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Assessment of mouse strain differences in baseline esterase activities and toxic response to sarin

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ABSTRACT

Genetics likely play a role in various responses to nerve agent (NA) exposure, as genetic background plays an important role in behavioral, neurological, and physiological responses. This study uses different mouse strains to identify if mouse strain differences in sarin exposure exist. In Experiment 1, basal levels of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and carboxylesterase (CE) were measured in different strains of naïve mice to account for potential pharmacokinetic determinants of individual differences. In Experiment 2, median lethal dose (MLD) levels were estimated in 8 inbred mouse strains following subcutaneous (s.c.) administration of sarin. Few strain or sex differences in esterase activity levels were observed, with the exception of erythrocyte AChE activity in the C57BL/6J strain. Both sex and strain differences in toxicity were observed, with the most resistant strains being the BALB/cByJ and FVB/NJ strains and the most sensitive strain being the DBA/2J strain. These findings can be expanded to explore pathways involved in NA response, which may provide an avenue to develop therapeutics for preventing and treating the damaging effects of NA exposure.

1. Introduction

NAs are organophosphorus compounds that inhibit AChE, leading to accumulation of acetylcholine at synapses and effector organs, and produce a physiological cascade that results in a variety of toxic effects (King and Aaron, 2015). The current treatments, pralidoxime (2-PAM) and atropine, are useful for treating the peripheral effects, but not as effective at treating the central effects of NAs (Nachon et al., 2013). To treat NA-induced seizures, diazepam is currently the standard prehospital treatment, but has a relatively narrow therapeutic window (McDonough and Shih, 1997). Therefore, it is essential to find new and better treatments for NA exposure. A strategy that has yet to be widely capitalized on is to discover therapeutics by investigating the pathways involved in genetically determined susceptibility or resistance to NA exposure. This strategy involves investigating if individual differences in response to NAs exist, which will provide information about resistant and sensitive phenotypes. As current discovery platforms for NA-induced toxicity continue to produce pharmacotherapies with similar mechanisms of action, studying the mechanisms of genetic susceptibility may yield information regarding novel targets for therapeutic development. The current study examined if strain differences exist in baseline esterase activity levels or in sarin-induced toxicity.

There is a long history of research on genetic differences in response to chemicals, but less information is available following exposure to NAs. One method for looking at individual differences in preclinical research is to use multiple inbred strains, because all same-sex individuals within a strain are homozygous at all alleles. Therefore, in carefully controlled environments, observed differences between inbred strains of mice can be attributed to genetic influences, while differences within a strain can be attributed to environmental differences (Crabbe et al., 1990; Hegmann and Possidente, 1981). Thus, it is possible to determine if there is genetic variability between different strains in the response to NA exposure. Heritability is the proportion of variability due to genetic differences, and is calculated using a ratio of estimated additive genetic variance divided by estimated environmental and genetic variance. Heritability estimates are useful because they provide an

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estimate of genetic influence on physiological or behavioral traits. Further, it is important to determine which strains are the extremes with regard to the normal distribution of a particular trait, which requires testing a panel of inbred strains. Therefore, using 8 or more inbred strains provides sufficient power to estimate heritability and to identify strains that aresensitive or resistant to NAs.

Prior to searching for other mechanisms of NA response, pharmacokinetic factors should be evaluated as determinants of inbred strain differences. Although it is involved in the pharmacodynamic effects of NAs as well, AChE is a membrane-bound enzyme that binds all NAs in tissue. In addition, CE and BChE non-specifically bind NAs in plasma and liver (King and Aaron, 2015; Myers, 1959); thus it is important to determine if there are variations in the levels of each enzyme across different strains of mice. Although various mouse and rat strains have been tested for differing levels of AChE, CE, and BChE, there are deficits in the literature (Matson et al., 2018). In the cases where differences in AChE or BChE exist across strains, interpreting them is difficult because of supplier differences, how the enzymes were measured, or what statistics were used. Although studies have measured CE levels in different rat strains (Clement and Erhardt, 1990; Overstreet et al., 1990), there appear to be no published studies comparing inbred mouse strains. To provide answers to deficits in the literature, plasma activity levels of CE and BChE, cortical brain levels of AChE, and erythrocyte (RBC) activity levels of AChE were measured in 10 inbred mouse strains. Based on the available literature, we did not expect to observe extensive variation in esterase activity levels across inbred mouse strains.

The literature is also somewhat lacking in its assessment of strain differences in toxicity following NA or other types of organophosphate exposure (Matson et al., 2018). Rat studies have uncovered strain differences in response to tri-ortho-cresyl phosphate and diisopropylfluorophosphate (DFP) (Carrington and Abou-Donia, 1988; Kacew et al., 1995; Overstreet and Russell, 1984; Overstreet et al., 1979). Overstreet and colleagues (Overstreet and Russell, 1984; Overstreet et al., 1979) selectively bred Flinders Sensitive and Flinders Resistant lines for response to the anticholinesterase DFP. These researchers were able to investigate the mechanisms involved in DFP sensitivity, though the Flinders Resistant Line DFP response was not significantly different from a sample of outbred rats. Therefore, it was difficult to investigate the mechanisms involved in DFP resistance (Overstreet, 1993). As reviewed by Matson et al. (2018), several mouse studies have observed statistically significant inbred strain differences in both LD50 s and other behavioral and physiological responses to organophosphates. In two studies, MLD values were determined for DFP and it was observed that the DBA/2Ibg was the most resistant (had the highest MLD value), C3H/2Ibg was intermediate, and C57BL/6Ibg was the most sensitive strain (Smolen et al., 1985; Wehner et al., 1987). The second study also found that BALB/cByJ mice were the most resistant compared to the three other strains. Clement et al. (1981) determined differences in MLD levels for soman across 8 inbred strains, and observed that the BALB/c strain was the most resistant, the C3H strain was intermediate, and the C57BL/6 strain was the most sensitive. Recently, Furman et al. (2014) assessed MLD levels following sarin exposure in C57BL/6, DBA/2, and FVB/N mice. These authors found again that C57BL/6 mice were the most sensitive (had the lowest MLD level) followed by DBA/2 and FVB/N mice. As the only published study using sarin also only used male mice from 3 inbred strains and 2 outbred lines of mice (Furman et al., 2014), we sought to expand these results by testing both sexes of 8 inbred mouse strains from the same supplier. Based on the reviewed literature of MLD values to other organophosphates, we hypothesized that of the strains tested in the current study, the C57BL/6J would be the most sensitive (exhibit a lower MLD value), DBA/2J would have an intermediate response, and FVB/2J and BALB/cByJ mice would be the most resistant (exhibit a higher MLD value) strains.

2. Methods

2.1. Experiment 1: Determination of AChE, BChE, and CE levels in 10 inbred strains of mice

In Experiment 1, plasma levels of CE and BChE and regional brain levels and RBC levels of AChE were measured in 160 female and male 6-week-old mice from 10 inbred strains (A/J, BALB/cByJ, BTBR T + Itpr3tf/J, C3H/HeJ, C57BL/6 J, CBA/J, DBA/2 J, FVB/NJ, 129S1/SvImJ, and MRL/MpJ) to identify pharmacokinetic factors that may influence the results of Experiments 2. Strains were chosen based on genetic variability, availability, and commonality across studies, as determined by resources on the Jackson Laboratories website (www.jax.org). Mice in Experiments 1 and 2 were obtained from Jackson Laboratory (Bar Harbor, Maine), and were group-housed with ad libitum access to food and water except during the experimental procedures. All mice were deeply anesthetized using carbon dioxide (CO_2), blood sampled via cardiac puncture and decapitated. Vaginal smears were taken immediately following anesthesia induction in female mice to determine estrus status, as Smith et al. (2015) observed that estrous status affects AChE activity levels. Cytological staining was performed using crystal violet, and analyzed using the method described by McLean and colleagues (McLean et al., 2012) to determine the stage of estrus (proestrus, estrus, metestrus, or diestrus). For the analyses, estrus or non-estrus was used to conserve power for testing across multiple strains.Following decapitation, the cerebral cortex was dissected, weighed, and the temporal cortex was diluted 1:20 in 1% Triton X-100 and homogenized. The sample was then centrifuged for 20 min at 31,000 RCF, and supernatant was collected and frozen at −80 °C for later analysis. Blood was collected in a 1.0 ml microcentrifuge tube containing 50 µl heparin sodium solution (15 U/ml) and centrifuged for 5 min at 16,000 RCF to separate plasma and packed RBCs. A 10 µl aliquot of the RBCs was diluted 1:50 in 1% Triton X-100, flash frozen and stored at −80 °C.

AChE activity in blood and brain samples was analyzed using a modified Ellman colorimetric assay and a BCA protein assay (Thermo Scientific Pierce), as described by Shih et al. (2009). Briefly, 7 µl of cortex sample (10 µl for RBC), 20 µl of DI water (17 µl for RBC), and 200 µl of DTNB reagent (0.424 M, pH 8.2) were added to each well of a 96-well plate. This was allowed to incubate (37 °C) and shake for 10 min before addition of 30 µl acetylthiocholine iodide substrate (17.1 mM). AChE activity was then measured and calculated utilizing a Spectramax Plus 384 microplate reader and SoftMax Pro GxP 5.4 software (Molecular Devices). All samples were measured in triplicate. AChE activity in each cortex sample was normalized to its corresponding total protein concentration, as interpolated from a standard curve, giving a final value in µmol substrate hydrolyzed/min/g of protein. For RBCs, activity is expressed as µmol substrate hydrolyzed/min/ml.

Plasma BChE and CE activities were assessed using an *in vitro* activity assay. In short, plasma from each animal was diluted ten-fold in 0.1 M potassium phosphate buffer at pH 7.0. A portion of this dilution was tested for activity by measuring the change in absorbance at 405 nm in a solution containing 10 mM *p*-nitrophenyl butyrate. The activity measurement was conducted in either buffer alone or buffer with 20 µM eserine to selectively inhibit the activity of BChE. Activity for each plasma sample in each condition was measured in triplicate. BChE activity was calculated by subtracting CE activity from overall sample activity.

2.2. Experiment 2: MLD determinations in 8 inbred strains of mice

Experiment 2 utilized males and females of the 8 inbred strains that had the most comparable esterase levels. In this experiment, toxicity differences in sarin response were assessed using a panel of 8 inbred strains identified in Experiment 1 (A/J, BALB/cByJ, BTBR T + Itpr3tf/

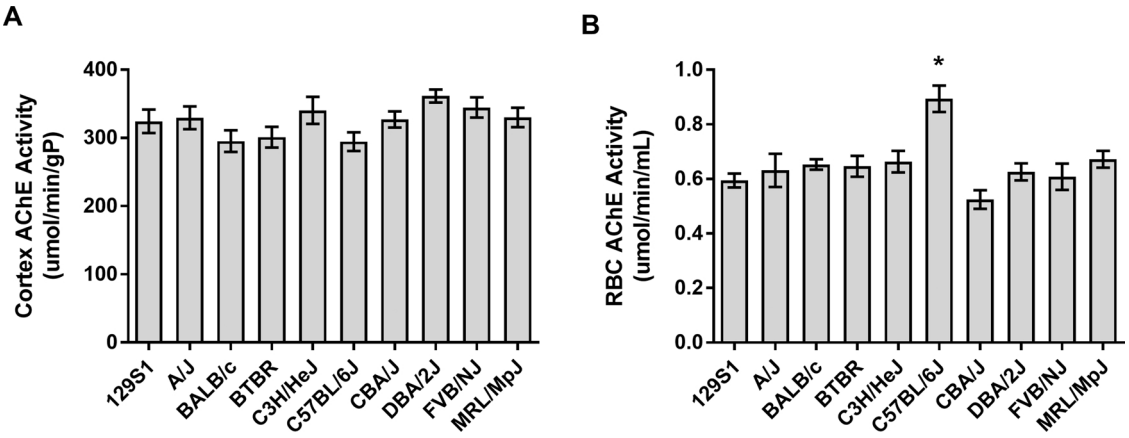

Fig. 1. Acetylcholinesterase (AChE) activity in 10 inbred mouse strains (n = 8 for each strain and sex) including129S1/SvImJ (129S1), A/J, BALB/cByJ (BALB/c), BTBR T + Itpr3tf/J (BTBR), C3H/HeJ, C57BL/6 J, CBA/J, DBA/2 J, FVB/NJ, and MRL/MpJ. Asterisk (*) indicates significant difference from all other strains, *ps* < .05. (A) Cortical AChE activity; (B) Red blood cell (RBC) AChE activity.

J, CBA/J, DBA/2 J, FVB/NJ, 129S1/SvImJ, and MRL/MpJ). For all strains, 24 h MLD levels were determined using a stage-wise, adaptive dose experimental design (Feder et al., 1991) in which 1–3 mice were allocated randomly to each of 1–6 agent challenge doses per stage. Previously published toxicity data were used to inform the selection of sarin doses for the first stage. In the first stage, a range of sarin doses was selected to span the predicted range of lethality from 0 to 100%. On the day of exposure, animals were weighed and single-housed prior to NA exposure, and sarin (42 μg/ml in saline) was administered via s.c. injection with doses based on body weights (μg/kg). Animals were continuously monitored for one hour after agent administration, followed by checks at four hours and 24 h post-exposure. All surviving animals were weighed and euthanized 24 h post-exposure. The results of the first stage were used to select sarin doses for the next stage, which allowed animals in this and later stages to be placed at doses that allowed better estimation of the MLD. The stage process continued until the width of the 95% confidence interval (defined as [upper bound dose – lower bound dose]/ [2 * MLD (for the 24-hour MLD)]) was less than 0.40.

2.3. Analyses

Statistical significance was defined as a *p* value less than .05 for all tests. For Experiment 1, a two-factor analysis of variance (ANOVA) was first conducted using Strain and Estrus Status as fixed factors to evaluate if estrus status influenced esterase activities. Another two-factor ANOVA using Strain and Sex as fixed factors was conducted to assess if differences exist in cortex AChE, RBC AChE, total plasma esterase, plasma CE, and plasma BChE. A Tukey's multiple comparison test was performed to test for differences between the strains. Narrow-sense trait heritability for each esterase phenotype was calculated using the ratio of between strain variance, a measure of additive genetic variance, to the total within strain variance, an estimate of environmental variance, plus between strain variance (Falconer and MacKay, 1996). This was performed by conducting a one-factor ANOVA using strain as a between subjects variable and calculating the ratio of the sum of squares between strains and the adjusted total sum of squares for each esterase measure.

In Experiment 2, the dose-response curve for each agent and strain was estimated using the methods of Feder et al. (1991) based on the stage-wise adaptive dose design results. A SAS program, PROBSEP, which uses a nonlinear regression analysis on the probit of the responses, estimated the dose-response curve along with the MLD, slope and their 95% confidence intervals (CI). MLD levels for each strain within agent were compared using the output from the PROBSEP program in a SAS program, PRORATIO, which compares the ratio of pairs of MLDs and estimates the 95% CI for the ratio. This is analogous to a Z-test, and if the 95% CI of the MLD ratio (CR) does not include the value of one, then the pair of MLDs is considered significantly different. Slopes and standard errors of the slopes from the dose-response curves were used in a one-factor ANOVA to compare slopes among strains within sex, followed by a Tukey's multiple comparison test comparing all pairs of strains. Graph Pad Prism was used for this analysis. It was not feasible to calculate heritability for MLD levels calculated using the stage-wise adaptive dose procedure, so the heritability estimates are only presented for the esterase phenotypes.

Genotypic correlations were performed using Pearson correlations of strain means for the described measures (Crabbe et al., 1990; Hegmann and Possidente, 1981). Correlated strain means in any given pair of esterase or MLD assays are an index of genetic correlation between these assays, and hence, an indication of common physiological mediation.

3. Results

3.1. Experiment 1

In females, neither strain nor estrus status significantly influenced cortex AChE or plasma CE activity levels (*Fs* < 1.7, *ps* > .05). There was a significant effect of strain for RBC AChE, $F(1, 9) = 2.49$, $p < .05$, $\eta_p^2 = .27$ and follow-up testing for this effect is described below. There was a significant interaction between strain and estrus status for BChE activity levels, $F(1, 8) = 2.17$, $p < .05$, $\eta_p^2 = .22$. Estrus was not controlled for across strains; therefore, not all estrus statuses were present in each strain, and it was not feasible to conduct post-hoc comparison tests. The mean activity level and 95% confidence intervals are divided by estrus or nonestrus and provided across strains in Supplementary Fig. 1.

AChE activity levels differed across strains as is indicated in Fig. 1, but there were no effects of sex on AChE activity. Cortex AChE activity varied across strains, $F(1, 9) = 2.12$, $p < .05$, $\eta_p^2 = .12$. Tukey's test indicated that there was a nearly significant difference between the C57BL/6 J and DBA/2 J strains ($p = .065$). RBC AChE also varied across strains, $F(1, 9) = 5.76$, $p < .001$, $\eta_p^2 = .27$. Tukey's test indicated that the C57BL/6 J strain had significantly higher activity than all other strains tested (*ps* < .01). As is indicated in Fig. 2, there were no differences in total plasma esterase, CE, or BChE activities across the strains or sexes (*Fs* < 1.3, *ps* > .05).

Overall, heritability estimates were low for all esterase measures. Heritability was 11% for cortex AChE activity, 26% for RBC AChE activity, 5% for total plasma esterase, 6% for plasma CE, and 3% for BChE activity.

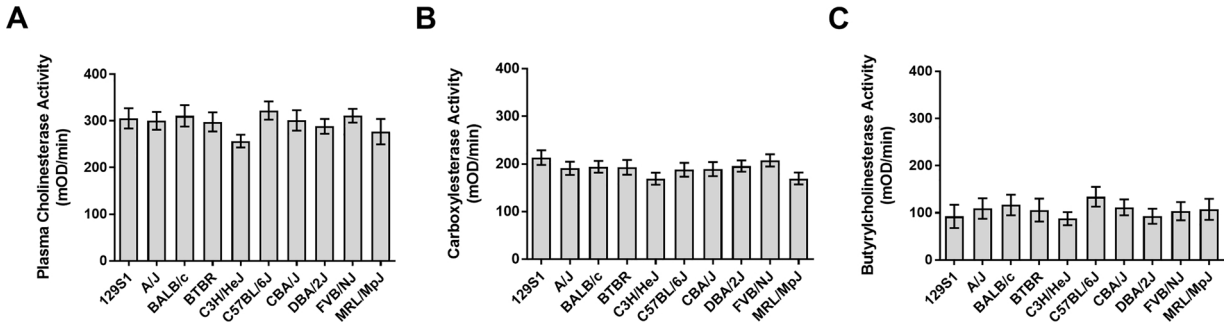

Fig. 2. Esterase activity in 10 inbred mouse strains. There were no significant differences in esterase activity across the tested strains. (A) Total plasma esterase activity; (B) Carboxylesterase activity; (C) Butyrylcholinesterase activity.

Table 1
MLD (μg/kg) and 95% CI by Strain and Sex. Asterisk (*) indicates significant difference in MLDs between sexes, $p < .05$.

Inbred Strain	Females	Males
129S1	277 (250, 307) *	238 (219, 257)
A/J	275 (216, 350)	255 (239, 273)
BALB/c	334 (304, 368)	287 (248, 332)
BTBR	300 (243, 371) *	223 (189, 263)
CBA/J	276 (243, 314) *	230 (220, 240)
DBA/2 J	216 (182, 256)	208 (191, 226)
FVB/NJ	378 (342, 418) *	276 (233, 327)
MRL/MpJ	373 (335, 416) *	243 (180, 329)

Table 2
Comparison of Strain MLDs within Sex. F * indicates significant difference between females of each strain, $p < .05$. M *: Significant difference between males of each strain, $p < .05$. NS indicates no significant difference for both sexes between strains.

Species	A/J	BALB	BTBR	CBA	DBA	FVB	MRL
129S1	NS	F */ M *	NS	NS	F */ M *	F *	F *
A/J		NS	NS	M *	M *	F *	F *
BALB/c			M *	F */ M *	F */ M *	NS	NS
BTBR				NS	F *	NS	NS
CBA/J					F */ M *	F */ M *	F *
DBA/2 J						F */ M *	F *
FVB/NJ							NS

3.2. Experiment 2

MLD estimates were consistently higher for females than males in all eight mouse strains tested. Significant differences between females and males were observed in five of the eight strains tested: 129S1/SvImJ, BTBR T + Itpr3tf/J, CBA/J, FVB/NJ, and MRL/MpJ, *ps* < .05 (Table 1). As evidenced in Fig. 3, there were significant differences in MLDs between several of the male and females of each strain.

Comparison of mouse strain MLDs was made within each sex, females to females and males to males. Table 2 provides the comparisons of strain MLDs within each sex. Strain differences were observed in 14 of the 28 (50%) paired strain comparisons in females and 10 of the 28 (36%) paired strain comparisons in males. Comparison of dose-response curve slopes for each strain and sex did not result in significant differences between strains within each sex (Fs < 1.3), indicating that the mechanism of NA toxicity is similar across strains.

3.3. Genotypic correlations

No genotypic correlations were present for the following comparisons: combined BChE/CE and RBC AChE activity, combined BChE/CE and cortex AChE activity, RBC and cortex AChE activity, and BChE and CE activity. Male and female MLD levels did not correlate with RBC AChE, cortex AChE, or combined BChE/CE activities. Therefore, none of the present phenotypes appear to be genetically related traits.

4. Discussion

Together, Experiments 1 and 2 indicate that there are strain differences in susceptibility to sarin-induced toxicity, but that pharmacokinetic responses influenced by enzyme activity levels are likely not the mechanisms underlying sarin toxicity differences in the eight tested strains. Minimal esterase activity differences were observed across the 10 inbred strains described in this study. The exception was the C57BL/

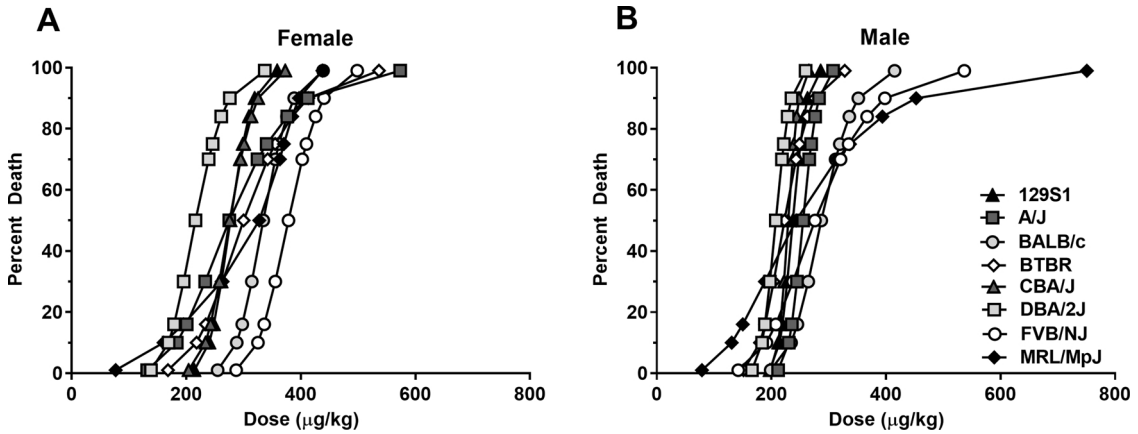

Fig. 3. Probit curve estimations in 8 inbred mouse strains. There were no significant differences in the probit curve slopes across the strains in both females and males. (A) Probit curve estimations in female mice; (B) Probit curve estimations in male mice.

6J AChE RBC activity, which was significantly higher than many of the other mouse strains. There was also a tendency for the C3H/HeJ strain to exhibit lower plasma esterase activities; therefore, these two strains were removed from subsequent MLD determinations. MLD levels varied across males and females of a majority of the strains. Strain comparisons performed for each sex indicated that there were also MLD differences across several of the strains. Heritability, or the genetic effect size, was also low for esterase activities, suggesting that the variance across strains was influenced more by environmental factors than genetic factors. Finally, there were no correlations between the measured phenotypes, indicating that they are not genetically related traits. The results from Experiment 1 confirm previous observations that there are few differences in esterase levels across different mouse strains. The exception was the C57BL/6J strain, which had higher RBC AChE activity than any of the other tested inbred strains. Particularly because the C57BL/6 strain is a very commonly used mouse strain, it should be taken into account that this strain may have a differing response to NA or other organophosphates compared to other mouse strains. This is supported by findings in the C57BL/6J strain which determined the MLD $\pm$ 95% confidence interval for sarin to be 96.4 (77.9, 119.3) μg/kg for males and 187.4 (149.5, 234.8) μg/kg for females (Cadieux et al., 2018). Therefore, experimental results using the C57BL/6 strain should not be interpreted as being representative of the general population. Additionally, the current enzyme activity data are novel because there are currently no published studies that compare CE levels across different inbred mouse strains. This is important because CE greatly influences the response to G-type NAs, such as sarin or soman (Sweeney and Maxwell, 1999). Thus, CE activity differences between the strains would likely cause differences in toxicity response to G-type NAs, which was not observed in the current study.

The MLD levels significantly differed across the sexes for most of the tested inbred strains. Interestingly, females exhibited higher MLD levels than males across all tested strains, demonstrating that female mice are more resistant to sarin-induced lethality than male mice. Specifically, the 129S1/SvImJ, BTBR T + Itpr3tf/J, CBA/J, FVB/NJ, and MRL/MpJ male and female MLD levels differed, indicating that either hormonal or genetically based sex differences contribute to toxicity response. The current results are contradictory to other reports using cyclosarin, sarin, and soman in rats, which observed increased sensitivity in females compared to males (Anthony et al., 2004; Mioduszewski et al., 2002; Sket, 1993). Hormonal fluctuations as a result of the estrous cycle influence sarin lethality in rats (Smith et al., 2015); therefore, it has been established that hormonal differences between males and females can influence sarin MLD levels. Experiment 2 did not take into account estrous cycle or hormonal levels; therefore, additional research is needed to determine the mechanisms of female resistance in mice.

There were also significant MLD level differences across several mouse strains, indicating that genetic differences contribute to sarin-induced toxicity response. The fact that esterase activity differences did not differ across strains suggests that there are other mechanistic factors to be considered across the assessed strains. The current data are limited by the fact that protein levels and inhibited enzyme activity levels were not assessed. Therefore, it is possible that if the amount of esterase or interaction with sarin differed across mouse strains, it could contribute to MLD differences across strains. Alternatively, unrecognized mechanisms of resistance or sensitivity may influence sarin-induced toxicity. Therefore, further research is required to understand the mechanisms underlying strain differences in sarin-induced toxicity response.

Specifically, the most resistant strain in males was BALB/cByJ strain, which is in agreement with two previous studies comparing soman and DFP toxicity, respectively, across inbred strains (Clement et al., 1981; Wehner et al., 1987). The current results suggest that the BALB strain is generally a resistant strain, across different organophosphorus agents and across sub-strains. It is unknown what the mechanisms of resistance are in this strain, and further research is warranted. The most resistant strain in females was the FVB/NJ strain, which had a significantly higher MLD value than 4 of the other tested strains. This finding is in agreement with Furman et al. (2014), which observed that the FVB males were most resistant among 5 tested mouse inbred strains or outbred lines. Although the sex differed in the present study, FVB/NJ mice of both sexes generally had higher MLD levels than most of the other strains. Further inquiry is also needed to discover the mechanisms underlying sarin resistance in the FVB/NJ strain. Finally, DBA/2J males and females had a significantly lower MLD level than almost all other strains. This finding is somewhat contradictory to previous results with organophosphatess that observed an intermediate or resistant toxicity response in DBA mice (Furman et al., 2014; Smolen et al., 1985; Wehner et al., 1987). The current findings may be due to differences across substrains, which have been shown to differ in toxicity response (Muller et al., 2009), or may be due to differences in chemical agents used. Further research is required to understand the mechanisms underlying sarin toxic sensitivity in DBA/2J mice.

In an attempt to understand mechanisms of resistance or sensitivity across the tested inbred strains, the Mouse Phenome Project Website (Jackson Laboratories, https://phenome.jax.org) was surveyed for relevant phenotypes to correlate with the current data. Unfortunately, there were not enough strain overlaps to run correlations between the current data and the website strain data. Though there were no toxicity or lethality studies with direct relevance, a search for phenotypes in which resistant and sensitive strains were outliers uncovered potentially interesting information to examine in follow-up studies. In studies where the BALB/cByJ strain was an outlier, the urine puddle count in both open field and light-dark box testing was high and fecal boli count in the elevated zero maze was high (Lipkind et al., 2004; O'Leary et al., 2013). Normally, defecation and urination are under parasympathetic control, but can be influenced by stress hormones in anxiety-producing situations (Sanger et al., 2000; Smith et al., 2011). Therefore, it is feasible that the BALB/cByJ strain has a high counter-parasympathetic network that is protective when the strain is exposed to parasympathomimetics. This observation is speculative, and would require further testing and verification. The FVB/NJ strain performed worse than other strains on the Morris water maze and Barnes circular maze, both of which are working memory tasks that rely heavily on cholinergic signaling. Although it would require additional testing, the FVB/NJ may have low baseline cholinergic signaling, which requires a larger dose of NA or other AChE inhibitor to result in toxicity or death. Finally, the DBA/2J strain had lower mean RBC volume and hemoglobin content as well as a lower monocyte differential compared to other strains. A decreased availability for NA binding to RBC AChE, and thus, increased likelihood for saturation of available RBC may influence the DBA/2J strain's susceptibility. Additionally, other blood-related abnormalities may contribute to the DBA/2J strain's increased sensitivity to NA-induced toxicity.

In conclusion, the current study demonstrates that strain differences exist in susceptibility to sarin-induced toxicity. Pharmacokinetic response influenced by enzyme activity levels is likely not the critical mechanism underlying observed strain differences in toxicity, because minimal esterase activity differences were observed across the 10 inbred strains described in this study. MLD levels were higher in females than males in a majority of the tested strains, which is contradictory to previous rat studies, and requires further inquiry. Strain differences in MLD levels were observed across several of the strains, and future studies should examine the mechanisms underlying sensitivity or resistance to NAs. Genetic differences may influence the severity of response to NAs, efficacy of countermeasures, and the long-term effects of exposure. Further, discovery platforms for NA-induced toxicity and seizures continue to produce pharmacotherapies with the same or similar mechanisms of action. Therefore, taking genetic sensitivity and resistance into account in the study of NA response is important and may yield information regarding novel targets for therapeutic development.

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Conflict of interest

The authors report no conflict of interests or competing financial interests. None of the authors has during the last five years appeared in any legal or regulatory proceedings related to the contents of the paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tox.2018.08.016.

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