

Maternal Exposure to Mercury Chloride During Pregnancy and Lactation Affects the Immunity and Social Behavior of Offspring

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Developmental HgCl₂ exposures of F1 offspring (H-2^{q/s}) from unsociable SJL/J (H-2^s) dams with high susceptibility to Hg-induced autoimmunity (SFvF1) and from highly sociable FVB/NJ (FVB; H-2^d) dams with lower susceptibility to Hg-induced autoimmunity (FvSF1) were investigated. Hg exposure increased the serum IgG levels of all offspring at postnatal day 21 (pnd21) and of SJL/J dams but not of FVB dams. Serum IgG anti-brain antibody (Ab) levels of pnd21 SFvF1 offspring and SJL dams were higher than those of the FvSF1 offspring and FVB dams, but Hg only increased the titers of the FVB dams and their offspring. Hg significantly elevated the presence of IgG in all brain regions of the pnd21 SFvF1 offspring, and the SFvF1 offspring had greater amounts of IgG in the brain than the FvSF1 offspring, which had Hg-induced increases in only two brain regions. Cytokine levels were elevated in the brain regions of Hg-treated pnd21 SFvF1 but not of FvSF1 offspring, and SFvF1 females had more brain regions expressing cytokines than the males. At pnd70, the serum IgG, serum anti-brain Abs, amounts of brain IgG, and brain cytokine levels of all of the Hg-treated offspring were equivalent to those of their appropriate controls, suggesting that developmental Hg exposure did not induce chronic immunological effects. However, the social behaviors of Hg-exposed SFvF1 offspring at pnd70 were significantly impaired, and SFvF1 females displayed greater decline in social behaviors than males, suggesting that the higher neuroinflammation of SFvF1 females earlier in life is associated with the altered behavior. Thus, developmental Hg exposure induces long-lasting effects on social behavior of offspring, which is dependent on sex and genetics and the induction of neuroinflammation.

Key Words: mercury; mouse social behavior; IgG anti-brain antibodies; maternal influences.

Mercury (Hg) has been demonstrated to induce renal autoimmunity due to autoantibody (autoAb)-induced nephritis, which is dependent on the major histocompatibility complex (MHC) haplotype (Henry *et al.*, 1988; Hultman and Eneström, 1988; Hultman *et al.*, 1993). The different forms of Hg, e.g., methyl Hg (MeHg), ethyl Hg, and inorganic Hg (Hg²⁺),

differentially affect immunity (Havarinasab and Hultman, 2005), and although MeHg is more toxic than Hg²⁺, Hg²⁺ generated more anti-brain Abs in A.SW (H-2^s) mice than MeHg (Zhang *et al.*, 2011). Mice carrying H-2^s genes have been shown to be the most sensitive to Hg-induced autoimmunity, whereas H-2^d mice are slightly less sensitive, and mice carrying H-2^a or H-2^b genes are low to nonresponders (Hultman *et al.*, 1992; Nielsen and Hultman, 2002; Zhang *et al.*, 2011). In addition, Hg is well known to be neurotoxic (Clarkson and Magos, 2006; Farina *et al.*, 2011; Myers and Davidson, 1998). Both MeHg and Hg²⁺ are able to directly interact with neurons and cause oxidative stress-induced apoptosis; however, neurons are much more sensitive to MeHg compared with Hg²⁺ (Clarkson and Magos, 2006; Gassó *et al.*, 2001). Neurobehavioral changes induced by MeHg were also observed in mice carrying H-2 haplotypes resistant to Hg-induced autoimmunity (Cooper and Kusnecov, 2007; Goulet *et al.*, 2003; Kim *et al.*, 2000), suggesting that the neurotoxicity of MeHg is at least partially through its direct interaction with neurons and/or other cells including astrocytes and microglia (Shanker *et al.*, 2003). At subclinical doses, neurobehavioral changes of mice exposed to Hg²⁺ were only observed in strains carrying H-2 haplotypes sensitive to Hg-induced autoimmunity (Zhang *et al.*, 2011). Although high levels of exposure to Hg²⁺ have direct neurotoxic effects, indirect neurotoxic effects at low levels of Hg exposures are posited to occur through the induction of anti-brain Abs and neuroinflammation. Because MeHg could be demethylated to form Hg²⁺ *in vivo* (Ishitobi *et al.*, 2010), the induction of anti-brain Abs by Hg²⁺ might also contribute to the MeHg-induced neurological alterations, dependent on the MHC haplotypes and the amount of Hg²⁺ released.

Because various studies have demonstrated that immature neurons are more sensitive to Hg toxicity, and Hg exposure can induce neurobehavioral alterations, a concern for the neurotoxicity of dietary exposure to MeHg in children remains (Koren and Bend, 2010; Monnet-Tschudi *et al.*, 1996; Myers *et al.*, 2009). The incidence of impaired social behavior has

been substantially increased during the past decades of years (Center for Disease Control and Prevention [CDC], 2012; Gurney *et al.*, 2003), questioning whether exposure to Hg may be involved (Schultz 2010). In animal models, the effects of developmental Hg exposure on the social behavior (sociability) had not been previously measured. Herein, the study was designed to investigate whether exposure to Hg during fetal development affects social behavior and whether any effects were dependent on maternal influences, including genetic susceptibilities and innate and adaptive immunity that could affect neuroinflammation. Although anti-brain Abs likely may not cause impaired social behavior in all mouse strains, they have been demonstrated to be crucial in some models (Martin *et al.*, 2008; Singer *et al.*, 2009).

This study was designed to assess whether gestational Hg²⁺ exposure of pregnant dams expressing certain H-2 haplotypes are induced to develop maternal anti-brain Abs, which could impair the social behavior of offspring. We hypothesize that a central mechanism for reduced social behavior is environmental toxicant induction of maternal anti-brain Ab deposition in the brain of developing fetuses, which causes detrimental developmental neural functions due to inflammation.

MATERIALS AND METHODS

Animals. SJL/J (H-2^b) and FVB/NJ (H-2^d) mice, 7–9 week of age from the Jackson Laboratory (Bar Harbor, ME), were bred for these experiments. Mice were housed in the AAALAC-approved Wadsworth Center Animal Facility with controlled temperature (23°C), humidity, and an artificial 12-h light-12-h dark cycle, and this study was approved by Wadsworth Center's IACUC. The two parental SJL/J and FVB strains have different sensitivities to Hg-induced immunomodulation (Abedi-Valuggerdi and Möller, 2000), whereas the reciprocal F1 offspring (SFvF1 and FvSF1) have similar genetic compositions with the exception of their sex chromosomes and mitochondria. Therefore, we selected the reciprocal F1 offspring to evaluate maternal influences on social behavior through immune modulation by Hg exposure.

Experimental groups. Two females were mated with one male per cage with free access to water and food. At gestational day (gd) 8 (based on the appearance of vaginal plug), females were taken out and placed in individual cages. A previous study indicated that developmental exposure to the subclinical dose of 50 µM HgCl₂ in maternal drinking water from gd8 to postnatal day (pnd) 21 induced IgG anti-brain Abs both in the sera of dams and pups of the A.SW (H-2^s) strain (Zhang *et al.*, 2011). Thus, our experiments utilized 50 µM HgCl₂ in maternal drinking water, which was provided *ad libitum* from gd8 to pnd21. Control animals were treated with normal drinking water. For the Hg-treated groups, 7 SJL/J dams and 6 FVB dams were used; for the control groups, 7 SJL/J dams and 7 FVB dams were used. SFvF1 and FvSF1 offspring were developmentally exposed to HgCl₂ and were evaluated for anti-brain Ab levels and altered social behavior.

Blood and tissue collection. Serum IgG, serum IgG anti-brain Abs, IgG presence in brain sections, and multiple cytokines in the brain were measured at pnd21 and pnd70. Dams and randomly selected pnd21 offspring (one male and one female per litter) at weaning were euthanized by CO₂ exposure and assayed. Bloods were collected by cardiac puncture; thereafter, brains were perfused with PBS and then collected. Brains of offspring were excised and dissected to obtain substantia nigra (SN), hypothalamus (HT), frontal cortex (FCX), striatum (STR), cortex (CX), hippocampus (HC), and cerebellum (CB). Thereafter, the brain regions were homogenized immediately. The brain

homogenates were sonicated for 1 min and then centrifuged at 12,000 × g for 30 min at 4°C. Supernatants, which were for brain IgG and cytokine assays, as described later, were collected and stored at –80°C until use. Bloods were stored at 4°C for 24 h, and sera were collected after centrifugation at 12,000 × g for 10 min, and then stored at –80°C until use. HgCl₂ exposure was stopped at pnd21. At pnd70, all of the F1 offspring were assayed for sociability and urine protein analysis; thereafter, bloods and brains were harvested as described above. One offspring of each sex was randomly selected from each litter for brain IgG and cytokine assays as described later. In addition, within each strain, sex, and treatment at pnd1, pnd21, and pnd70, respectively, one offspring was selected randomly to make whole brain homogenate as antigens (Ags) for serum anti-brain Ab detection as described later. Whole brain homogenates were also made from one pnd21 SJL/J mouse and one pnd21 FVB mouse, respectively.

Urine protein analysis. Urine protein analysis of SFvF1 and FvSF1 offspring was performed at pnd70. Mice were first anesthetized with CO₂; thereafter, urine was harvested and tested immediately with urinalysis strips (SIEMENS, Tarrytown, NY). Protocol was according to manufacturer's instruction.

ELISA. ELISA was used to measure levels of serum IgG, serum IgG anti-brain Abs, and IgG in brain regions. For measurement of serum IgG, goat anti-mouse (GAM) IgG γ-chain (Sigma, St Louis, MO) was used as capture Abs. Peroxidase-conjugated GAM IgG whole molecule (Sigma) was used as detection Abs; 3,3',5,5'-tetramethylbenzidine (Sigma) was used as substrate. One mole of H₂SO₄ was used to stop the peroxidase-substrate reaction. The ELISA plates were read at OD450 on an ELISA analyzer (Bio-Tek, Winooski, VT). For the analysis of serum IgG anti-brain Abs, brains of offspring were homogenized in the presence of homogenization buffer containing 1% NP-40, 50 mM Tris-Cl (pH 7.6) (Sigma), 150 mM NaCl, 2 mM EDTA, 1 mM Na-orthovanadate, 5 mM NaF, and 10 µg/ml of proteinase inhibitor cocktail (Sigma). Brain homogenates from both Hg-treated and water-treated offspring (pnd1, pnd21, and pnd70) and normal FVB and SJL/J mice (pnd21) were used as Ags. Protein concentrations were assayed with a BCA Protein Assay Kit (Pierce, Rockford, IL) according to the manufacturer's instructions. Homogenates (200 µg of protein/ml) were coated to ELISA plates as brain Ags. Rat anti-mouse CD16/CD32 (Fc block; BD Pharmingen, San Jose, CA) was used to incubate with the coated brain Ags prior to the addition of serum samples. Peroxidase-conjugated GAM IgG γ-chain (Sigma) was used as detection Abs. Thereafter, the protocol was the same as described earlier. Because we did not observe any differences of brain Ags due to sex, age, or treatment under our setting, to compare the serum anti-brain Ab levels, we used the same pnd1, pnd21, and pnd70 homogenates from the control (water) groups for analysis of all sera of each strain. For the quantification of the IgG concentration in different brain regions, the protocol was the same as serum IgG detection as described earlier. The concentration of IgG in the homogenate of each brain region was calculated as ng IgG/mg protein.

Luminex analysis. Cytokines were measured in the brain sections of SFvF1 and FvSF1 offspring. Because autoAbs might cause elevation of multiple types of cytokines derived from multiple types of immune cells, we performed Luminex analysis to measure brain cytokines. At pnd21, classical proinflammatory cytokines (interleukin [IL]-1β, IL-6, and tumor necrosis factor [TNF]-α) and other myeloid cell or dendritic cell-related cytokines (granulocyte-macrophage colony-stimulating factor [GM-CSF], monocyte chemoattractant protein [MCP]-1, macrophage inflammatory protein [MIP]-2, and IL-12p70), Th1 cytokines (interferon [IFN]-γ and IL-2), Th2 cytokines (IL-4, IL-5, IL-10, and IL-13), and Th17 cytokines (IL-17) were assayed at pnd21. Based on the data from pnd21 offspring, at pnd70, IL-1β, IL-6, MCP-1, IL-12p70, IL-4, IL-10, IL-13, and IL-17 were quantified. Fluorokine MAP multiplex mouse cytokine panel (R&D, Minneapolis, MN) was used. Protocol followed the manufacturer's instructions. Regional brain homogenate preparation and protein assay were as described above. Plates were run on a Luminex 100 IS analyzer. The cytokine concentration was calculated as pg cytokine/mg protein.

Sociability test. The sociability behaviors of the F1 offspring were tested using the three-chamber testing system (Dold Labs & Engineering, Seguin, TX) developed by Crawley and colleagues (Moy *et al.*, 2007). The procedure was performed, as described (Moy *et al.*, 2007), except that BALB/c mice (Taconic Farms, Inc., Hudson, NY) were used. The time spent in each of the three chambers and the migration among the three chambers were recorded by the affiliated social interaction software; the time sniffing the stimulus mouse and the time engaged in self-grooming behavior were recorded by hand-held timers. To avoid any left/right bias, for each test, the stimulus mouse was alternated in the left or the right chamber. Only the data collected from the second 10 min were used for behavioral analysis. If more than one male or female per litter was utilized, the time in each chamber was averaged.

Statistics. All the data were in the form of mean \pm SEM. Data for the dams were first analyzed using two-way ANOVA (strain and treatment); data for the offspring were first analyzed using three-way ANOVA (strain, sex, and treatment). If there was a significant difference, *post hoc* Bonferroni or Holm-Sidak tests were performed as stated. A value of $p < 0.05$ was selected as the level of significance. All statistical analyses were performed with SigmaStat 3.1.

RESULTS

Hg Effects on Litters

The dose of HgCl₂ used (50 μ M) was selected because it was previously shown to have no detrimental effect on A.SW offspring, a strain known to be sensitive to Hg-induced immunomodulation; there were no effects on litter size, birth weights, and growth (Zhang *et al.*, 2011). For the SFvF1 or FvSF1 offspring, there also were no significant differences with regard to litter sizes or body weights due to the Hg treatment; the average litter sizes were 6–7 pups. Birth weights did not significantly differ (data not shown), all animals looked healthy, and no abnormal appearances were observed after Hg treatment.

Urine Protein Levels Were Not Altered at Pnd70 After Hg Treatment

Because SJL/J mice are susceptible to Hg-induced renal autoimmunity (Abedi-Valuggerdi and Möller, 2000; Hultman *et al.*, 1992), we assessed whether developmental Hg exposure could induce proteinuria in F1 offspring. The urine protein levels were measured at pnd70 when the mice were assessed for behavioral activity because poor kidney functions might affect behavior. The gestational Hg treatment did not compromise adult kidney function, in that urine protein was not changed either in SFvF1 (100 \pm 0.1 [control] vs. 98.6 \pm 33.6 mg/dl) or in FvSF1 offspring (98.8 \pm 29.1 [control] vs. 107.1 \pm 19.1 mg/dl).

Serum IgG Levels

Serum IgG levels of the dams were measured at weaning of the offspring, and those of the offspring were measured at pnd21 and pnd70. For dam serum IgG, two-way ANOVA of dams indicated significant treatment differences ($F_{(1,24)} = 4.686$, $p = 0.042$) with no strain differences. No significant interactions were indicated. *Post hoc* Bonferroni test indicated that serum IgG levels were significantly elevated in the Hg-treated SJL/J dams ($t = 3.087$, $p = 0.005$) (Fig. 1A), but not in the

FVB dams (Fig. 1B). For pnd21 offspring serum IgG, three-way ANOVA indicated significant treatment ($F_{(1,43)} = 127.188$, $p < 0.001$) and strain ($F_{(1,43)} = 4.281$, $p = 0.045$) differences but no sex differences. *Post hoc* Bonferroni test indicated that Hg treatment significantly increased serum IgG levels of both the SFvF1 (male: $t = 9.208$, $p < 0.001$; female: $t = 7.746$, $p < 0.001$) and FvSF1 offspring (male: $t = 4.411$, $p < 0.001$; female: $t = 3.645$, $p < 0.001$) (Figs. 1A and B). At pnd70, the serum IgG levels of the offspring were no longer elevated by the Hg exposure (Fig. 1).

Serum IgG Antibrain Abs

Within each strain, brain Ags from untreated pnd1 offspring were used to test the sera of the dams for IgG antibrain Abs; brains from untreated pnd21 offspring and pnd70 offspring were used as Ags for pnd21 and pnd70 offspring testing, respectively. The sera from each group of Hg-treated mice and the counterpart water (control) group were tested using

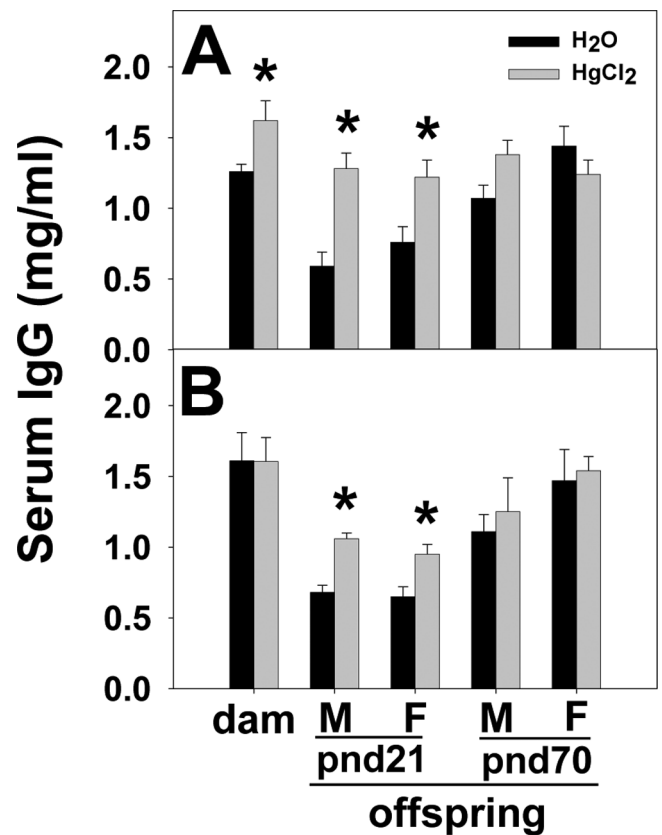


FIG. 1. Serum IgG levels of dams and pnd21 and pnd70 offspring. GAM IgG γ -chain Abs were used as capture Abs, and peroxidase-conjugated GAM IgG whole molecule Abs were used as detection Abs. Serum IgG was quantified by ELISA for SJL/J dams and SFvF1 offspring (A) and for FVB dams and FvSF1 offspring (B). M, male; F, female. * indicates a significant difference of the Hg group compared with the counterpart water group ($p < 0.05$). Number of mice used (H₂O; Hg): SJL/J dam (7; 7), pnd21 M (5; 7) and F (5; 7) SFvF1, pnd70 M (6; 7) and F (5; 7) SFvF1; FVB dam (7; 6), pnd21 M (7; 6) and F (7; 6) FvSF1, pnd70 M (6; 6) and F (6; 6) FvSF1.

the same brain homogenate. For serum anti-brain Abs of dams, two-way ANOVA indicated significant strain ($F_{(1,24)} = 9.637$, $p = 0.005$) and treatment differences ($F_{(1,24)} = 4.368$, $p = 0.043$). *Post hoc* Bonferroni test indicated no significant treatment differences for the SJL/J dams; both groups of the SJL/J dams had high levels of anti-brain Abs (Fig. 2A). However, the levels of anti-brain Abs of Hg-treated FVB dams were significantly increased compared with those of the water group ($t = 2.041$, $p = 0.048$) (Fig. 2B). For serum anti-brain Abs of pnd21 offspring, three-way ANOVA indicated significant strain ($F_{(1,43)} = 7.139$, $p = 0.011$) and treatment differences ($F_{(1,43)} = 4.374$, $p = 0.043$) but no sex differences. *Post hoc* Bonferroni test indicated no significant treatment differences between water and Hg-treated SFvF1 offspring; both groups had relatively high levels of serum anti-brain Abs (Fig. 2A). The levels of anti-brain Abs were significantly higher for the Hg-treated pnd21 FvSF1 offspring (male: $t = 2.430$, $p = 0.019$; female: $t = 2.163$, $p = 0.036$) compared with those of the water group (Fig. 2B). In addition, the levels of serum IgG anti-brain Abs of pnd21 offspring were similar to those of their dams for both of the two strains, in that the SFvF1 dams and offspring had high levels and no differences due to Hg, whereas the FvSF1 dams and offspring had lower levels that were elevated by Hg. At pnd70, the levels of serum anti-brain Abs returned to relatively low levels in the SFvF1 offspring, indicating that the pnd21 SFvF1 levels likely reflect mainly the levels of the SJL/J dams. There were no significant differences between the water and Hg groups of pnd70 offspring of either strain (Fig. 2). The serum anti-brain Abs from the SFvF1 and FvSF1 offspring and their dams also reacted to brain Ags from pnd21 SJL/J or FVB mice, and serum IgG anti-brain Abs from SJL/J dams also reacted with pnd70 SFvF1 brain Ags (data not shown). No significant differences were observed for Abs binding to brain Ags from water-treated mice and Hg-treated mice (data not shown). No significant differences were observed in brains from pnd1, pnd21, or pnd70 offspring when anti-brain Abs from dam sera were tested (data not shown). No sex differences of brain Ags were observed (data not shown).

Deposition of IgG in the Brain

Homogenates of brain regions from the pnd21 and pnd70 offspring were quantified for the presence of IgG. At pnd21, three-way ANOVA indicated significant treatment differences for all brain regions (SN: $F_{(1,40)} = 32.096$, $p < 0.001$; HT: $F_{(1,40)} = 37.334$, $p < 0.001$; FCX: $F_{(1,40)} = 36.907$, $p < 0.001$; STR: $F_{(1,40)} = 20.790$, $p < 0.001$; CX: $F_{(1,40)} = 52.970$, $p < 0.001$; HC: $F_{(1,40)} = 26.452$, $p < 0.001$; CB: $F_{(1,40)} = 35.827$, $p < 0.001$) and strain differences for many regions (HT: $F_{(1,40)} = 9.280$, $p = 0.004$; FCX: $F_{(1,40)} = 18.332$, $p < 0.001$; STR: $F_{(1,40)} = 7.677$, $p = 0.009$; CX: $F_{(1,40)} = 13.212$, $p < 0.001$), but no sex differences. *Post hoc* Bonferroni test indicated that the Hg-treated SFvF1 offspring had significantly elevated amounts of IgG (ng/mg protein) in all tested brain regions compared with the water group for both males and females (Fig. 3A).

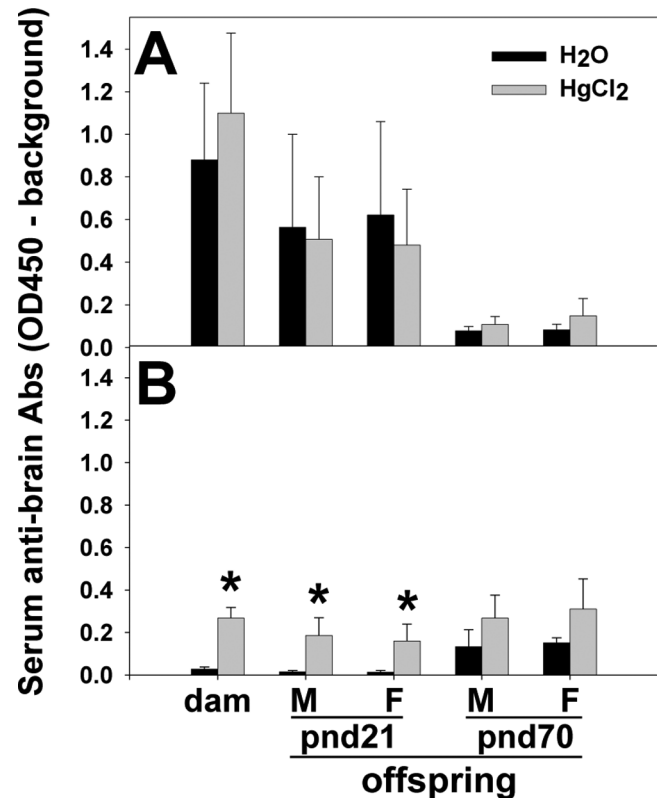


FIG. 2. Serum anti-brain IgG Ab levels of dams and pnd21 and pnd70 offspring. Brain homogenates from normal pnd21 offspring and pnd70 offspring were used as Ags to detect serum anti-brain Abs in pnd21 and pnd70 offspring, respectively; normal pnd1 brain homogenate was used as brain Ags to detect dam anti-brain IgG Abs. Peroxidase-conjugated GAM IgG γ -chain Abs were used as detection Abs. ELISA was used for assessment of serum anti-brain IgG Abs for SJL/J dams and SFvF1 offspring (A) and FVB dams and FvSF1 offspring (B). M, male; F, female. * indicates a significant difference of the Hg group compared with the counterpart water group ($p < 0.05$). Number of mice used (H₂O; Hg): SJL/J dam (7; 7), pnd21 M (5; 7) and F (5; 7) SFvF1, pnd70 M (6; 7) and F (5; 7) SFvF1; FVB dam (7; 6), pnd21 M (7; 6) and F (7; 6) FvSF1, pnd70 M (6; 6) and F (6; 6) FvSF1.

For the pnd21 FvSF1 offspring, the amount of IgG in some brain regions also was significantly increased by exposure to Hg (Fig. 3B). At pnd70, the significant differences between water and Hg groups of all offspring were no longer present (data not shown).

Effects of Developmental HgCl₂ Exposure on Sociability

At pnd70, the social behaviors of the offspring were tested, as previously described (Moy *et al.*, 2007). Three-way ANOVA compared the social behavior of male and female SFvF1 and FvSF1 offspring in the H₂O and HgCl₂ treatment groups. Both male SFvF1 and FvSF1 offspring demonstrated more interaction with the stimulus mouse than the females regardless of the Hg treatment (H₂O, $p = 0.002$; HgCl₂, $p < 0.001$; Fig. 4). The greater social interactions of the males also were apparent based on increased entrances to the chamber of the stimulus mouse and increased sniffing time (Table 1). SFvF1

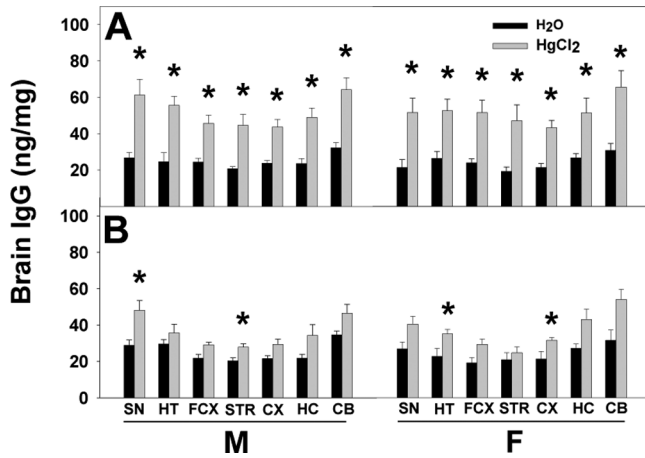


FIG. 3. IgG levels in brain regions of pnd21 offspring. Whole brains of pnd21 SFvF1 (A) and FvSF1 (B) offspring were dissected into SN, HT, FCX, STR, CX, HC, and CB, and homogenates of each region were used to assess presence of IgG by ELISA. GAM IgG γ -chain Abs were used as capture Abs, and peroxidase-conjugated GAM IgG whole molecule Abs were used as detection Abs for quantification of brain IgG (ng/mg protein). M, male; F, female; * indicates a significant difference of the Hg group compared with the counterpart water group ($p < 0.05$). Number of mice used (H₂O; Hg): M (5; 7) and F (5; 7) SFvF1, M (7; 6) and F (4; 6) FvSF1.

($p < 0.001$) and FvSF1 ($p = 0.045$) males had more sociability than the females, and female FvSF1 offspring had more sociability than the SFvF1 females ($p = 0.013$). The HgCl₂ treatment significantly lowered interaction with the stimulus mouse for both males ($p = 0.047$) and females ($p = 0.005$), but the HgCl₂ significantly lowered social behavior only for the SFvF1 offspring ($p < 0.001$) (Fig. 4). To address whether there was a trafficking/mobility difference, that is entrances and exits among chambers, we totaled the number for each group. Interestingly, the SFvF1 offspring migrated more than the FvSF1 offspring ($F_{(1,41)} = 9.12$, $p = 0.004$) (Table 1), but there were no sex, treatment, or interaction differences. Thus, the lower sociability of the SFvF1 mice was not due to less trafficking. Hg treatment did not significantly change the self-grooming behavior of either strain (data not shown).

Levels of Cytokines in the Brains of the Hg-Treated Pnd21 SFvF1 Offspring Were Elevated

Because Hg enhanced the amount of IgG in multiple brain regions of male and female SFvF1 offspring but significantly lowered social behavior only of the female SFvF1 offspring, as a measure of neuroinflammation, cytokine expression in various brain regions was assayed. Hg did enhance the expression of some cytokines in different brain regions of the SFvF1 offspring, and females had more regions with cytokine levels enhanced by Hg (Fig. 5). There were no Hg effects on the cytokine levels of the FvSF1 offspring (Supplementary fig. 1). Additionally, at pnd70, brain cytokine levels of both SFvF1 and FvSF1 offspring were equivalent between the Hg-treated and the water control groups (data not shown). GM-CSF, IFN- γ ,

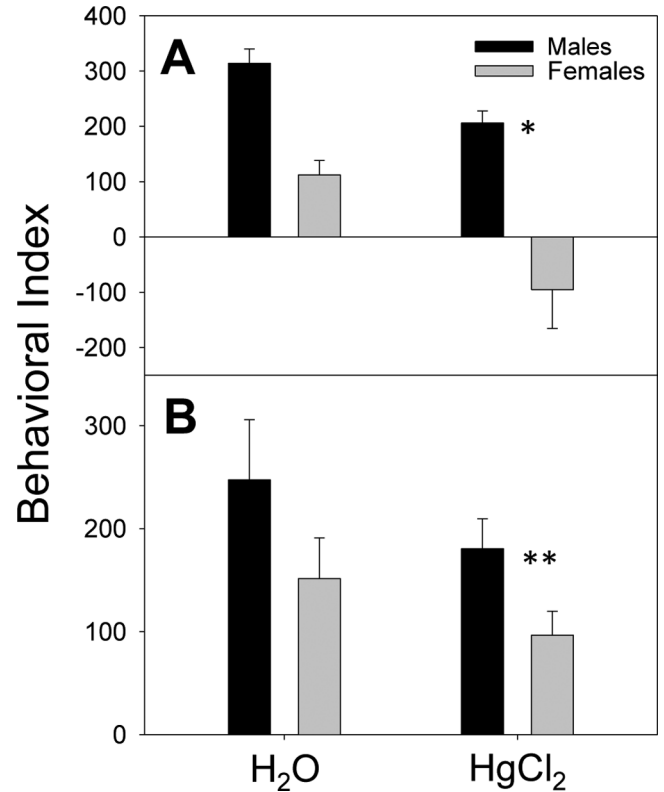


FIG. 4. Social behavior of pnd70 offspring. Three-chamber mouse social behavior testing was performed. BALB/c mice of the same age and sex were used as stimulus mice. A 20-min test was performed in which only the behavior in the second 10min was analyzed. The behavioral index is the time (s) in the side chamber with the novel mouse of same sex minus time in the side chamber with the novel object for the SFvF1 offspring (A) and the FvSF1 offspring (B). Three-way ANOVA (sex, treatment, and strain) indicated a significant sex difference ($F_{(1,42)} = 30.68$, $p < 0.001$), HgCl₂ effect ($F_{(1,42)} = 12.57$, $p < 0.001$) and a significant interaction ($p = 0.012$) between sex and strain. Pairwise multiple comparisons (Holm-Sidak method) indicated significant differences for the following: H₂O versus HgCl₂ for males ($p = 0.047$) and females ($p = 0.005$), male versus female for H₂O ($p = 0.002$) and HgCl₂ ($p < 0.001$), female SFvF1 versus female FvSF1 ($p = 0.013$), male versus female FvSF1 ($p = 0.45$), male versus female SFvF1 ($p < 0.001$), and H₂O versus HgCl₂ for SFvF1 offspring ($p < 0.001$). Number litters assayed for the treatment groups (H₂O; Hg) were M (6; 7) and F (5; 7) SFvF1, M (7; 6) and F (6; 6) FvSF1; * indicates significant sex and treatment difference and ** indicates only sex difference.

IL-2, IL-5, MIP-2, and TNF- α were not detectable (data not shown). The following cytokine results were obtained for the pnd21 brain regions. For IL-1 β , three-way ANOVA indicated significant treatment differences (FCX: $F_{(1,36)} = 7.103$, $p = 0.012$; CX: $F_{(1,36)} = 5.223$, $p = 0.028$), strain differences (SN: $F_{(1,36)} = 18.519$, $p < 0.001$; HT: $F_{(1,36)} = 48.896$, $p < 0.001$; FCX: $F_{(1,36)} = 52.780$, $p < 0.001$; STR: $F_{(1,36)} = 45.642$, $p < 0.001$; CX: $F_{(1,36)} = 53.105$, $p < 0.001$; HC: $F_{(1,36)} = 34.684$, $p < 0.001$; CB: $F_{(1,36)} = 19.297$, $p < 0.001$), and sex differences (STR: $F_{(1,36)} = 5.725$, $p = 0.022$; CB: $F_{(1,36)} = 5.234$, $p = 0.028$). *Post hoc* Bonferroni tests indicated that Hg treatment significantly increased IL-1 β in the FCX for SFvF1 females. For IL-13, three-way ANOVA indicated significant treatment differences

TABLE 1
Behavior Analysis of SFvF1 and FvSF1 Offspring After Developmental Exposure to HgCl₂

Strain	Chamber entrances ^a				Sniffing ^b			
	Males		Females		Males		Females	
	H ₂ O	HgCl ₂	H ₂ O	HgCl ₂	H ₂ O	HgCl ₂	H ₂ O	HgCl ₂
SFvF1	2.50±0.67 (58.5±7.43) ^c	2.86±0.99 (67.7±5.85)	0.80±0.49 (72.8±10.80)	-2.86±1.28 (55.5±4.36)	89.67±16.47	57.29±5.79	57.40±4.94	35.43±6.44
FvSF1	4.29±2.29 (44.1±5.12)	2.83±1.33 (52.0±5.33)	2.00±2.13 (47.0±9.97)	0.83±1.11 (53.3±4.28)	76.60±13.27	66.33±7.89	55.17±10.00	38.5±5.97

Notes. All results are reported as mean ± SEM. For chamber entrances (^a), the number of times entering the chamber with the empty cage was subtracted from the entrances into the chamber with the novel mouse. All entrances and exits from chambers also were totaled (^c); the SFvF1 mice differed from the FvSF1 mice ($F_{(1,41)} = 9.12, p = 0.004$) by three-way ANOVA, but there were no significant sex, treatment, or interaction differences. Three-way ANOVA analysis of the chamber entrances indicated a sex difference ($F_{(1,41)} = 7.75, p = 0.008$) but no treatment differences and no significant interactions. For sniffing (^b), the time (s) in direct contact with the novel mouse was measured. Three-way ANOVA analysis of sniffing indicated significant sex ($F_{(1,41)} = 14.40, p < 0.001$) and treatment ($F_{(1,41)} = 9.06, p = 0.004$) differences. There were no strain differences or significant interactions.

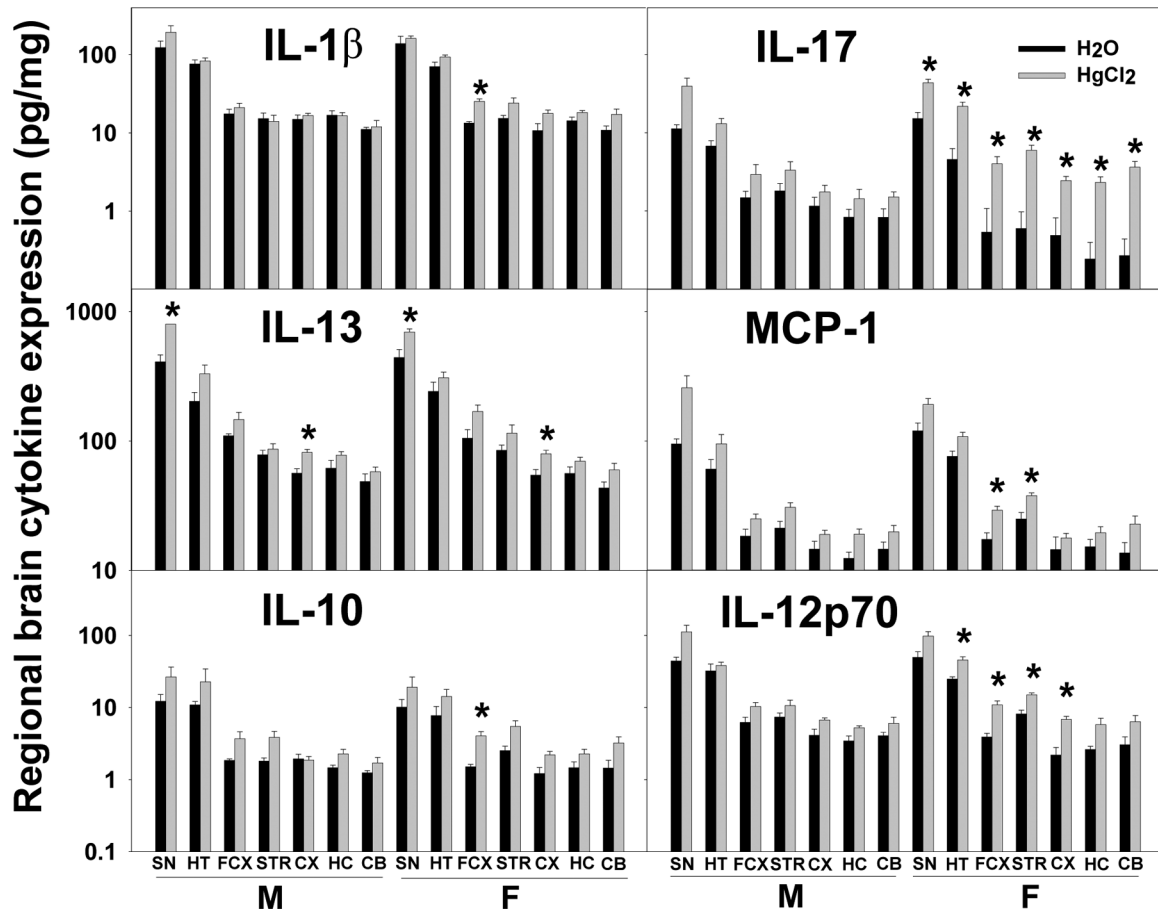


FIG. 5. Expression of brain cytokine levels of offspring at pnd21. Brains from pnd21 SFvF1 offspring were dissected into SN, HT, FCX, STR, CX, HC, and CB, and their homogenates were assayed for cytokine expression (pg/mg protein). M, male; F, female. * indicates a significant difference of the Hg group compared with the counterpart water group ($p < 0.05$). Number of mice used (H₂O; Hg): M (5; 6) and F (5; 6) SFvF1.

(SN: $F_{(1,36)} = 4.573, p = 0.04$; CX: $F_{(1,36)} = 6.928, p = 0.012$) and strain differences (SN: $F_{(1,36)} = 9.039, p = 0.005$; FCX: $F_{(1,36)} = 5.040, p = 0.031$; STR: $F_{(1,36)} = 64.032, p < 0.001$; CX: $F_{(1,36)} = 17.083, p < 0.001$; HC: $F_{(1,36)} = 20.022, p < 0.001$;

CB: $F_{(1,36)} = 91.057, p < 0.001$) but no sex differences. *Post hoc* Bonferroni tests indicated that Hg treatment significantly increased IL-13 in the SN and CX for both SFvF1 males and females. For IL-10, three-way ANOVA indicated significant

treatment differences (FCX: $F_{(1,36)} = 10.553, p = 0.003$; STR: $F_{(1,36)} = 10.190, p = 0.003$; HC: $F_{(1,36)} = 4.150, p = 0.048$), strain differences (SN: $F_{(1,36)} = 15.646, p < 0.001$; HT: $F_{(1,36)} = 9.413, p = 0.004$; FCX: $F_{(1,36)} = 40.313, p < 0.001$; STR: $F_{(1,36)} = 48.618, p < 0.001$; CX: $F_{(1,36)} = 60.398, p < 0.001$; HC: $F_{(1,36)} = 13.765, p < 0.001$; CB: $F_{(1,36)} = 20.261, p < 0.001$), and sex differences (CB: $F_{(1,36)} = 5.261, p = 0.028$). *Post hoc* Bonferroni tests indicated that Hg treatment significantly increased IL-10 in the FCX for SFvF1 females. For IL-17, three-way ANOVA indicated significant treatment differences (SN: $F_{(1,36)} = 18.315, p < 0.001$; HT: $F_{(1,36)} = 26.686, p < 0.001$; FCX: $F_{(1,36)} = 8.065, p = 0.007$; STR: $F_{(1,36)} = 14.127, p < 0.001$; CX: $F_{(1,36)} = 6.955, p = 0.012$; HC: $F_{(1,36)} = 7.510, p = 0.010$; CB: $F_{(1,36)} = 14.664, p < 0.001$), strain differences (SN: $F_{(1,36)} = 35.302, p < 0.001$; HT: $F_{(1,36)} = 45.707, p < 0.001$; FCX: $F_{(1,36)} = 13.988, p < 0.001$; STR: $F_{(1,36)} = 21.288, p < 0.001$; CB: $F_{(1,36)} = 5.726, p = 0.022$), and sex differences (CB: $F_{(1,36)} = 5.976, p = 0.020$). *Post hoc* Bonferroni tests indicated that Hg treatment significantly increased the inflammatory cytokine IL-17 in all of the brain regions for SFvF1 females. For MCP-1, three-way ANOVA indicated significant treatment differences (SN: $F_{(1,36)} = 8.353, p = 0.007$; FCX: $F_{(1,36)} = 7.943, p = 0.008$; STR: $F_{(1,36)} = 4.544, p = 0.04$; CB: $F_{(1,36)} = 10.034, p = 0.003$), strain differences (HT: $F_{(1,36)} = 11.463, p = 0.002$; FCX: $F_{(1,36)} = 25.595, p < 0.001$; CX: $F_{(1,36)} = 6.811, p = 0.013$; HC: $F_{(1,36)} = 14.922, p < 0.001$; CB: $F_{(1,36)} = 12.387, p = 0.001$), and sex differences (STR: $F_{(1,36)} = 5.899, p = 0.02$; HC: $F_{(1,36)} = 6.829, p = 0.013$). *Post hoc* Bonferroni tests indicated that Hg treatment significantly increased MCP-1 in the FCX and STR for SFvF1 females. For IL-12p70, three-way ANOVA indicated significant treatment differences (SN: $F_{(1,36)} = 10.616, p = 0.002$; HT: $F_{(1,36)} = 6.332, p = 0.017$; FCX: $F_{(1,36)} = 17.436, p < 0.001$; STR: $F_{(1,36)} = 9.435, p = 0.004$; CX: $F_{(1,36)} = 15.590, p < 0.001$; HC: $F_{(1,36)} = 5.865, p = 0.021$; CB: $F_{(1,36)} = 5.608, p = 0.024$), strain differences (SN: $F_{(1,36)} = 69.432, p < 0.001$; HT: $F_{(1,36)} = 171.972, p < 0.001$; FCX: $F_{(1,36)} = 141.127, p < 0.001$; STR: $F_{(1,36)} = 123.719, p < 0.001$; CX: $F_{(1,36)} = 77.515, p < 0.001$; HC: $F_{(1,36)} = 59.344, p < 0.001$; CB: $F_{(1,36)} = 54.475, p < 0.001$), and sex differences (STR: $F_{(1,36)} = 5.812, p = 0.021$). *Post hoc* Bonferroni tests indicated that Hg treatment significantly increased IL-12p70 in the HT, FCX, STR, and CX for SFvF1 females.

Although Hg did not affect the expression of IL-4 or IL-6 in the brain, IL-6 expression and, to a lesser extent, IL-4 expression were significantly greater in the SFvF1 offspring compared with the FvSF1 offspring, and SFvF1 females expressed higher IL-6 levels in the STR and HC than the males (Table 2).

DISCUSSION

The parental strains utilized herein were selected based on their H-2 haplotypes (H-2^s and H-2^g) and their known social behaviors. Mice carrying H-2^s genes (SJL/J mice) are known to be susceptible to Hg-induced autoimmune disease,

whereas mice of H-2^g haplotype (FVB mice) are less susceptible (Abedi-Valuggerdi and Möller, 2000; Hansson and Abedi-Valuggerdi, 2003; Hultman *et al.*, 1992; Warfvinge *et al.*, 1995). The parental strains were posited to have different sensitivities to Hg-induced autoimmunity, in that although Hg induces anti-nucleolar autoAbs in SJL and FVB mice, the Ab levels of FVB mice were lower compared with those of SJL/J mice; additionally, Hg treatment did not induce renal IgG deposition in FVB mice (Abedi-Valuggerdi and Möller, 2000), which is suggested to be critical for Hg-induced nephritis (Hultman and Eneström, 1988). With regard to social behavior, we anticipated that the highly social FVB strain would dominate or at least lessen the unsocial nature of SJL mice without any treatment but would be sensitive to environmentally induced impairment of social behavior. As expected, the untreated SFvF1 and FvSF1 offspring were more sociable compared with SJL/J mice. Thus, the SFvF1 and FvSF1 strains are a good model to test HgCl₂ effects on social behavior because both parental strains are sensitive to HgCl₂-induced autoimmunity, and the F1 offspring have similar genetics and normal social behavior.

Maternal autoantibody induction by Hg was considered to have potential detrimental consequences to fetal brains because more IgG can enter fetal brains, in that the blood-brain barrier (BBB) is absent or less intact in the developing central nervous system (Lossinsky *et al.*, 1986; Vorbrod *et al.*, 1986). However, there is evidence supporting the concept of an existing BBB during the fetal period (Ek *et al.*, 2012). The higher amounts of IgG in the brains of the Hg-treated SFvF1 and FvSF1 offspring do not seem to just reflect higher serum antibrain Ab levels because the SFvF1 mice had equivalent serum antibrain Ab levels in the water and Hg groups. The higher amounts of brain IgG in Hg-treated SFvF1 offspring relative to those in control SFvF1 or Hg-exposed FvSF1 mice suggest that the BBB may have been compromised or delayed in formation of tight junctions by the Hg, thus allowing more maternal IgG to enter. Additionally, brain vascular integrity may be different between the two strains. However, the male and female SFvF1 mice had equivalent levels of IgG in the brain, but the females had more cytokine expression and lower sociability. Thus, the differential effects of Hg on sociability likely are related to parameters in addition to BBB integrity.

The increased amounts of IgG in the Hg-exposed mice are related to the ability of Hg to enhance lymphocyte activation (Badou *et al.*, 1997; Jiang and Möller, 1995). The elevated levels of maternal SJL/J IgG could account for the elevated serum levels of the pnd21 SFvF1 offspring. However, the FvSF1 offspring also had elevated levels of serum IgG at pnd21 even though their dams did not have increased IgG due to the Hg exposure. This suggests that the higher levels at pnd21 could relate to increased neonatal expansion of B cells or increased early activation of the B cells or T cells (Johansson *et al.*, 1997) and/or the differences in genetics. The normal serum IgG levels of the offspring at pnd70 might be due to the absence of any Hg exposure after pnd21 and/or an induction of CD8⁺ suppressive

TABLE 2
IL-6 and IL-4 Expression in Different Brain Regions of SFvF1 and FvSF1 Offspring at Pnd21 (pg/mg protein)

Brain sections	Treatment	IL-6				IL-4			
		SFvF1		FvSF1		SFvF1		FvSF1	
		Males	Females	Males	Females	Males	Females	Males	Females
SN	H ₂ O	48.6±7.6	57.4±8.6	7.3±1.2	6.5±1.5	1.6±1.3	1.9±1.8	4.8±1.0	4.8±0.9
	HgCl ₂	98.7±30.3	79.1±11.4	8.3±1.5	6.8±0.8	0.8±0.8	6.0±6.0	4.6±0.7	5.5±0.5
HT	H ₂ O	33.5±8.8	36.0±8.6	3.9±0.7	3.8±0.6	4.5±0.5	3.6±2.3	4.2±0.6	3.6±0.4
	HgCl ₂	21.8±3.6	74.4±23.1	4.1±0.3	2.8±0.4	5.6±4.8	4.8±2.8	4.2±0.8	4.3±0.4
FCX	H ₂ O	12.0±5.2	6.2±4.9	0.6±0.2	0.6±0.4	1.1±0.4	0.8±0.1	2.1±0.3	2.4±0.3
	HgCl ₂	6.2±0.4	8.2±3.1	0.5±0.1	0.5±0.1	0.7±0.5	2.5±0.9	2.7±0.6	2.9±0.5
STR	H ₂ O	3.0±1.2	5.1±0.5 ^a	2.7±1.3	1.2±0.5	1.2±0.4	0.7±0.1	1.8±0.7	1.7±0.2
	HgCl ₂	3.2±0.1	7.6±0.9 ^a	1.8±0.7	1.0±0.3	0.5±0.3	1.2±0.9	2.5±0.4	2.4±0.4
CX	H ₂ O	2.0±0.7	7.9±4.2	1.0±0.3	0.8±0.3	1.2±0.3	1.0±0.3	1.7±0.6	1.6±0.3
	HgCl ₂	3.0±0.2	7.9±3.0	0.7±0.3	0.7±0.5	1.5±0.4	2.3±0.7	2.0±0.3	2.3±0.3
HC	H ₂ O	2.2±0.9 ^b	4.0±0.9 ^{ab}	0.7±0.4 ^b	0.8±0.3 ^b	1.2±0.3	0.9±0.2	2.0±0.8	2.3±0.3
	HgCl ₂	1.6±0.1 ^b	4.6±0.3 ^{ab}	1.4±0.7 ^b	0.9±0.7 ^b	0.5±0.3	2.4±0.7	2.1±0.3	2.3±0.2
CB	H ₂ O	3.6±1.2	4.0±1.4	0.8±0.4	1.1±0.3	1.3±0.2 ^c	0.9±0.2	2.1±0.3	2.0±0.3
	HgCl ₂	10.9±5.0	5.3±0.9	2.3±1.5	1.6±0.3	0.8±0.2 ^c	2.7±0.9	2.1±0.3	2.3±0.2

Notes. All results are reported as mean ± SEM. For IL-6, three-way ANOVA indicated significant strain differences for all regions with $p < 0.001$ – 0.006 ; ^aindicates sex difference of SFvF1 (STR: $t = 2.75$, $p = 0.012$; HC: $t = 3.16$, $p = 0.004$) and females of each strain (STR: $t = 4.73$, $p = 0.001$; HC: $t = 5.12$, $p = 0.001$); ^bindicates strain difference (HC for H₂O, $t = 3.53$, $p = 0.002$; HgCl₂, $t = 2.78$, $p = 0.01$). For IL-4, three-way ANOVA indicated significant strain differences for FCX, STR, HC, and CB ($p = 0.003$ – 0.024); ^cindicates that males of each strain differ ($t = 2.48$, $p = 0.018$).

T cells (Vas and Monestier, 2008). It is not surprising that the levels of IgG in the brain are elevated when there is early production of anti-brain Abs due to delay in BBB development. The pnd70 SFvF1 and FvSF1 offspring had higher levels of serum IgG compared with the counterpart pnd21 offspring, which is due to the immune system not being fully developed at pnd21, and over time the Hg-enhanced Ab levels can accumulate in tissues. The pnd70 FvSF1 offspring also had similar anti-brain Ab levels compared with Hg-treated pnd21 FvSF1, which may relate to the normal baseline levels for adult animals with H-2^{s/q} because pnd70 SFvF1 offspring also have comparable adult levels of serum anti-brain Abs. Many mouse strains and humans develop Abs that can react with self-Ags, but these Abs do not necessarily induce any pathology. The Hg-treated pnd21 FvSF1 offspring had significantly increased serum anti-brain Abs, whereas their brain cytokines and social behavior were not altered by Hg exposure, indicating this level of serum anti-brain Abs or their specificities have less or no pathophysiological significance. In addition, the pnd70 FvSF1 offspring had similar or higher levels of IgG in the brain compared with the pnd21 offspring. Higher serum IgG levels lead to higher brain IgG levels within each strain even in control mice, but more IgG in the brain does not necessarily mean a detrimental change in behaviors.

IgG anti-brain Abs have been detected in the sera of humans and mice with impaired social behaviors (Heo *et al.*, 2011), and aberrant behaviors have been induced in animals after fetal exposure to IgG from mothers who had children with impaired social behavior (Braunschweig *et al.*, 2008; Cabanlit *et al.*, 2007; Singer *et al.*, 2009; Zimmerman *et al.*, 2007).

Because the SFvF1 and FvSF1 offspring have the same H-2 haplotype, the commonality of the immune response genes suggests that the specificities of the Abs could be similar. This also suggests the majority of anti-brain Abs detected in pnd21 SFvF1 offspring might be from SJL/J dams, and SJL/J Abs likely could be more detrimental to behavior than FVB Abs because SJL/J mice have low sociability. Thus, it is likely that the higher levels of serum anti-brain Abs detected in SFvF1 offspring or the dam's Ab specificities have a greater influence than the Ab specificities of the F1 offspring or the FVB dams. In addition, it is interesting to note that the control SJL/J dams also had high levels of serum anti-brain Abs, which are posited to relate to the impaired social behaviors of SJL/J mice (Moy *et al.*, 2008). Because Hg treatment induced serum anti-brain Abs in A.SW mice (H-2^s) (Zhang *et al.*, 2011) and FVB mice, it is likely that anti-brain Abs were also induced by Hg in SJL/J dams, but the pre-existing high levels of serum anti-brain Abs in SJL/J dams may cause the undetectable change between Hg-treated SJL/J dams and control dams. The greater elevation of brain cytokine levels of the SFvF1 mice than those of the FvSF1 mice may be due to the slightly higher and more brain-reactive IgG in the brains of the Hg-exposed SFvF1 mice or due to Hg causing greater activation of cytokines unrelated to IgG levels. Ganor *et al.* (2005) reported that in healthy and epileptic monozygotic twins, both twins had serum autoAbs to some brain Ags, indicating that the presence of autoAbs in the sera may not be directly responsible for their neurobehavioral differences. Thus, we suggest that the presence of anti-brain Abs in the brain and/or increased sensitivity to the Abs due to Hg modifications influence the behaviors. Because there were no

differences in the Ab levels or cytokine levels in the brains of the SFvF1 mice at pnd70, the developmental Hg exposure and levels of IgG antibrain Abs and brain cytokines during development until pnd21 seem to be the critical parameters affecting the social behavior impairment.

Brain inflammation has been suggested to be involved in many neurodevelopmental diseases (Gao *et al.*, 2011; Lee *et al.*, 2010; Mondal *et al.*, 2008). Elevated antibrain Ab deposition during early brain development has been posited to initiate neuroinflammation, which subsequently affects behavior (Heo *et al.*, 2011). The absence of brain IgG and cytokine differences of the H₂O and Hg-exposed pnd70 SFvF1 offspring, but presence of aberrant behavior for those females developmentally exposed to Hg, indicates that the elevated brain cytokines observed at pnd21 may be responsible for the altered behaviors. Interestingly, SFvF1 mice may be more susceptible to neuroinflammation modulating behavior, in that their brain levels of IL-6, a cytokine associated with stress (Atzori *et al.*, 2012), are high without any treatment. In pnd21 female SFvF1 offspring, Th1, Th2, and Th17 cytokines were increased in many brain regions. IFN- γ , a key cytokine of Th1 cells, was not detectable in the brain, suggesting that inflammatory glial, Th2, and Th17 cells seem to be more involved in the behavioral changes observed in SFvF1 offspring. Further experiments are required to identify the source of these cytokines in the brain. The self-grooming assessment, a mouse behavior which might resemble the repetitive behaviors observed in humans with impaired social behaviors, was not changed in Hg-treated pnd70 SFvF1 offspring, suggesting that the balance of direct pathway and indirect pathway of STR was not altered (Lewis and Kim, 2009; Tanimura *et al.*, 2011).

There have been studies indicating that Hg effects on the dam should be considered with regard to strain differences of Hg toxicity (Ekstrand *et al.*, 2010; Hultman and Nielsen, 1998). We did not observe any significant differences in water consumption between SJL/J and FVB dams; the mice drank 4–5 ml per day. The parental SJL/J and FVB dams do have different social behaviors. Hg treatment might alter the behavior of the dams, which might affect the behavior of the offspring, but no obvious differences in pup rearing were noticed. Yang *et al.* (2007) reported that cross-fostering of BTBR and B6 offspring with B6 and BTBR dams did not affect their social behavior development. As a result, we suggest that the reduced social behavior of Hg-treated SFvF1 offspring observed in our study was less connected to the pre-existing SJL/J dams' impaired social behavior or their possible change in behaviors due to Hg exposure. Although yet inconclusive, we speculate that the Hg-induced impairment of the social behavior of SFvF1 offspring was due to the neural damage from heightened brain cytokines. We suggest that certain autoAbs in the brain enhance activation of microglia, as evidenced by the increase in IL-1 β and IL-12. Additionally, we suggest that Hg enhances the cellular sensitivity to the increased brain cytokines. A similar IgG-related response has been described for loss of dopaminergic neurons of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice; the

IgG activation of microglia via their Fc receptors has been implicated in the pathology (Lira *et al.*, 2011). The Hg-induced decline of sociability was most evident in the SFvF1 female offspring, which did not have more brain IgG, but they did have more brain regions with elevated cytokines and more cytokine expressions in some brain regions, especially IL-17. IL-6 and IL-17 have been implicated in maternal immune activation causing autism-like behaviors (Hsiao *et al.*, 2012). Multiple brain regions may contribute to the decreased mouse sociability as elevated cytokine levels were not restricted to any one region. However, the sex differences observed for the elevation of IL-17 in CB of Hg-treated pnd21 SFvF1 offspring are interesting because there have been studies indicating that CB is altered in humans with impaired social behaviors (Bolduc *et al.*, 2012; Webb *et al.*, 2009). It appears that only the early Hg-induced elevated levels of cytokines in the brain affect the sociability.

Because Hg is well known to affect renal function, kidney failure could have caused brain damage through a high level of nitrogen compounds retained in the blood or a build-up of other metabolites that could alter neuronal functions (Buchman *et al.*, 2009; Khatri *et al.*, 2007; Yakushiji *et al.*, 2010). However, unchanged urine protein levels in Hg-treated SFvF1 and FvSF1 mice at pnd70 indicate that any change in social behavior after Hg treatment was less likely to be linked with any kidney failure.

In conclusion, developmental exposure to HgCl₂ significantly decreased the sociability of SFvF1, but not of FvSF1, offspring. Moreover, the Hg exposure affected the female SFvF1 offspring to a greater extent than their male littermates. The decreased sociability appears to be best correlated with the genetics of the dam and the increased presence of certain cytokines in the brains of the SFvF1 offspring, which may partially have been elevated by the antibrain Abs. Although performed with mice, our study provides evidence that Hg exposure might contribute to increased prevalence of low social behavior through maternal influences in a genetic- and sex-dependent manner.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

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