced blood and brain oxidative stress and its response to some thiol chelators in rats. Life Sci 77(18), 2324-2337.
Flora, S.J., Mittal, M., Mishra, D., 2009. Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic amines and DNA damage in mouse brain. J Neurol Sci 285(1-2), 198-205.
Gold, P.E., 1986. The use of avoidance training in studies of modulation of memory storage. Behav Neural Biol 46(1), 87-98.
Gross, C.T., Canteras, N.S., 2012. The many paths to fear. Nat Rev Neurosci 13(9), 651-658.

Gumilar, F., Lencinas, I., Bras, C., Giannuzzi, L., Minetti, A., 2015. Locomotor activity and sensory-motor developmental alterations in rat offspring exposed to arsenic prenatally and via lactation. Neurotoxicol Teratol 49, 1-9.

Gurer, H., Ercal, N., 2000. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med 29(10), 927-945.

Hall, M., Gamble, M., Slavkovich, V., Liu, X., Levy, D., Cheng, Z., van Geen, A., Yunus, M., Rahman, M., Pilsner, J.R., Graziano, J., 2007. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. Environ Health Perspect 115(10), 1503-1509.

Hasselmo, M.E., 2006. The role of acetylcholine in learning and memory. Curr Opin Neurobiol 16(6), 710-715.

Hasselmo, M.E., Fransen, E., Dickson, C., Alonso, A.A., 2000. Computational modeling of entorhinal cortex. Ann N Y Acad Sci 911, 418-446.

Herrera, A., Pineda, J., Antonio, M.T., 2013. Toxic effects of perinatal arsenic exposure on the brain of developing rats and the beneficial role of natural antioxidants. Environ Toxicol Pharmacol 36(1), 73-79.

Hood, R.D., Vedel-Macrander, G.C., Zaworotko, M.J., Tatum, F.M., Meeks, R.G., 1987. Distribution, metabolism, and fetal uptake of pentavalent arsenic in pregnant mice following oral or intraperitoneal administration. Teratology 35(1), 19-25.

Izquierdo, I., 1994. Pharmacological evidence for a role of long-term potentiation in memory. FASEB J 8(14), 1139-1145.

Izquierdo, I., da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M.B., Medina, J.H., 1992. Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. Behav Neural Biol 58(1), 16-26.

Izquierdo, I., Medina, J.H., 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem 68(3), 285-316.

Izquierdo, I., Vianna, M.R., Izquierdo, L.A., Barros, D.M., Szapiro, G., Coitinho, A.S., Muller, L., Cammarota, M., Bevilaqua, L.R., Medina, J.H., 2002. Memory retrieval and its lasting consequences. Neurotox Res 4(5-6), 573-593.

Jerusalinsky, D., Kornisiuk, E., Izquierdo, I., 1997. Cholinergic neurotransmission and synaptic plasticity concerning memory processing. Neurochem Res 22(4), 507-515.

Jin, Y., Xi, S., Li, X., Lu, C., Li, G., Xu, Y., Qu, C., Niu, Y., Sun, G., 2006. Arsenic speciation transported through the placenta from mother mice to their newborn pups. Environ Res 101(3), 349-355.

Johansen, J.P., Tarpley, J.W., LeDoux, J.E., Blair, H.T., 2010. Neural substrates for expectation-modulated fear learning in the amygdala and periaqueductal gray. Nat Neurosci 13(8), 979-986.

Jovanovic, D.D., Paunovic, K., Manojlovic, D.D., Jakovljevic, B., Rasic-Milutinovic, Z., Dojcinovic, B.P., 2012. Arsenic in drinking water and acute coronary syndrome in Zrenjanin municipality, Serbia. Environ Res 117, 75-82.

Kannan, G.M., Flora, S.J., 2004. Chronic arsenic poisoning in the rat: treatment with combined administration of succimers and an antioxidant. Ecotoxicol Environ Saf 58(1), 37-43.

Kapaj, S., Peterson, H., Liber, K., Bhattacharya, P., 2006. Human health effects from chronic arsenic poisoning--a review. J Environ Sci Health A Tox Hazard Subst Environ Eng 41(10), 2399-2428.

Kim, S., Takeuchi, A., Kawasumi, Y., Endo, Y., Lee, H., Kim, Y., 2012. A Guillain-Barre syndrome-like neuropathy associated with arsenic exposure. J Occup Health 54(4), 344-347.

Kopf, S.R., Benton, R.S., Kalfin, R., Giovannini, M.G., Pepeu, G., 2001. NO synthesis inhibition decreases cortical ACh release and impairs retention of a conditioned response. Brain Res 894(1), 141-144.

Kozul-Horvath, C.D., Zandbergen, F., Jackson, B.P., Enelow, R.I., Hamilton, J.W., 2012. Effects of low-dose drinking water arsenic on mouse fetal and postnatal growth and development. PLoS One 7(5), e38249.

Lawrence, R.A., Burk, R.F., 1976. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 71(4), 952-958.

Lindgren, A., Danielsson, B.R., Dencker, L., Vahter, M., 1984. Embryotoxicity of arsenite and arsenate: distribution in pregnant mice and monkeys and effects on embryonic cells in vitro. Acta Pharmacol Toxicol (Copenh) 54(4), 311-320.

Liu, L.Z., Jiang, Y., Carpenter, R.L., Jing, Y., Peiper, S.C., Jiang, B.H., 2011. Role and mechanism of arsenic in regulating angiogenesis. PLoS One 6(6), e20858.

Lu, C., Zhao, F., Sun, D., Zhong, Y., Yu, X., Li, G., Lv, X., Sun, G., Jin, Y., 2014. Comparison of speciated arsenic levels in the liver and brain of mice between arsenate and arsenite exposure at the early life. Environ Toxicol 29(7), 797-803.

Luo, J.H., Qiu, Z.Q., Shu, W.Q., Zhang, Y.Y., Zhang, L., Chen, J.A., 2009. Effects of arsenic exposure from drinking water on spatial memory, ultra-structures and NMDAR gene expression of hippocampus in rats. Toxicol Lett 184(2), 121-125.

Ma, N., Sasoh, M., Kawanishi, S., Sugiura, H., Piao, F., 2010. Protection effect of taurine on nitrosative stress in the mice brain with chronic exposure to arsenic. J Biomed Sci 17 Suppl 1, S7.

McGaugh, J.L., 2002. Memory consolidation and the amygdala: a systems perspective. Trends Neurosci 25(9), 456.

McGaughy, J., Everitt, B.J., Robbins, T.W., Sarter, M., 2000. The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins. Behav Brain Res 115(2), 251-263.

Micheau, J., Marighetto, A., 2011. Acetylcholine and memory: a long, complex and chaotic but still living relationship. Behav Brain Res 221(2), 424-429.

Morris, R.G., 1989. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. J Neurosci 9(9), 3040-3057.

Muir, J.L., 1997. Acetylcholine, aging, and Alzheimer's disease. Pharmacol Biochem Behav 56(4), 687-696.

Myhrer, T., 2003. Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. Brain Res Brain Res Rev 41(2-3), 268-287.

Nagaraja, T.N., Desiraju, T., 1994. Effects on operant learning and brain acetylcholine esterase activity in rats following chronic inorganic arsenic intake. Hum Exp Toxicol 13(5), 353-356.

Navas-Acien, A., Silbergeld, E.K., Streeter, R.A., Clark, J.M., Burke, T.A., Guallar, E., 2006. Arsenic exposure and type 2 diabetes: a systematic review of the experimental and epidemiological evidence. Environ Health Perspect 114(5), 641-648.

Olton, D.S., Papas, B.C., 1979. Spatial memory and hippocampal function. Neuropsychologia 17(6), 669-682.

Paxinos, G., Watson, C., 1986. The rat brain in stereotaxic coordinated. San Diego, CA: Academic Press.

Piao, F., Li, S., Li, Q., Ye, J., Liu, S., 2011. Abnormal expression of 8-nitroguanine in the brain of mice exposed to arsenic subchronically. Ind Health 49(2), 151-157.

Rodriguez, V.M., Carrizales, L., Jimenez-Capdeville, M.E., Dufour, L., Giordano, M., 2001. The effects of sodium arsenite exposure on behavioral parameters in the rat. Brain Res Bull 55(2), 301-308.

Rodriguez, V.M., Limon-Pacheco, J.H., Carrizales, L., Mendoza-Trejo, M.S., Giordano, M., 2010. Chronic exposure to low levels of inorganic arsenic causes alterations in locomotor activity and in

the expression of dopaminergic and antioxidant systems in the albino rat. Neurotoxicol Teratol 32(6), 640-647.
Sanderson, D.J., Good, M.A., Seeburg, P.H., Sprengel, R., Rawlins, J.N., Bannerman, D.M., 2008. The role of the GluR-A (GluR1) AMPA receptor subunit in learning and memory. Prog Brain Res 169, 159-178.
Schliebs, R., Arendt, T., 2011. The cholinergic system in aging and neuronal degeneration. Behav Brain Res 221(2), 555-563.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3(6), 1101-1108.
Sepand, M.R., Razavi-Azarkhiavi, K., Omidi, A., Zirak, M.R., Sabzevari, S., Kazemi, A.R., Sabzevari, O., 2016. Effect of Acetyl-L-Carnitine on Antioxidant Status, Lipid Peroxidation, and Oxidative Damage of Arsenic in Rat. Biol Trace Elem Res 171(1), 107-115.
Shi, H., Hudson, L.G., Liu, K.J., 2004. Oxidative stress and apoptosis in metal ion-induced carcinogenesis. Free Radic Biol Med 37(5), 582-593.
Shila, S., Subathra, M., Devi, M.A., Panneerselvam, C., 2005. Arsenic intoxication-induced reduction of glutathione level and of the activity of related enzymes in rat brain regions: reversal by DL-alpha-lipoic acid. Arch Toxicol 79(3), 140-146.
Smith, A.H., Steinmaus, C.M., 2011. Arsenic in drinking water. BMJ 342, d2248.
Steinmaus, C., Yuan, Y., Kalman, D., Rey, O.A., Skibola, C.F., Dauphine, D., Basu, A., Porter, K.E., Hubbard, A., Bates, M.N., Smith, M.T., Smith, A.H., 2010. Individual differences in arsenic metabolism and lung cancer in a case-control study in Cordoba, Argentina. Toxicol Appl Pharmacol 247(2), 138-145.
Tsai, S.Y., Chou, H.Y., The, H.W., Chen, C.M., Chen, C.J., 2003. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 24(4-5), 747-753.
Tseng, C.H., 2009. A review on environmental factors regulating arsenic methylation in humans. Toxicol Appl Pharmacol 235(3), 338-350.
Vianna, M.R., Szapiro, G., McGaugh, J.L., Medina, J.H., Izquierdo, I., 2001. Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. Proc Natl Acad Sci U S A 98(21), 12251-12254.
Wang, S.X., Wang, Z.H., Cheng, X.T., Li, J., Sang, Z.P., Zhang, X.D., Han, L.L., Qiao, X.Y., Wu, Z.M., Wang, Z.Q., 2007. Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. Environ Health Perspect 115(4), 643-647.
Warburton, E.C., Koder, T., Cho, K., Massey, P.V., Duguid, G., Barker, G.R., Aggleton, J.P., Bashir, Z.I., Brown, M.W., 2003. Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. Neuron 38(6), 987-996.
Wess, J., 2004. Muscarinic acetylcholine receptor knockout mice: novel phenotypes and clinical implications. Annu Rev Pharmacol Toxicol 44, 423-450.
WHO, 2013. (World Health Organization) Guidelines for Drinking-water quality: incorporating First and Second addenda to Third Edition. In e commendations, Vol. 1. Geneva: WHO Press. Available at: http://www.who.int/water_sanitation_health/dwq/gdwq3/en/index.
Xi, S., Guo, L., Qi, R., Sun, W., Jin, Y., Sun, G., 2010a. Prenatal and early life arsenic exposure induced oxidative damage and altered activities and mRNA expressions of neurotransmitter metabolic enzymes in offspring rat brain. J Biochem Mol Toxicol 24(6), 368-378.
Xi, S., Jin, Y., Lv, X., Sun, G., 2010b. Distribution and speciation of arsenic by transplacental and early life exposure to inorganic arsenic in offspring rats. Biol Trace Elem Res 134(1), 84-97.
Xi, S., Sun, W., Wang, F., Jin, Y., Sun, G., 2009. Transplacental and early life exposure to inorganic arsenic affected development and behavior in offspring rats. Arch Toxicol 83(6), 549-556.

Yadav, R.S., Chandravanshi, L.P., Shukla, R.K., Sankhwar, M.L., Ansari, R.W., Shukla, P.K., Pant, A.B., Khanna, V.K., 2011. Neuroprotective efficacy of curcumin in arsenic induced cholinergic dysfunctions in rats. Neurotoxicology 32(6), 760-768.

Yoshihara, T., Ichitani, Y., 2004. Hippocampal N-methyl-D-aspartate receptor-mediated encoding and retrieval processes in spatial working memory: delay-interposed radial maze performance in rats. Neuroscience 129(1), 1-10.

Zarazua, S., Rios, R., Delgado, J.M., Santoyo, M.E., Ortiz-Perez, D., Jimenez-Capdeville, M.E., 2010. Decreased arginine methylation and myelin alterations in arsenic exposed rats. Neurotoxicology 31(1), 94-100.

Zoli, M., Pucci, S., Vilella, A., Gotti, C., 2017. Neuronal and extraneuronal nicotinic acetylcholine receptors. Curr Neuropharmacol.

Captions

Fig. 1. Food intake was performed every 3 days coinciding with the record body weight of dams during pregnancy and offspring during lactation. Drink consumption of dams during pregnancy and lactation was recorded daily. All results are expressed as Mean ± SEM of 12 animals per group.

Fig. 2. Latencies measured in the step-down inhibitory avoidance test during the training session (0 min) and the test sessions for the evaluation of STM (90 min after training) and LTM (24 h after training) in the adult female offspring exposed to 0.05 and 0.10 mg/L iAs during pregnancy and lactation. Bars represent median (±interquartile range) of step-down latencies in seconds of 10 animals per group. *$p < 0.01$, compared to control group.

Fig. 3. Expression of α7-nAChR subunit at mRNA level in hippocampus from 90-day-old female offspring exposed during pregnancy and lactation to 0.05 and 0.10 mg/L iAs. Quantitative real-time PCR was performed to determine mRNA level. The values are shown as the means ± SD. $n = 5$-6. *$p < 0.001$, compared to control group.

Fig. 4. Transaminase levels in hippocampus from 90-day-old female offspring exposed during pregnancy and lactation to 0.05 and 0.10 mg/L iAs. The values are shown as the means ± SD of $n = 5$. *$p < 0.001$, compared to control group.

Fig. 5. Activities of CAT (A) and GPx (B), and MDA content (C) in the complete brain homogenates of adult female offspring exposed to 0.05 and 0.10 mg/L iAs during the pregnancy and lactation. The values are shown as the means ± SD. n = 5. * $p < 0.05$; ** $p < 0.001$ compared to the control group.

Fig. 6. Activities of CAT (A) and GPx (B) in the hippocampus of 90-day-old female offspring exposed to 0.05 and 0.10 mg/L iAs during the pregnancy and lactation. The values are shown as the means ± SD. n = 5. * $p < 0.001$, compared to control group.