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Learning deficits in C57BL/6J mice following perinatal arsenic exposure: Consequence of lower corticosterone receptor levels?

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ABSTRACT

Most studies on arsenic as a drinking water contaminant have focused on its carcinogenic potential but a few suggest that arsenic can adversely affect cognitive development. One parameter of the hypothalamic–pituitary–adrenal axis, the corticosterone receptor (CR) has been shown to be altered by arsenic. These receptors are found throughout the central nervous system, particularly in the hippocampus, an area of the brain of central importance for learning and memory. We examined the impact of perinatal exposure to 50 parts per billion (ppb) sodium arsenate on CRs and learning and memory behavior in the C57BL/6J mouse. Measurements of CRs revealed that arsenic-exposed offspring have significantly lower levels of these receptors in the nucleus than controls. Exposed offspring showed longer latency to approach a novel object than controls in an object recognition task. In the 8-way radial arm maze, arsenic offspring had a significant increase in the number of entry errors compared to controls. Results suggest that moderate exposures to perinatal arsenic can significantly reduce CR levels in the hippocampus and can have adverse effects on learning and memory behavior.

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1. Introduction

Arsenic is one of the most toxic naturally occurring contaminants found in the environment. A common source of human exposure to inorganic arsenic is through drinking water contamination. Exposure has been associated with skin, lung and bladder cancers, vascular diseases, hypertension, genotoxicity, cellular disruption and diabetes (ATSDR Arsenic, 2007). While most studies on arsenic have focused on its carcinogenic potential, studies in rodents pioneered by Rodriguez et al. suggest that arsenic can adversely affect cognitive development (Rodriguez et al., 2001, 2002), however, these effects on nervous system development and function have received less attention (Rodriguez et al., 2003). Literature describing the effects of arsenic in drinking water on human cognitive development is sparse, and has been best characterized through epidemiological studies, often based on research from countries with significantly higher arsenic concentrations than the United States (Calderon et al., 2001; von Ehrenstein et al., 2007; Wasserman et al., 2004). The majority of these studies have focused on children; and deficits in learning and memory, particularly in hippocampal-dependent tasks, following acute and chronic arsenic exposure have been reported. However, the cellular and molecular mechanisms involved in this process are still poorly understood.

Events that disrupt maturation and development of the hypothalamic–pituitary–adrenal (HPA) stress axis have been shown to permanently alter CR expression in the adult (de Kloet et al., 1998; Matthews, 2000; Welberg and Seckl, 2001). CRs are found in heterooligomeric complexes in the cytoplasm and upon binding corticosterone (CORT) are translocated to the nucleus where they influence diverse gene transcription. The glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) are two forms of the CR which are activated by binding of CORT. Arsenic has been shown to perturb components of the HPA stress axis, like the GR (Bodwell et al., 2004) and alter gene expression of inducible genes (Hamilton et al., 1998). Perturbation of the HPA stress axis has been implicated in both cognitive damage and the promotion of carcinogenesis. Thus far, no studies of the effects of perinatal arsenic on GR and MR have been reported.

Neurons in the hippocampus contain both GRs and MRs. GRs are involved in consolidation of learned information (de Kloet et al., 1999) and MRs are involved in interpretation of novel information (Berger et al., 2006), memory retrieval (Conrad et al., 1997), and visuospatial learning (Yau et al., 1999). Further, spatial learning and memory deficits have been shown in GR and MR knockout mice (Berger et al., 2006; Steckler et al., 1998) and sustained activation of GR by chronically elevated CORT impairs hippocampal function and memory processes (McEwen and Sopolsky, 1995). The connection between the hippocampus and learning and memory has been well established for decades. De Kloet et al. (1998)

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showed that both the presence of CORT and a functional GR are critical to normal hippocampal function. Stress, through the actions of CORT and CRs, can either facilitate or impair learning and memory processes (Joels, 2006). Studies have shown that, at intermediate levels, CORT enhances learning and memory but low or high levels adversely affect cognition (Lupien and McEwen, 1997). We have previously shown that perinatal arsenic-exposed offspring have elevated levels of circulating CORT into adolescence and young adulthood (Martinez et al., 2008). Evidence from prenatal lead exposure studies suggest that excess fetal CORT levels can program pathologies in adult life (Cory-Slechta et al., 2004; Seckl and Meaney, 2004). High levels of circulating CORT have been frequently shown to be associated with a series of clinical diseases including neurodegenerative diseases, major depression and schizophrenia as well as aging (Alderson and Novack, 2002; Belanoff et al., 2001; Lupien et al., 1999).

Given our previous report of high basal CORT levels in perinatal arsenic-exposed offspring (Martinez et al., 2008), we examined hippocampal GR and MR translocation to determine if this parameter was affected by the arsenic-induced changes in CORT level. We also examined spatial and non-spatial learning and memory behavior in our perinatal mouse model to determine if deficits were attributable to the role of glucocorticoids.

2. Methods

Note: Arsenic is classified as a probable human carcinogen (ATSDR, 2007). All arsenicals were handled as potentially highly toxic compounds.

2.1. Perinatal arsenic exposure paradigm

The arsenic exposure paradigm and behavioral tasks employed in these studies were approved by the UNM Health Sciences Center Institutional Animal Care and Use Committee. All mice were bred and maintained on a reversed 12-h light/dark cycle (lights on from 17:00 to 09:00 h) with food and water *ad libitum* in a temperature controlled (22 °C) room in the Animal Resource Facility. All dosing suspensions were prepared approximately weekly. Arsenic water (sodium arsenate, Sigma, St. Louis, MO) was prepared using standard tap water. Control mice were administered untreated tap water. Tap water at UNM contains approximately 5 ppb arsenic. Female C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were assigned to either a control or 55 ppb arsenic water treatment group. After a 2-week acclimation period on the treated waters, male breeder mice were introduced into each female's cage. Three days later, the males were removed from the cages and nesting material was placed in the female's cage. Mouse dam water consumption was monitored throughout pregnancy and the dams continued to drink the treated waters until their offspring were weaned. Offspring were weaned at 23 days of age and maintained in same-sex, litter-mate housed cages with *ad libitum* access to untreated tap water and mouse chow (7004 Harlan Teklad S-2335, Denver, CO) until they were used in experimental procedures at 35–40 days of age. Mice (two per litter; 5–10 litters total per treatment group) were assigned to either one of the biochemical assay procedures or the behavioral testing methods. Offspring used in behavioral studies were not used in biochemical studies. A sample size of five represents the total number of litters and not individual animals used.

2.2. Drinking water analysis and data

Arsenic and control water bottles were monitored and recorded every other day for water consumption. Bottles from individual cages were weighed to the nearest tenth of a gram. Water samples (10 mL) from each dosing suspension were sent in their liquid forms to the laboratory of Dr. Abdul-Mehdi Ali (UNM Department of Earth and Planetary Sciences) for determination of arsenic concentration according to US EPA method 200.8.

2.3. Offspring whole brain arsenic concentrations

To evaluate whole brain concentrations of arsenic, samples were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Perkin Elmer Elan DRC II, Waltham, MA) by Dr. Ali. The internal standard was indium (In) at mass 115. The analysis was performed comparable to method US EPA 200.8. Exactly 10 mL of the standards, samples, and QC samples were spiked with 1 mL of 100 mg/L indium (In) as internal standard to correct for drifts in the signal that may be caused by sample matrix, viscosity, and/or peristaltic pump (sample) pulsing. The system was calibrated using NIST traceable calibration standards (stock solution) and QC solutions (stock solution). The system is sensitive down to the parts per trillion level. Three mg brain samples were taken from one cerebellar lobe from arsenic-exposed and control mice. Samples were digested, using 2 mL nitric acid, at 90 °C. After digestion was completed, digests were brought up to 10 mL final volume and transferred into Inductively Coupled Plasma (ICP) plastic tubes. Results were then calculated using the starting weight and the final volume after digestion. Results were expressed as μg/g and then converted to ppb based on the standard curve. Samples were run in duplicate.

2.4. Western immunoblotting

2.4.1. Tissue preparation

Adolescent offspring, 35–40 days of age, were sacrificed by decapitation and whole hippocampal formation was rapidly dissected. Subcellular fractions are prepared exactly as described by Buckley et al. (2004). Protein determinations were performed as described in Weeber et al. (2001) and were used to load equal amounts of protein in each well. To determine the lack of cytoplasmic contamination in the nuclear fraction, the membrane was probed between 68 kDa and 21 kDa for anti-A-Raf (1:500; Santa Cruz) and anti-H-Ras (1:500; Santa Cruz) antibodies, respectively.

2.4.2. Glucocorticoid and mineralocorticoid receptors

Cytosolic and nuclear fractions from seven or eight hippocampi from arsenic-exposed and control animals were analyzed for GR and MR via western blot. The linear range of the total protein was calculated to ensure that the protein was not saturating the blot (data not shown). Control samples were run on the same blot as arsenic-exposed samples. The amount of total GR and MR of the sample was corrected for beta-actin and data analyzed by *t*-test. Extracts were thawed on ice, diluted in 4× SDS-PAGE sample buffer (Invitrogen, Carlsbad, CA) and heated at 70 °C for 10 min. Samples (2 μg protein per well (GR); 3 μg protein per well (MR)) were separated using 4–12% NuPAGE Bis–Tris gels (Invitrogen) and transferred to 0.45-μm-thick nitrocellulose membranes (Invitrogen). The GR and MR membranes were blocked with 0.25% I-block (Applied Biosystems, Foster City, CA) in TBS-T (25 mM Tris–HCl pH 7.2, 150 mM NaCl and 0.05% Tween-20) for 1 h at room temperature. Blots were then cut and incubated with either a polyclonal primary antibody to GR (1:3 000; M-20, Santa Cruz Biotechnology, Santa Cruz, CA), MR (1:500; H-300 Santa Cruz) or beta-actin (1:2 000; Cell Signaling, Boston, MA) overnight at 4 °C. The reaction was stopped with four consecutive 5-minute washes in TBS-T. A goat anti-rabbit IgG (H + L):HRP (1:50 000, Thermo Fisher Scientific, Rockford, IL) was used for the secondary antibody incubation in 0.25% I-block 1 h at room temperature. The reaction was stopped with four consecutive 5-minute washes in TBS-T. Membranes were then incubated in Supersignal West Pico Working Solution (Thermo Scientific) for 5 min and exposed to F-BX57 film (Phenix Research Products, Candler, NC). Film was developed in Kodak D-19 developer then washed and fixed in Kodak fixer. The developed film was scanned (Hewlett Packard Scan Jet 5P)

and immunoreactivities quantified by measurements of optical densities using BioRad Quantity-One analysis software. Each protein sample was run in duplicate and the average optical density taken.

2.5. Novel object exploration

The novel object exploration task was conducted in a plexiglas open field apparatus (43 cm × 43 cm × 21 cm high, designed by Allan laboratory) using a protocol described previously by Grailhe et al. (1999). The floor of the apparatus was black and divided into five areas: four equal quadrants and one center area 14 cm in diameter. The experiments were conducted in a dimly lit room with the aid of a video camera to minimize subject stress and anxiety. The floor and walls of the open field were wiped with 70% isopropanol before each test session. Each test consisted of two videotaped five-minute sessions. During the first session, mice were placed in the center area and allowed to explore and acclimate to the apparatus. A novel object, consisting of a pink and green striped gray cube (2.5 cm^3) with an open side (designed by Allan laboratory), was placed in the center area with the open side facing the mouse. Response to the novel object during the second five-minute session was videotaped. Subsequently, a trained observer blinded to experimental group identity analyzed the video tapes and measured latency to approach the object and total number of center line crosses in the presence of the novel object.

2.6. Eight-way radial arm maze

Radial arm maze testing was conducted using the mouse runway system from Coulbourn Instruments (Whitehall, PA) using a protocol modified from Egashira et al. (2002). Plastic food cups containing sweetened condensed milk as a reinforcer, were placed approximately 2/3rds the way down in each arm. The maze was located in a room containing many extra-maze visual cues. Arm entry was recorded by an observer seated several feet away from the maze. All mice were habituated to the maze for 20 min on the day prior to testing. One week before testing, mice were placed on a caloric restriction diet where they received 3 g of lab chow (7004 Harlan Teklad S-2335, Denver, CO) each day. Untreated tap water was available *ad libitum*. Testing was conducted over 3 consecutive days. For each 8-minute testing session, the mouse was placed in the center of the maze. For each testing session; the number of correct choices in the initial eight chosen arms and the number of errors which was defined as choosing arms which had already been visited were scored.

2.7. Statistical analysis

All data were analyzed by *t*-test comparing the perinatal arsenic to the control on each of the dependent measures. Unless otherwise noted, all behavioral data points were taken from two animals per litter and this average treated as a single result. A repeated measures ANOVA was used in the eight-way radial arm maze behavioral test. Significance was set at $p \leq 0.05$.

3. Results

3.1. Arsenic exposure paradigm

No signs of overt toxicity (i.e. ataxia, redness, swelling, fetal malformations, death) were observed in the mice throughout the duration of the study. Absence of structural malformations is in accordance with what has been shown previously (Holson et al., 2000). The doses of arsenic used in present study were well tolerated and did not alter maternal body weight or body weights of the newborn pups (Martinez et al., 2008), consistent with what has been reported by others (Bardullas et al., 2009). Arsenic exposure did not change whole brain weight ($t < 1$, not significant) or hippocampal wet weight ($t < 1$, not significant) of offspring at day 35.

3.2. Drinking water analysis and data

Samples from six arsenic treated and six control water samples from throughout the span of the study were sent to Earth and Planetary Sciences for analysis. Water arsenic concentrations, analyzed by ICP-MS, were ~56 ppb and untreated tap water (control group) concentrations were ~6 ppb. The average daily litter water consumption, monitored every other day, and averaged over the entire study period was 13.09 ± 0.93 g/day for the control group and 15.98 ± 2.97 g/day for the perinatal arsenic group. There were no significant differences in the amount of water consumed by the litters in either treatment condition ($t < 1$, not significant). We did not measure the amount of arsenic present in mother's breast milk because this amount has been shown to be negligible according to several papers. One example comes from a study of Andean women exposed to high concentrations (about 200 ppb) of inorganic arsenic in drinking water. The concentrations of arsenic in their breast milk ranged from about 0.0008 to 0.008 ppm (Concha et al., 1998). Similarly, a World Health Organization study detected arsenic at concentrations of 0.00013–0.00082 ppm in human breast milk (Somogyi and Beck, 1993). Although these studies were not conducted in animals, extrapolation of the data would suggest that the concentration of arsenic that is present in the breast milk in our mice after exposure to arsenic in their drinking water is extremely low.

3.3. Brain arsenic concentrations

Total inorganic arsenic concentrations detected in brain tissue of offspring at 35 days of age were measured. Control brain levels were 1.0 ± 0.24 ppb and perinatal arsenic offspring levels were 2.24 ± .02 ppb. Levels of arsenic in the perinatal exposure group significantly exceeded controls ($t(6) = 3.87$, $^{**}p < 0.008$). The source of arsenic in the brains of control animals may be either organic or inorganic given that untreated tap water at UNM has ~6 ppb arsenic and approximate levels of arsenic within the chow are 0.16 ppm, based on analyses done at Harlan Labs (personal communication).

3.4. Glucocorticoid and mineralocorticoid receptors

Due to the high basal CORT levels in the perinatal-exposed mice that we previously reported (Martinez et al., 2008); we decided to examine the intracellular distribution of the GR and MR proteins. Intracellular distribution of the GR and MR is important for its function.

Fig. 1, panels A–B and C–D, shows a representative western blot, together with results of the densitometric quantitation of the nuclear and cytosolic glucocorticoid receptor bands, respectively. The rabbit polyclonal antibody against the mouse glucocorticoid receptor recognized a prominent band at ~95 kDa, consistent with the molecular weight of the glucocorticoid alpha receptor. Perinatal arsenic exposure reduced levels of both nuclear and cytosolic GR receptors. Fig. 1B shows the quantification of the nuclear compartment where there was significantly less GR in the perinatal mice than controls ($t(13) = 3.81$, $^{**}p < 0.002$). The cytosolic fraction (Fig. 1D) revealed a similar significance ($t(13) = 2.11$, $^{*}p < 0.05$). This finding is curious as we would have predicted higher nuclear GR levels in the perinatal offspring due to their higher basal CORT levels.

Fig. 2 shows a representative western blot, together with results of the densitometric quantitation of the mineralocorticoid receptor bands. The rabbit polyclonal antibody against the mouse mineralocorticoid receptor recognized a prominent band at ~102 kDa, consistent with the molecular weight of the mineralocorticoid receptor. In the perinatal arsenic exposed mice there was significantly less MR in the nuclear fraction compared to controls (Fig. 2B) ($t(13) =$

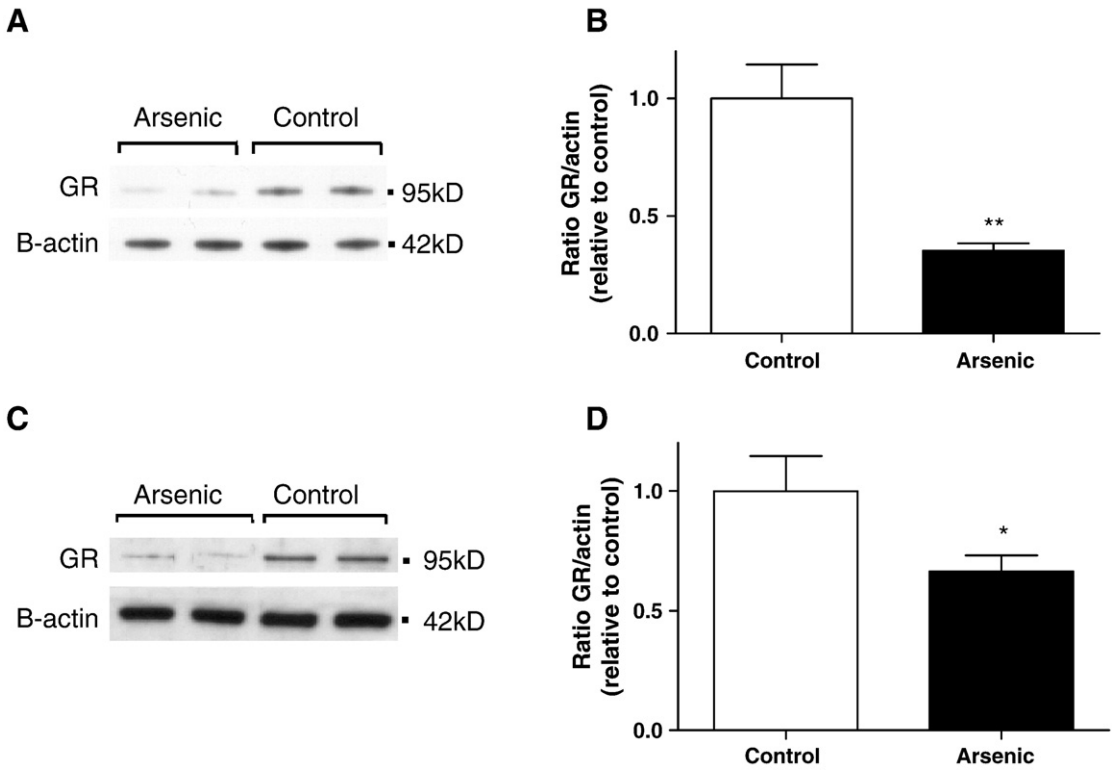

Fig. 1. Nucleocytoplasmic trafficking of GRs and densitometric quantitation of GR bands. (A) Representative western blot of GR in the hippocampal nuclear compartment of arsenic-exposed mice. The anti-GR polyclonal antibody recognized a prominent band at ~95 kDa, representative of GRα. (B) Quantitation of GR bands in nuclear subcellular fraction of arsenic-exposed mice was based on densitometric analysis (**$p<0.002$ vs. control). (C) Representative western blot of GR in the hippocampal cytosolic compartment of arsenic-exposed mice. (D) Quantitation of GR bands in cytosolic subcellular fraction of arsenic-exposed mice was based on densitometric analysis (*$p<0.05$ vs. control). Results are expressed as a percentage of the GR signal to beta-actin signal, relative to controls and are presented as mean ± SEM of seven–eight litters.

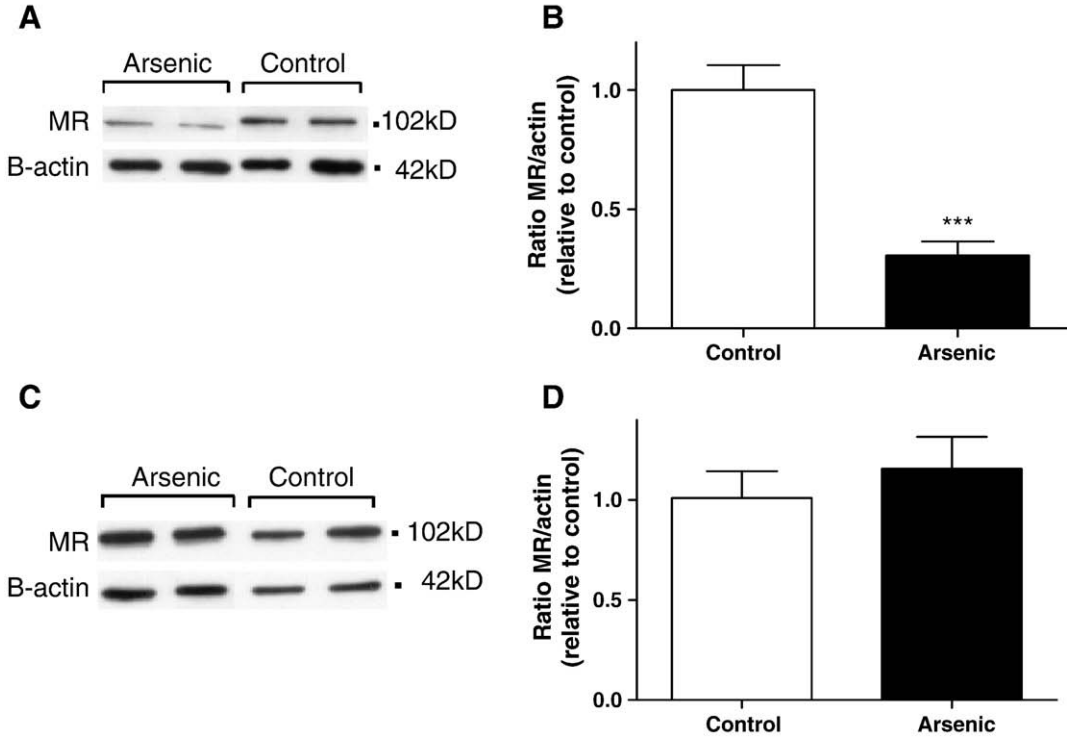

Fig. 2. Nucleocytoplasmic trafficking of MRs and densitometric quantitation of MR bands. (A) Representative western blot of MR in the nuclear compartment of arsenic-exposed mice. The anti-MR polyclonal antibody recognized a prominent band at ~102 kDa. (B) Quantitation of MR bands in hippocampal nuclear subcellular fraction of arsenic-exposed mice was based on densitometric analysis (***$p<0.0001$ vs. control). (C) Representative western blot of MR in the cytosolic compartment of arsenic-exposed mice. (D) Quantitation of MR bands in hippocampal cytosolic subcellular fraction of arsenic-exposed mice was based on densitometric analysis ($p>0.05$ vs. control). Results are expressed as a percentage of the MR signal to beta-actin signal, relative to controls, and are presented as mean ± SEM of six–eight litters.

5.95, $^{***}p<0.0001$). Fig. 2D shows the cytosolic fraction, where there is no difference in relative amount of MR ($t<1$, not significant).

3.5. Novel object exploration

Behavior is an informative end-point for assessing the neurobiological effects of arsenic exposure. Mineralocorticoid receptors have been shown to be associated with ability to learn in a novel situation. We used the novel object task as a measure of learning and memory. Fig. 3, panels A and B, shows the latency to novel object and entries to the center in the presence of novel object, respectively during a second five-minute period. Perinatal-exposed mice took significantly longer to recognize the presence of the novel object, measured as latency to approach the novel object ($t(12)=13.79$, $^{***}p<0.0001$). Compared to controls, perinatal mice made significantly fewer center entries in the presence of the novel object ($t(12)=4.61$, $^{***}p<0.0006$). The increase in latency and center entries may be confounded by an anxiety response; therefore we conducted an additional behavioral test to rule out this variable.

3.6. 8-way radial arm maze

We used the 8-way radial arm maze to determine if the arsenic exposure was associated with decreased hippocampal-dependent spatial learning and memory. Because variations in maternal care within the same strain of animals can lead to differences in behavioral performance including spatial memory (Barha et al., 2007) we pulled offspring from different litters to preclude the litter effect. It has also been reported that males inherently perform better on spatial memory tasks than do females (Brandeis et al., 1989; Vorhees et al., 2004), thus only males were used on the behavioral tasks. All mice were acclimated to the testing field prior to test days. All control mice showed a progressive decline in number of entry errors over the three testing days. Arsenic offspring errors did not change significantly with test day (Fig. 4). There was a significant effect of treatment ($F(1,18)=50.79$, $^{***}p<0.0001$) and these results indicate that perinatal arsenic exposure in mice might induce spatial learning and memory impairment.

4. Discussion

The persistence of arsenic in drinking water raises the importance of assessing the neurobiological consequences of exposure to low levels of arsenic. Our perinatal exposure model provides a unique opportunity to examine the effects of arsenic on the developing brain at an environmentally relevant level, magnitudes lower than what has been previously studied. Arsenic has been shown to accumulate in target organs (Kenyon et al., 2008) and at 35 days of age, two weeks after weaning and absence of arsenic, our model revealed arsenic (total arsenicals) content in the brains of our adolescent offspring. We are currently seeking to determine which areas of the brain, if any, are preferentially affected. We previously showed elevated basal CORT levels in offspring perinatally exposed to 55 ppb arsenic compared to their control counterparts (Martinez et al., 2008). These findings led us to evaluate the status of the receptors responsible for mediating changes in CORT levels. Results from the present experiments revealed lower abundance of hippocampal cytosolic GRs in perinatal arsenic-exposed offspring compared to controls but no difference in abundance of cytosolic MRs. Nuclear levels of both GR and MR were significantly lower than those of controls. In addition to this receptor deficit perinatal-exposed mice exhibited behavioral underperformance in the learning and memory tests.

CORT binds and activates the cytosolic GR and MR hormone receptors initiating translocation of activated receptor to the nucleus. Although there is sufficient CORT to activate these receptors in our animals (Martinez et al., 2008), the amount of nuclear hippocampal GR and MR is significantly less in the arsenic-exposed offspring than in the controls (Figs. 1AB–3AB). A plausible explanation for this is failure of the receptors to traffic to the nucleus. While other groups have investigated this possibility and found that the trafficking of these receptors to the nucleus is unaffected by arsenic (Kaltreider et al., 2001) these studies were conducted using a hepatoma cell line and the results may not be the same in the brain, as is the case with many studies in other systems. In addition, the cell line fails to maintain the contribution of the intact HPA axis and does not account for genes that may have previously been affected. For example, high CORT levels have been shown to reduce dynein cytosplasmic intermediate chain 1 gene expression, reducing motor protein activity and axonal transport (Morsink et al., 2006), a result that would not have been taken into account in the hepatoma cell line. In accordance

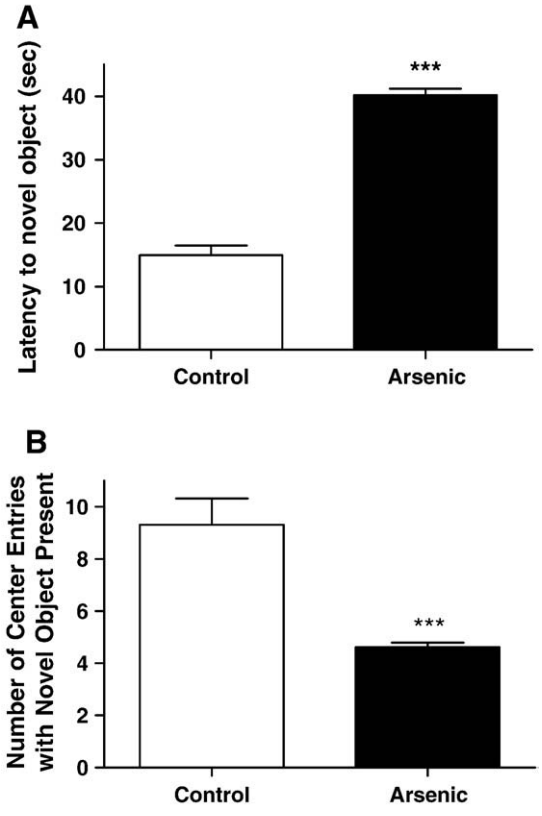

Fig. 3. Effects of arsenic exposure to 50 ppb arsenic on novel object exploration at D35. (A) Latency to approach novel object after acclimation period ($^{***}p<0.0001$ vs. control). (B) Entries to the center in the presence of the novel object ($^{***}p<0.0006$ vs. control). Data are presented as mean ± SEM of seven litters.

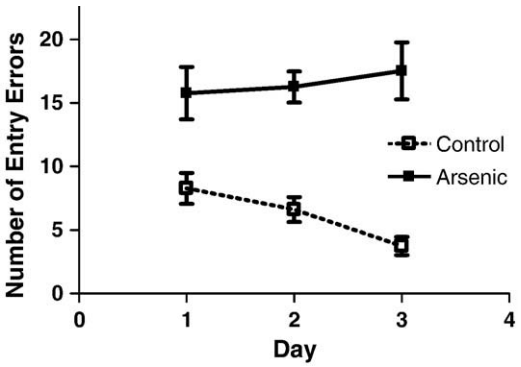

Fig. 4. Effects of arsenic exposure on number of entry errors in 8-way radial arm maze at D35. Day 1 represents first day after acclimation period. There was a significant effect of treatment ($^{***}p<0.0001$ vs. control). Data are presented as mean ± SEM of ten litters.

with our high CORT, the translocation of GR to the nucleus by dynein may be reduced. Lower levels of nuclear GR and MR would have far reaching consequences on gene transcription. It has been shown that, in particular for GR, nuclear translocation of the activated receptor and its ability to induce transcriptional activity are intimately correlated (de Kloet et al., 2009). Binding of GRs to promoter glucocorticoid response elements (GREs) regulates gene transcription both positively and negatively and approximately 20% of the genome is under this control. In support of this possibility, we previously showed increases in 5-HT_{1A} receptor density in the dorsal hippocampus of our perinatal arsenic-exposed mice (Martinez et al., 2008). The 5-HT_{1A} receptor is negatively regulated by corticosteroids through the GR (Chalmers et al., 1993; Datson et al., 2001; de Kloet et al., 1986; Meijer et al., 1997) and reduced GR binding to negative GREs would allow for transcription and ultimately upregulation of the 5-HT_{1A} gene and may be one example of the consequences of reduced nuclear GRs.

The finding of no difference in cytosolic MR protein levels compared to controls could be explained by the presence of two different promoters (P1 and P2) for the MR gene. P1 and P2 can both be activated by glucocorticoids but P2 can also be activated by aldosterone with the same affinity (Viengchareum et al., 2007). Thus GR and MR although both activated by glucocorticoids are also differentially regulated. Activation of P2 by aldosterone, which is independent of the HPA axis, could be enough to keep MR levels constant in the cytoplasm. These differences in transcription regulation could help explain the differences in protein levels seen in our animals.

The reductions in GR are important as studies using GR germline knockouts have shown that knocking down the receptor in all organs results in an inviable animal that dies approximately 1 week after birth due to atelectasis of the lungs (Erdmann et al., 2008). However, conditional knockouts and loss of the receptor in whole brain result in animals that are viable and exhibit many of the characteristics that we have seen in our animals. For example, GR knockout mice are reported to have elevated CORT levels, impaired spatial memory and reduced anxiety behavior (Erdmann et al., 2008). Our perinatal arsenic-exposed mice exhibit slight reductions in anxiety in the elevated plus maze (unpublished observations) and depression-like behavior in a learned helplessness task (Martinez et al., 2008). Dysregulation within the HPA axis has long been suggested to be part of depressive pathophysiology. The high CORT levels at the adolescent time point at which our measurements were taken suggest that there is dysregulation of the axis. We are currently investigating this possibility. GR expression is detectable at embryonic day 14.5 and HPA axis activity and regulation is assumed to be established around this time during development (Michelsohn and Anderson, 1992). Our perinatal exposure period could be affecting the establishment of regulation of the axis, enabling sustained or cumulative damage with further high CORT levels resulting in an inability to return to homeostasis and prevent a maladaptive overshoot. GRs are important for terminating the stress response via negative-feedback control and MRs are thought to control the basal circadian rhythm of plasma CORT secretion (Sapolsky et al., 1986). A reduction in MRs and GRs, coupled with reduced nuclear transport, could affect the maintenance of homeostasis via the HPA axis and HPA response to stressful situations. Alterations in the HPA axis response to stress and changes in transcription mediated by GR are likely to play an important role in mechanisms underlying neural plasticity, providing a possible basis for linking neuroendocrine dysregulation and cognitive decline.

It is well established that chronically elevated levels of CORT, mediated by the binding to GRs and MRs, produce cognitive deficits (de Kloet et al., 2005, Joels et al., 2007; McEwen, 2007). Many epidemiological studies have focused on the issue of deficits in learning and memory associated with exposure to arsenic (Calderon et al., 2001; von Ehrenstein et al., 2007; Wasserman et al., 2004). Our perinatal arsenic-exposed offspring performed poorly on learning and memory tests. Results from the novel object exploration test revealed deficits in latency to the novel object and in number of center entries in perinatal-exposed animals suggesting that arsenic animals have learning and memory deficits (Fig. 3). This impaired performance could be explained by loss of nuclear MR. Findings from mutant mice with inactivated MR resulted in impaired behavioral reactivity to novelty (Berger et al., 2006). The 8-way radial arm maze, an assessment of spatial working memory, illustrated that arsenic-exposed offspring have difficulties performing more arduous hippocampal-dependent learning tasks (Fig. 4). Arsenic-exposed offspring failed to make improvements in the task as the days progressed. This is in agreement with other findings that long periods of stress or CORT treatment impair performance on a variety of spatial tasks (Brinks et al., 2007; Elizalde et al., 2008; Schwabe et al., 2008). For example, hypercorticism, after exposure to restraint stress for 6 h per day for 3 weeks, was shown to result in impaired spatial memory in adult animals (Conrad et al., 1996; Luine et al., 1996). Furthermore, long-term high dose exogenous CORT treatment in Long–Evans rats was shown to produce disruptive effects in water-maze performance, a test for spatial memory similar to the 8-way radial arm maze (Bodnoff et al., 1995). MR appears to mediate the immediate effects of CORT on memory acquisition, while the presence of a functional GR is responsible for modulation of spatial memory (Oitzl et al., 1997). While a direct link between the poor performance in these behavioral tasks and reduced GR levels is not established by these studies the findings are consistent with the interpretation that memory performance is affected by arsenic-induced decreases in GR in the hippocampus.

In conclusion, the present study indicates a relationship between decreased CR levels and cognition. While other studies have examined CR levels and subcellular localization in other target organs, to our knowledge ours is the first report of the brain receptor levels after perinatal arsenic exposure. Changes in CR levels cannot fully explain the perinatal arsenic-induced impairments in cognitive performance. However, these lower CR levels are likely altering transcription of genes that are important for learning and memory. Overall these data suggest that moderate perinatal arsenic exposure can have long lasting effects on learning and memory well into adolescence, consistent with the Fetal Basis of Adult Disease hypothesis (White et al., 2007), which postulates that many adult diseases have a fetal origin. Further studies detailing the molecular effects of perinatal arsenic exposure on the central nervous system are essential to our understanding of arsenic production of learning and memory deficits.

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Conflicts of interest

The authors report no conflicting interests.

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