STUDIES ON THE NEUROTOXICITY OF ARSENIC IN RATS IN DIFFERENT EXPOSURE TIMING SCHEMES

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Running title: Neurotoxicity of arsenic in rats

Summary

Arsenic has long been recognized as human poison and, more recently, as an essential micronutrient. Here, the effects of low-level arsenic exposure on the central and peripheral nervous system functions were studied in rats, in a 4-8-12 week subchronic exposure scheme, and in a 3-generation scheme involving treatment of the parents and the offspring. From the rats, spontaneous and evoked activity of the sensory cortical areas, and compound action potential from the tail nerve, was recorded in urethane anesthesia, then dissection with organ weight measurement was done.

Body weight gain of the treated animals did not differ significantly from the control. There were, however, dose-dependent changes in the weight of the liver and other organs.

Latency of the cortical evoked potentials increased in the treated rats in both schemes. The change was significant after long exposure times and in the higher dose groups. A shift of the spontaneous cortical activity to higher frequencies was also observed, with similar dose and time dependence.

Low-level arsenic affected the behavioral and electrophysiological functions in the brain, indicating that long-lasting arsenic exposure can result in manifest alteration of the central and peripheral nervous system. Consequently, arsenic exposed populations may have a higher risk of behavioral and functional neurotoxic effects, potentially additive to the neurotoxicity of other environmental xenobiotics.

Key words

arsenic - neurotoxicity - cortical activity - behaviour - rat

Introduction

Arsenic is a ubiquitous element present in the rocks and soil, in the surface and especially in certain artesian waters [ATSDR 2000]. As an environmental pollutant, it originates from mining, smelting and refining of certain ores and also from burning of coal with higher As content [Pacyna et al. 1995]. Sea food can have naturally elevated As content [Eisler 1994] while cereals and meat can be contaminated by airborne As [Schoof et al. 1999]. In some regions, including South-East Hungary and the adjacent areas in Romania, there is an elevated As concentration in the drinking water produced from bedrock aquifers [Börzsönyi et al. 1992].

To our present knowledge, As is a micronutrient [Nielsen 1991], but in higher amounts it is a well-known human poison. In chronic intoxication, the target organs of arsenic are first of all the respiratory, gastrointestinal, cardiovascular and haemopoetic systems [ATSDR 2000] but data on As toxicity on the central and peripheral nervous system were also reported [Rodriguez et al. 2003]. In the central and peripheral nervous system of humans, As produces, among others, abnormal electromyography and altered nerve conduction velocity [Bernstam and Nrigau 2000]. Arsenic-induced neuropathy was reported in epidemiological studies [Ramirez-Campos et al. 1998]. In infants exposed to inorganic As, severe hearing loss and abnormal EEG was found 5 years after exposure [Liu et al. 1994]. Deafness due to environmental arsenic exposure was also reported [Bencko et al. 1988].

In our studies, human As exposure was modelled in rats, with relatively low oral doses given to adult animals, or during intra- and extrauterine development.

Methods

Wistar rats, obtained from the SPF breed of the University's Breeding Center, were used in the experiments. The animals were kept under GLP-certified* conditions (temperature 20-22 °C, humidity 60-70 %, 12 hours light-dark cycle) and fed with standard rodent chow. Food and water were continuously available ad libitum. The animals were observed daily for the symptoms of intoxication, their body weight was recorded weekly.

In the subchronic exposure model, ten weeks old adult males (in groups of 10) were treated by gavage with 6.6 (low dose), 13.2 (medium dose) or 26.4 (high dose) mg/kg b.w. of arsenic (in form of $NaAsO_2$, purity: 99.5 %) dissolved in distilled water to 1.0 ml/kg b.w. administration volume, for 4, 8 or 12 weeks in a 5 days per week system. Control rats received the same volume of distilled water. On the day following the last arsenic administration, the rats were prepared for electrophysiology (see below).

The three-generation exposure model was started with generation P mating at their age of 11-12 weeks (two female and one male rat put into one cage). The offspring (generation F1) was treated with arsenic from their age of 4 to the age of 11 weeks, using the regimen described above. Then, 10 males per group were separated for electrophysiology, and 10 female and 5 male animals per dose were kept to produce the next generation (F2 and F3). After mating, the pregnant females were placed in separate cages, and were treated 7 days per week until separation the offspring of the next generation at their age of 4 weeks. The number and body weight of the offspring were determined within 12 hours of birth and the litter size was reduced to eight (up to five males per litter) on the 4th day after parturition. The above schedule was applied also for the further generations.

*The laboratory and animal house of the Department is GLP certified for safety toxicological testing. Certification No. 3011/48/2003.

For electrophysiological recording, the rats were anaesthetized with urethane (1000 mg/kg ip.), and placed in a stereotaxic frame. The skull over the left hemisphere was opened and silver electrodes were placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) centers. Thirty minutes later, electrcorticogram (ECoG) was recorded simultaneously from these areas for 5 minutes. The software used for recording and analysis (Neurosys 1.11, Expermetria Ltd, Hungary) yielded the power spectrum by bands. From that, the ECoG index ([delta+theta]/[beta1+beta2]) was calculated and plotted. Then, cortical evoked potentials (EPs) were recorded from the same cortical sites by the above mentioned electrodes. The somatosensory stimuli were rectangular electric pulses (1 Hz, 3-4 V, 0.2 msec) delivered to the contralateral whisker pad. The visual stimuli were flashes (1 Hz, 60 lux) provided by a flashbulb device and conducted via an optical fiber to the contralateral eye. Acoustic stimulation was performed by clicks (1 Hz, 40 dB) lead directly into the ear of the rat. Of each modality, 50-50 evoked potentials were recorded and averaged by the mentioned computer program. Latency (and duration) of the averaged sensory evoked potentials were measured off-line. The conduction velocity of the tail nerve was measured by electric stimulation of the nerve and leading off the action potential, based on the distance between the site of stimulation and recording. For further details of the electrophysiological technique, see [Institóris et al. 2004].

Having finished electrophysiological investigations, the rats were sacrificed with an overdose of urethane, dissected, and internal organs weighed. The organ weights were expressed as relative to brain weight [Institóris et al. 2001].

All data were analyzed by one-way ANOVA and post-hoc LSD after the Kolmogorov-Smirnov normality check. During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

Results

Body and organ weights

The body weight gain of the subchronically treated animals did not differ significantly from the control. There was, however, a dose-dependent decrease in relative liver and thymus weight (Table 1). In the three-generation study, body weight effects were also minimal. The relative weight of the liver, but not of the thymus, was significantly diminished in the F1 and F2 rats treated with the high dose (Table 2).

Cortical activity

In the rats treated subchronically, ECoG index had a decreasing trend, corresponding to a shift to higher frequencies. In certain groups after 12 weeks treatment, the change was significant vs. control (Figure 1). In the three-generation model, the changes of the EGoC were also moderate. The decrease was significant in the F3 rats in the somatosensory, and F2 and F3 in the auditory area (Figure 2).

The latency of the cortical EPs increased in the all groups receiving subchronic As administration. The difference vs. control was significant only with the high dose and after 8 and 12 (somatosensory area) or 12 (visual and auditory area) weeks (Figure 3). In F1, all changes of the EPs were below significance. With the high dose, the latency increase was significant in the F2 and F3 rats in all three cortical areas. In the visual EP, the medium dose F3 animals also had a significant latency increase (Figure 4.).

Peripheral nerve activity

The only parameter of the tail nerve which showed significant alterations under influence of oral As was conduction velocity, see Table 3.

Discussion

The doses of As given in our study had no significant effect on body weight gain, indicating that the observed changes in the nervous system activity were probably not due to a general toxic effect. The effect on liver weight, seen in both exposure schemes, is explained by the organ tropism of As and the role of the liver in its detoxification [Klaassen 1996]. The changes in thymus weight indicate (most probably oxidative) stress [Thomas et al. 2001].

Several neurotransmitter systems, known to influence CNS activity, are affected by As. Cholinesterase activity was decreased in rats receiving oral As in a treatment scheme comparable to ours [Nagaraja and Desiraju 1994]. The decrease of slow/fast band activity ratio (ECoG index) seen in our study is in agreement with lowered AChE activity, regarding the cholinergic nature of the ascending activation [Metherate et al. 1992]. Decreased glutamic acid decarboxylase activity due to As treatment [Nagaraja and Desiraju 1993] may have led to desensitization in the specific afferent pathways. This, and the known relationship between cortical activity level and the intensity of evoked responses [Rémond and Lesévre 1967], plausibly explain the depression (increased latency) of the cortical evoked potentials in the As-treated rats.

The apparent dose-dependence of the effects obtained by subchronic As treatment is explained by good intestinal absorption [ATSDR 2000], and increased access to the brain by damage of the choroid plexus induced by methylated As generated in normal metabolism of this metal [Rodriguez et al. 2003]. In case of the multi-generation exposure model, two factors could have contributed to the increased functional damage in the 2^{nd} and 3^{rd} generations: the deposition of As in the mother rats and the increased sensitivity of fetal rat brain to As-induced apoptotic neuronal loss [Chattopadhay et al. 2002].

The changes in the neurophysiological parameters investigated in our work showed that the administered low-level arsenic doses affected the functions of brain. On the basis of the dose, time, and generation dependence of these effects, it can be supposed that long-lasting arsenic exposure can result in manifest alterations in the central and peripheral nervous system. Arsenic exposed populations may have a higher risk of behavioral and functional neurotoxic effects, potentially additive to the neurotoxicity of other environmental xenobiotics.

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Table 1. Body weights and relative organ weights (organ weight/brain weight) in subchronic oral As exposure. Mean ±SD, n=10. * p<0.05.

Weeks of treatment	Doses	Body weight (g)	Liver weight, relative	Thymus weight, relative
4	Control	320 ± 3.0	3.42 ± 0.10	0.17 ± 0.01
	Low	342 ± 13	3.07 ± 0.07*	0.13 ± 0.01*
	Medium	308 ± 9.1	2.98 ± 0.09*	0.15 ± 0.01
	High	313 ± 8.0	2.81 ± 0.05*	0.13 ± 0.01
8	Control	376 ± 10	3.51 ± 0.13	0.14 ± 0.01
	Low	383 ± 12	2.89 ± 0.08*	0.11 ± 0.01*
	Medium	395 ± 7.7	2.81 ± 0.07*	0.10 ± 0.01*
	High	366 ± 13	2.82 ± 0.10*	0.12 ± 0.01*
12	Control	455 ± 16	2.61 ± 0.18	0.09 ± 0.01
	Low	414 ± 13	2.57 ± 0.07	0.10 ± 0.01
	Medium	450 ± 12	2.70 ± 0.06	0.09 ± 0.01
	High	432 ± 18	2.35 ± 0.08	0.08 ± 0.01

Table 2. Body weights and relative organ weights (organ weight/brain weight) in three-generation oral As exposure. Mean ±SD, n=10. * p<0.05.

Generation	Doses	Body weight (g)	Liver weight, relative	Thymus weight, relative
F1	Control	312 ± 13	3.56 ± 0.49	0.23 ± 0.03
	Low	314 ± 18.5	3.50 ± 0.32	0.22 ± 0.04
	Medium	311 ± 16.6	3.47 ± 0.41	0.24 ± 0.01
	High	309 ± 11.2	3.43 ± 0.31*	0.23 ± 0.02
F2	Control	310 ± 11.3	3.54 ± 0.46	0.24 ± 0.04
	Low	312 ± 10.1	5.50 ± 0.28	0.23 ± 0.03
	Medium	309 ± 12.1	3.47 ± 0.33	0.21 ± 0.04
	High	310 ± 13.7	3.38 ± 0.27*	0.21 ± 0.03
F3	Control	316 ± 16.3	3.52 ± 0.35	0.22 ± 0.02
	Low	315 ± 17.4	3.50 ± 0.32	0.21 ± 0.04
	Medium	308 ± 14.4	3.49 ± 0.39	0.19 ± 0.06
	High	307 ± 14.2	3.42 ± 0.62	0.24 ± 0.03

Table 3. Conduction velocity, measured in the tail nerve, after subchronic (left columns) or three-generation (right columns) oral As exposure. Mean ±SD, n=10. * p<0.05.

Weeks of treatment	Doses	Conduction velocity (m/s)		Generation	Doses	Conduction velocity (m/s)
4	Control	23.9 ± 0.21		F1	Control	23.7 ± 0.30
	Low	23.8 ± 0.30			Low	23.55 ± 0.50
	Medium	23.6 ± 0.19			Medium	23.46 ± 0.35
	High	23.3 ± 0.28			High	22.8 ± 0.50*
8	Control	23.7 ± 0.25		F2	Control	23.8 ± 0.25
	Low	23.5 ± 0.10			Low	23.4 ± 0.35
	Medium	23.5 ± 0.19			Medium	23.5 ± 0.20
	High	23.2 ± 0.27			High	22.9 ± 0.45*
12	Control	23.8 ± 0.22		F3	Control	23.75 ± 0.35
	Low	23.5 ± 0.28			Low	23.37 ± 0.38
	Medium	22.8 ± 0.18*			Medium	22.9 ± 0.35*
	High	22.7 ± 0.27*			High	22.57 ± 0.45*

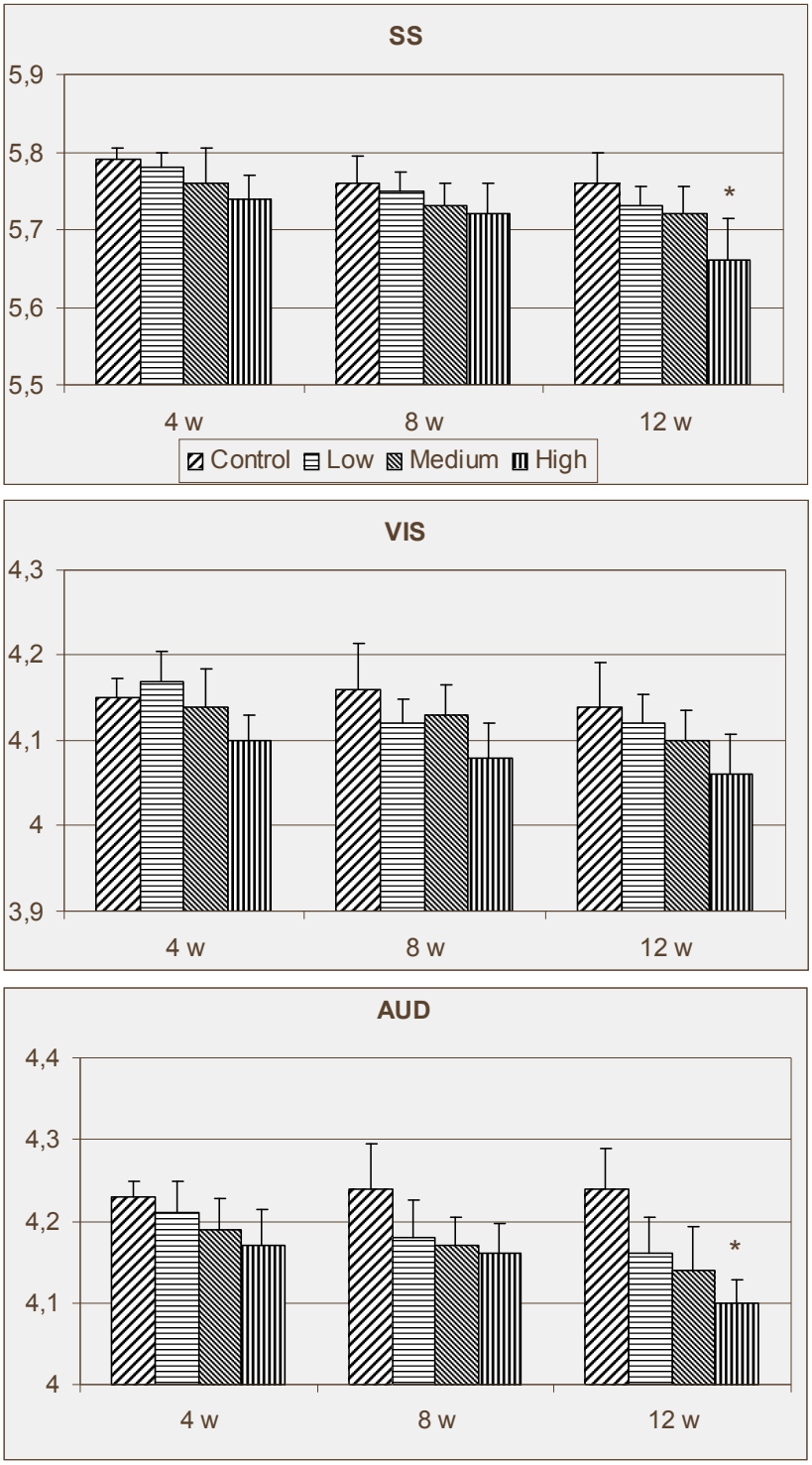

Figure 1. ECoG index values (ordinate) in the somatosensory (SS), visual (VIS) and auditory (AUD) cortical center after subchronic oral exposure to low (6.6 mg/kg b.w.), medium (13.2 mg/kg) and high (26.4 mg/kg) arsenic (see insert in the top graph). Abscissa: weeks of treatment.

Mean+SD, n=10, * $p<0.05$ vs. untreated control.

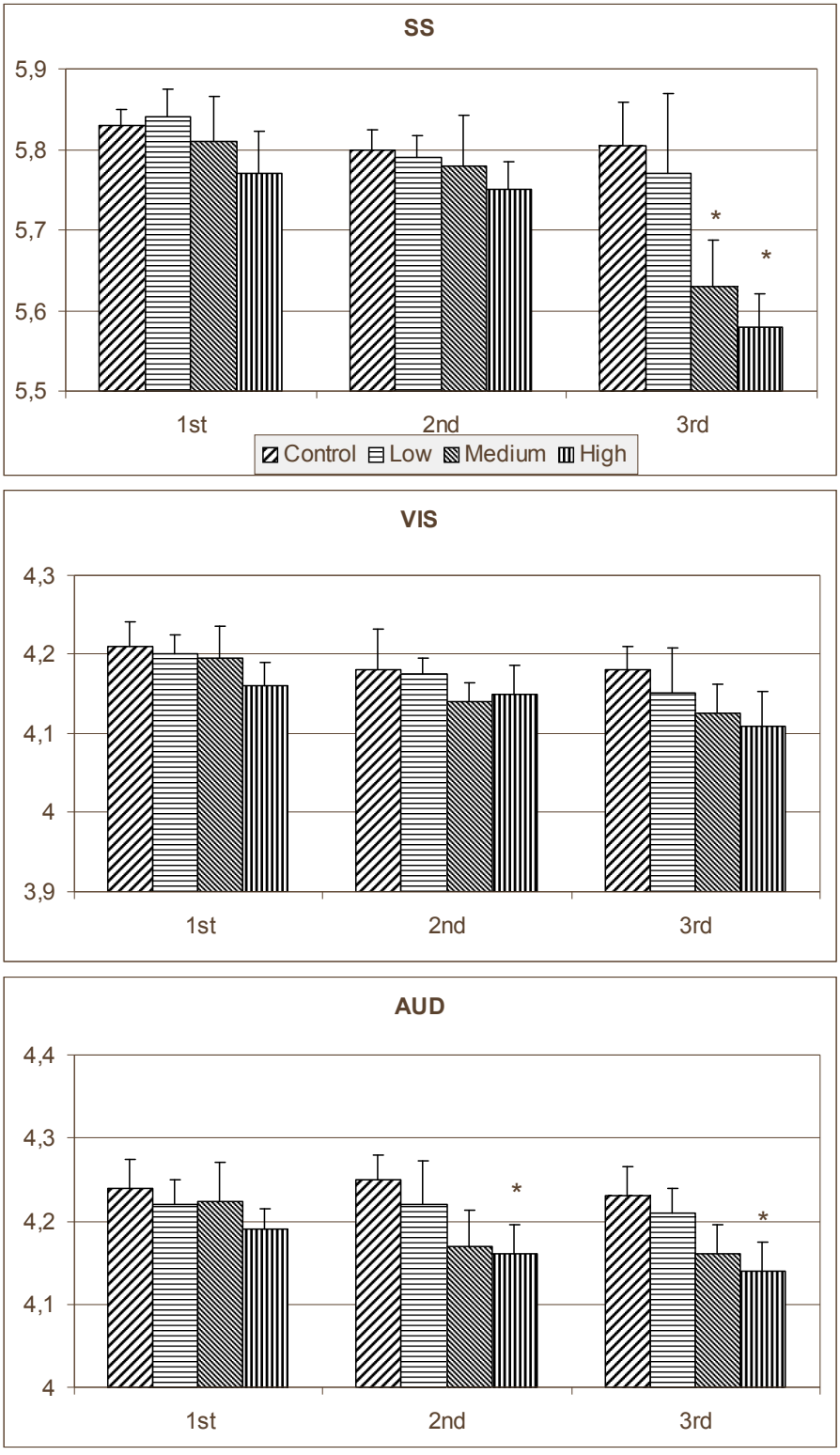

Figure 2. ECoG index values (ordinate) in the three cortical centers after oral exposure to arsenic in three generations (abscissa). Plotted as in Figure 1.

Mean+SD, n=10, * $p<0.05$ vs. untreated control.

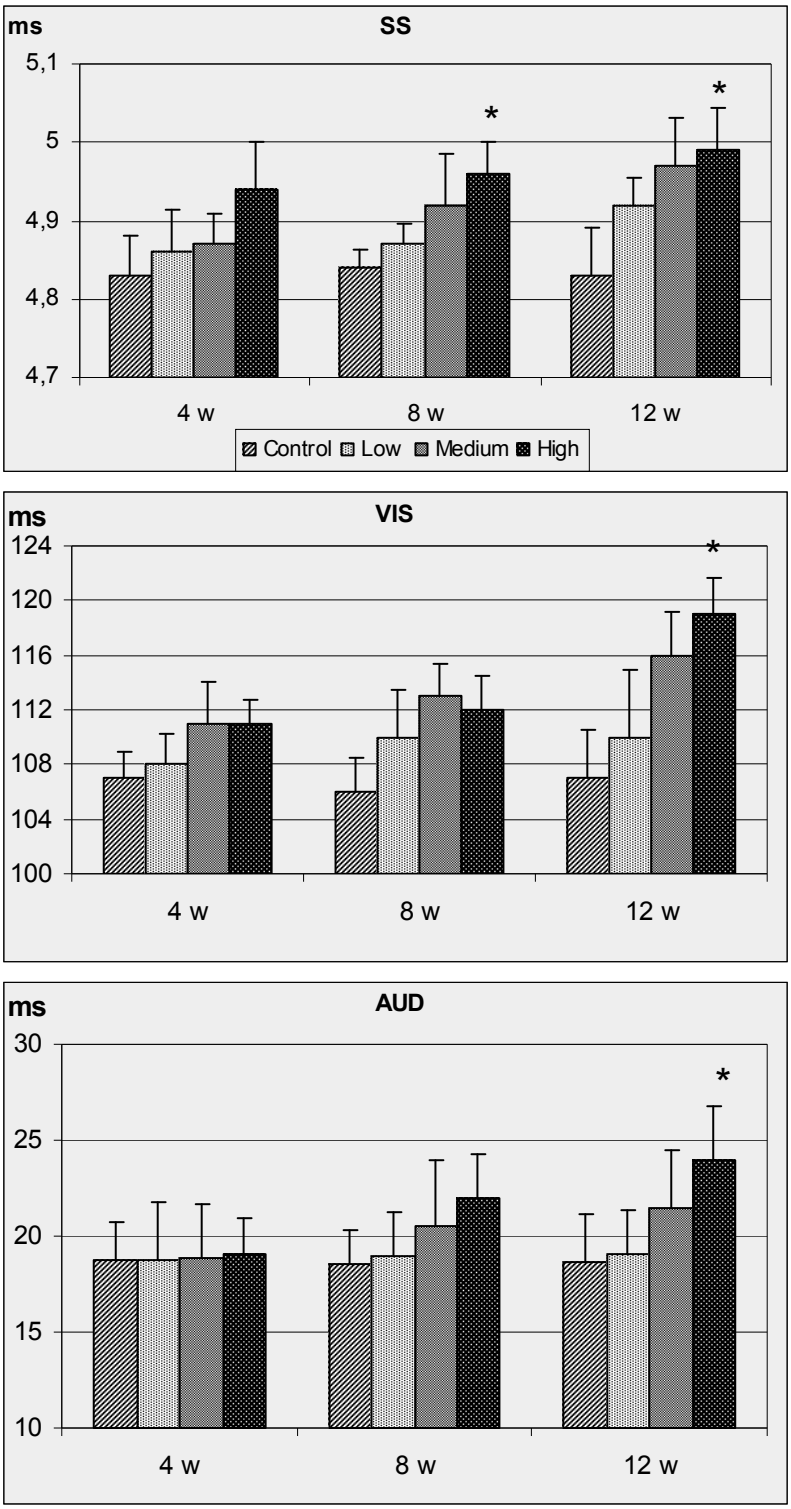

Figure 3. Latency (ordinate) of the sensory evoked potentials from the three cortical centers after subchronic arsenic exposure. Abscissa: weeks of treatment. Plotted as in Figure 1. Mean+SD, n=10, * $p<0.05$ vs. untreated control.

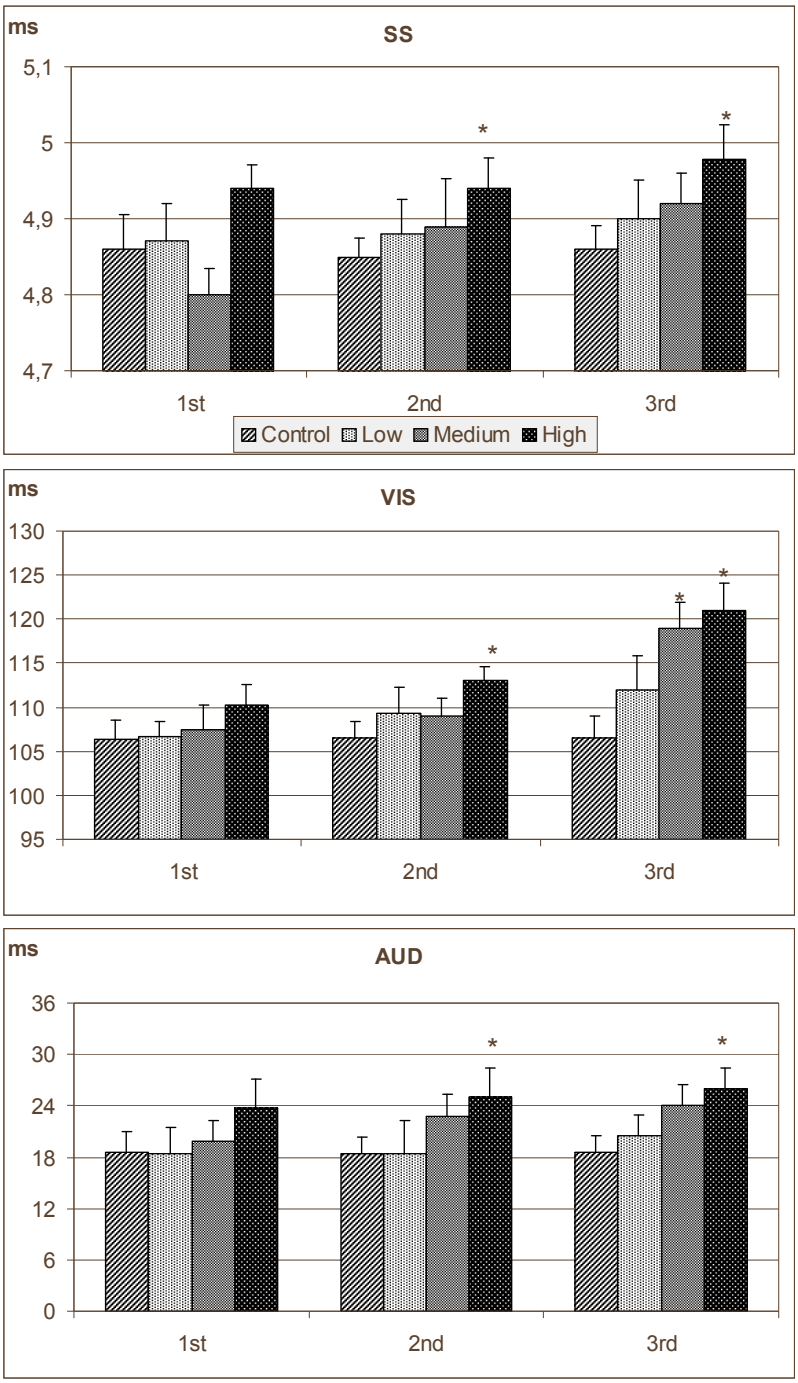

Figure 4. Latency (ordinate) of the cortical evoked potentials after oral exposure arsenic in three generations (abscissa). Plotted as in Figure 2.

Mean+SD, n=10, * $p<0.05$ vs. untreated control.