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Short Title: Developmental Perchlorate Exposure and Hippocampal Function

Keywords: brain, cognition, development, hippocampus, iodine, learning and memory, neurotoxicity, perchlorate, thyroid hormone

Abbreviations:

ADHD	Attention Deficit Disorder
ANOVA	analysis of variance
CDC	Center for Disease Control
cm	centimeter
CS	conditioned stimulus
dL	deciliter
EPSP	excitatory postsynaptic potential
GABA	γ-amino-butyric acid
GD	gestational day
gm	gram
h	hour
Hz	hertz
I/O	input/output
ip	interperitoneal
IPI	interpulse interval
IQ	intelligence quotient
kg	kilogram
LTP	long-term potentiation
m	minute
max	maximum
MDC	minimum detectable concentration
µA	microampere
mA	milliamp
mL	milliliter
mm	millimeter
mo	month
ms	millisecond
mV	millivolt

ng	nanogram
NHANES	National Health and Nutrition Survey
NIS	sodium-iodine symporter
PC	personal computer
PN	postnatal day
ppm	parts per million (milligrams per liter)
PS	population spike
PTU	propylthiouracil
S	second
SD	standard deviation
SE	standard error of the mean
T3	triiodothyronine
T4	thyroxine
TSH	thyroid stimulating hormone
US	unconditioned stimulus
U.S. EPA	United States Environmental Protection Agency

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ABSTRACT

Background: Perchlorate is an environmental contaminant that blocks iodine uptake into the thyroid gland and reduces thyroid hormones. This action of perchlorate raises significant concern over its effects on brain development. Objectives: The purpose of this study was to evaluate neurological function in rats following developmental exposure to perchlorate. Methods: Pregnant rats were exposed to 0, 30, 300 or 1000ppm perchlorate in the drinking water from gestational day 6 until weaning. Adult male offspring were evaluated on a series of behavioral tasks and neurophysiological measures of synaptic function in the hippocampus. Results: T3 and T4 were reduced in pups on PN21 at the highest dose. T4 in dams was reduced relative to controls by 16%, 28% and 60% in the 30, 300, 1000ppm dose groups, respectively. Reductions in T4 were associated with increases in TSH limited to the high dose group. No changes were seen in serum T3. Perchlorate did not impair motor activity, spatial learning, or fear conditioning. However, significant reductions in baseline synaptic transmission were observed in hippocamapl field potentials at all dose levels. Reductions in inhibitory function were evident at 300 and 1000ppm, and augmentations in long-term potentiation were observed in the population spike measure at the highest dose. Conclusions: Dose-dependent deficits in hippocampal synaptic function were detectable with relatively minor perturbations of the thyroid axis indicative of an irreversible impairment in synaptic transmission in response to developmental exposure to perchlorate.

Thyroid hormones play crucial roles in the development and maturation of the central nervous system. Severe reductions during critical periods in the pre- and early post- natal period produce stunted growth and mental retardation in children (for reviews see Anderson et al. 2003; Bernal 2002). However, recent reports indicate that children born to women experiencing modest subclinical perturbations of the thyroid axis during pregnancy have reduced IQ scores and subtle deficits in cognition, memory, and visuo-spatial ability and a higher incidence of activity dependent hyperactivity disorder, (ADHD) (Haddow et al. 1999; Zoeller and Rovet 2004). Neuroanatomical alterations have also been demonstrated in animal models of modest thyroid dysfunction in early development (Auso et al. 2004; Goodman and Gilbert 2007; Lavado-Autric et al. 2003). These structural deficits are accompanied by impairments in synaptic transmission, auditory function, behavioral and neurophysiological assessments of learning and memory, and increased seizure sensitivity (Auso et al. 2004; Gilbert et al. 2006; Gilbert and Sui 2006; Goldey et al. 1995; Lavado-Autrec et al. 2003; Sui and Gilbert 2003).

Perchlorate is an environmental contaminant that reduces thyroid hormone (Wolff 1998). Ammonium perchlorate is a salt primarily used in solid rocket fuel and propellants, explosives, pyrotechnics and blasting formulations. Improper disposal and use result in release of the salt to the environment where it rapidly dissociates to perchlorate anion. Perchlorate anion has been detected in drinking water supplies, fruits, vegetables, grain and dairy products (U.S. EPA 2002). Perchlorate blocks the uptake of iodide, an element critical for thyroid hormone synthesis, by competitive inhibition at the sodium-iodine symporter (NIS) in the thyroid gland (Wolff 1998). This action is sufficient to reduce circulating levels of thyroid hormone and may induce neurotoxicity

in developing organisms (U.S. EPA 2002; York et al. 2005a). Dohan et al (2007) recently demonstrated that the NIS transports perchlorate with a higher affinity than it does iodine and perchlorate accumulates in breast milk. As such, breast fed infants may be subjected to higher concentrations of perchlorate than previously thought, compromising hormone production by reducing concentration of maternal iodine in milk in addition to directly inhibiting thyroidal uptake of iodine.

Recent data from Center for Disease Control (CDC) National Health and Nutrition Survey (NHANES) reveal a strong association between perchlorate exposure in women in the general US population and circulating levels of thyroid hormone (Blount et al. 2006; Steinmaus et al. 2007). Evidence of neurodevelopmental sequelae in clinical studies and in animal models with low level thyroid disruption, and recent observations of the extent of perchlorate exposure in the general US population, have raised considerable public health concerns over the effects of low-level perchlorate ingestion (Ginsberg and Rice 2005; Ginsberg et al. 2007; U.S. EPA 2002).

The hippocampus is a brain structure necessary for some types of learning and memory, and its structural integrity is dependent upon adequate supplies of thyroid hormone during development (Auso et al. 2004; Madeira et al. 1991; 1992). The relationship between thyroid hormones insufficiencies during critical periods of hippocampal development and ensuing functional deficits has not been defined. Although structural and functional alterations in hippocampus are profound with severe thyroid hormone reductions (Akaike et al. 1991; Dong et al. 2005; Gilbert and Paczkowski 2003; Vara et al. 2002), recent findings indicate that severe hypothyroidism is not required for their induction. Alterations in the expression of thyroid hormone-responsive genes in

hippocampus and cortex, errors in neuronal migration of both excitatory and inhibitory neurons, changes in phenotypic expression of interneurons, and aberrant glial cell fate determinations have recently been demonstrated with modest degrees and brief episodes of thyroid hormone insufficiency (Auso et al. 2004; Gilbert et al. 2006; Goodman and Gilbert 2007; Lavado-Autric et al. 2003; Sharlin et al. 2008).

The present study was designed to examine the impact of perturbations of the thyroid axis associated with developmental exposure to perchlorate using electrophysiological and behavioral assessments of the hippocampus in rodents. Perchlorate induced a disruption of hormonal status in the neonate which recovered upon termination of exposure, yet a pronounced deficit in neurophysiological properties of the hippocampus was evident in adulthood. These findings indicate that neurological impairment is associated with modest degrees of thyroid hormone insufficiency and support previous animal studies of neurodevelopmental sequellae associated with low levels of perchlorate exposure (U.S. EPA 2002; York et al. 2005a).

EXPERIMENTAL PROCEDURES

Animal Treatment

Pregnant Long–Evans (n=106) rats were obtained from Charles River (Raleigh, NC) on gestational (GD) 2 and housed individually in standard plastic hanging cages in an approved animal facility. All animal treatments were in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All animals were treated humanely and with regard for alleviation of pain and suffering. Animal rooms were maintained on a 12:12 light:dark schedule and animals were

permitted free access to food (Purina rat chow, 5001) and tap water. Beginning on GD 6 and continuing until postnatal day (PN) 30, dams were administered 0, 30, 300 or 1000 ppm ammonium perchlorate (ClO_4, Sigma, St. Louis, MO) in the drinking water. The day of birth was designated PN0 and all litters were culled to 10 pups on PN4 equating as much as possible the number and pups of each gender in a given litter. Blood was collected from animals culled on PN4, pooled within a litter regardless of gender, and hormone determinations were performed when sufficient quantities of serum were available. One male and one female pup/litter were sacrificed on PN14 and PN21 for determinations of circulating levels of thyroid hormones, brain and hippocampal weights. On PN30, the offspring were weaned, transferred to plastic hanging cages (2-4/cage) and were permitted free access to food and tap water.

Water consumption was monitored twice weekly during gestation and lactation. Dam weights were monitored frequently throughout pregnancy and lactation and offspring weights were recorded during the first postnatal month and again in adulthood. Eye opening was examined by daily observation between PN15 and PN19 and the ratio of pups within a litter with both eyes open was determined. Behavioral and electrophysiological tests were performed on adult male offspring as described below and only a single animal/litter is represented in any of the assessments.

Hormone Analysis

Trunk blood from pups on PN4, PN14, PN21, PN80-90 and from dams at weaning on PN30 was collected following decapitation and allowed to clot on ice for a minimum of 30 m. Serum was separated via centrifugation of clotted samples and stored at -80°C for

later analyses. Serum concentrations of total thyroxine (T4) and total triiodothyronine (T3) were analyzed by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Thyroid stimulating hormone (TSH) was measured using standard double antibody assay as described by Thibodeaux et al. (2003). All samples were run in duplicate and the intra- and inter- assay variations ranged from 9-12%. Outliers were removed from the dataset if the value fell more than 2 standard deviation (SD) above or below the mean for the dose group. The lowest calibrator was 5ng/mL for the T4 and 10 ng/mL for the T3 assays. The minimum detectable concentration (MDC) for each assay was determined statistically (3 SDs above background). The MDC for was 4.9 ng/mL for T4 and 7.8 ng/mL for T3 assays. In those cases where the sample result fell below the level of the lowest calibrator, the result was set by default to the MDC for statistical purposes.

Behavioral Assessments. Three behavioral tasks, motor activity, spatial learning, fear conditioning, briefly described below, were conducted in adult male offspring. Additional methodological detail for these tasks is available online.

Motor Activity. Motor activity was assessed as a general test of neurotoxicity. Adult male offspring (n=8-11/group) were tested at 13 mo of age using six photocell devices. Five successive 6-m intervals of activity were recorded during a single 30-m session. All testing occurred between 7:00am and noon on the same day and order of testing was counterbalanced across dose groups.

Morris Water Maze. Spatial learning was assessed in the Morris Water maze as previously described (Gilbert and Sui 2006). At 3 mo of age, male offspring (11-17/dose

group) were administered 2 daily trials, 3-5 m between trials, for 15 consecutive days. The maze consisted of a water-filled circular tank located in a small room with salient and invariant visual cues posted on the walls. Four locations around the edge of the pool were defined as start points, and a circular escape platform was placed just below the surface of the water of one quadrant. Animals were placed into the tank and allowed to search for the escape platform for a maximum of 60 s. Latency to reach the escape platform was recorded and the animals permitted to rest on it for 15 s. A series of probe trials was conducted on trial 1 of test days 3, 6, 9, 12 and 15 in which the platform was removed and animals allowed to swim freely for 60 s. The percentage of time animals spent in each quadrant of the pool was recorded for each probe trial.

Fear Conditioning. Trace fear conditioning was examined as it requires the integrity of the hippocampus (Bangasser et al. 2006). Conditioning was assessed in a different subset of animals at 7-8 mo of age. Testing was conducted in two chambers of identical dimensions, one for fear training and context testing, and a second, located in different room, for cue testing. Conditioned stimulus (CS) and unconditioned stimulus (US) pairs were presented six times on day 1. A 30 s trace interval separated CS (15 s compound light/tone stimulus) offset and US (1 mA, 0.5 s footshock through grid floor) onset, with a 3 m intertrial interval. The chamber was dark and sound attenuating chamber doors remained closed during training. The following day context learning was assessed by returning animals to the training chamber and monitoring activity for 5 m via an infrared motion detector (Colbourn Instruments) mounted on the ceiling of the test chamber. Animals were returned to holding cages in a dimly-lit room for 1 h. Conditioning to cue was evaluated thereafter by placing animals in a test chamber located in a brightly lit

room, painted with black and white vertical stripes, with a smooth white plexiglass floor, and sprayed with apple-scented disinfectant to provide a distinct olfactory cue. Activity was monitored for 2 m before and after 6 CS presentations separated by a 2-m intertrial interval.

Electrophysiological Assessments.

Surgical Procedures. Adult male offspring (5-9 mo of age) were anesthetized with urethane (1-2 gm/kg, ip) and prepared for stereotaxic surgery according to procedures described previously (Gilbert and Sui 2006). Two animals were assessed each day and dose groups were counterbalanced over days to equate the mean age across groups. Data presented are comprised of 16, 17, 16, and 14 animals for the 0, 30, 300 and 1000ppm dose groups, respectively. Animals were mounted in a stereotaxic frame and a bipolar twisted stainless steel wire electrode lowered into the angular bundle of the perforant path according to flat skull stereotaxic coordinates (from bregma, -7.2mm posterior, 4.1mm lateral). A monopolar insulated nichrome wire was lowered into the ipsilateral dentate gyrus (-3.5mm posterior, 2.2 mm lateral) to record field potentials from the dentate gyrus. Nominal depths for stimulating and recording electrodes were 2.2 and 3.5 mm below dura, respectively, but optimal depth placement was achieved through electrophysiological monitoring of the response evoked in the dentate gyrus following single pulse perforant path stimulation.

The field potential is comprised of an initial positive component, the excitatory postsynaptic potential (EPSP) and a negative compound action potential, the population spike (PS). The positive component provides an index of synaptic activity comprising the

summed EPSPs at the level of the dendrites (Lomo 1971). The slope of the EPSP was calculated as the rate of amplitude change for the initial positive component of the dentate gyrus field potential prior to PS onset. The negative component, PS, provides a measure of cell excitability, the number of granule cells firing action potentials in response to EPSP (Lomo 1971). PS were estimated by the amplitude of a line connecting the lowest value of the negative potential to the point of intersection of a tangent connecting the two positive peaks of the potential.

Input/Output (I/O) Functions. Input-output (I/O) functions describe the relationship between the intensity of current applied to the perforant path (input=current) and the magnitude of the subsequent voltage change induced in the dentate gyrus (output=voltage). Examination of I/O functions across a range of stimulus strengths characterizes the efficiency of excitatory synaptic transmission across this monosynaptic connection. A series of 25 intensities were delivered ranging from 20-1500 μA (base-to-peak), 10 pulses/intensity (biphasic square wave pulses, 0.1 ms duration using a Grass S-88 stimulator and PSIU-6 constant current converters), 10 s between each pulse. Responses were amplified, digitized (33 kHz sampling rate), averaged using LabWindows (National Instruments, Austin, TX) and custom designed software, and stored on a PC for later analysis. Both EPSP and PS measures were taken from these averaged responses to generate a curve characterizing the current-voltage relationships between perforant path stimulation and response amplitude across dose groups.

Paired Pulse Depression and Facilitation. Somatic output of cortical networks is modulated by local circuit interneurons, the majority of which have gamma-amino-butyric acid (GABA) as their neurotransmitter (Fukuda et al. 1996). Estimates of the

integrity to somatic inhibition is derived by delivering pairs of stimulus pulses at various interpulse intervals (IPI) and expressing the amplitude of PS evoked by the second pulse relative to the first (see Burdette and Gilbert 1995). Paired pulse determinations of synaptic depression and enhancement were collected upon completion of baseline I/O functions in control and perchlorate-treated animals. Two pulses of equal strength were delivered (IPIs=10, 20, 30, 70 and 250 ms) at three stimulus intensities. Intensities were chosen to produce PS amplitudes of the first pulse (conditioning pulse) equivalent to 20%, 50% and 100% of the maximal PS amplitude recorded at 1500μA. Ten pulse pairs were averaged for each animal at each IPI and at each intensity. Data were expressed as a ratio of test pulse to conditioning pulse PS amplitude. A ratio of less than 1 reflects paired pulse depression, a ratio greater than 1, paired pulse facilitation.

Long Term Potentiation. Hippocampal long-term potentiation (LTP) is a form of synaptic plasticity that has been intensively studied as a cellular model of learning. It has been evaluated *in vitro* in hippocampal area CA1 and in the dentate gyrus of the intact animal to identify molecular substrates and link physiological to behavioral aspects of learning (Bliss and Collingridge 1993; Martinez and Derrick 1996). LTP was assessed following the collection of paired pulse functions and the baseline I/O function. A probe stimulus (intensity producing a PS 50% of maximal) was delivered before and after train delivery to monitor the magnitude of evoked LTP. LTP was induced by delivering three train-pairs (i.e., two 4-pulse bursts at 400 Hz with a 200 ms interval between each burst, repeated 3X at 10 s intervals) at a high stimulus strength (1500μA). Averaged responses were sampled at the probe stimulus intensity immediately and 15 m after train delivery. At completion of electrophysiological testing, animals were sacrificed by decapitation

and the brain was immersion fixed in 4% paraformaldehyde for histological verification of electrode placement.

Statistical Analyses.

All statistical analyses were conducted using SAS V6.12. Dam and pup body weights were evaluated with litter as the unit of analysis using repeated measures ANOVA with between subjects effects of Dose and Day, and a within Litter effect of Gender. Sufficient serum and samples from each gender from each litter were not always available so hormone data were first evaluated using ANOVA for the main effects of Dose and Gender, and Dose X Gender interactions at each age. As no main or interaction effects of gender were observed for any hormone estimates, the mean value of male and female pups from each litter was calculated and formed the data for subsequent analyses. These data were then subjected to ANOVAs using litter as the unit of analysis. Where significant effects were found in hormone measures, mean contrast tests were conducted using Dunnetts T (1-tailed tests, α=0.05) to compare each dose group with control.

Repeated measures ANOVA were conducted for motor activity, water maze learning, and fear conditioning to cue. One way ANOVAs were used to assess context conditioning and hippocampal LTP. Differences in baseline synaptic transmission were evaluated by subjecting EPSP slope and PS amplitudes collected in the baseline I/O function to a repeated measures ANOVA with one between- (Dose) and one within- (Stimulus Intensity) subjects factor. When significant interactions were found between Dose and Intensity, planned comparisons were performed contrasting each dose group to controls and evaluated using the Holm-Bonferroni correction to control for the number of

comparisons. Paired pulse functions were evaluated with repeated measures ANOVA with one between (Dose) and two within subjects factors (Interval and Intensity). In the event of significant interactions, step-down ANOVAs were performed, collapsing across intensity or interpulse interval where appropriate. When significant effects of Dose were obtained, mean contrast tests were conducted using Dunnetts T statistic. One-tailed tests for multiple comparisons of tests of excitatory and inhibitory synaptic transmission were employed based on predictions of diminished synaptic responsiveness as observed in previous work with low level thyroid hormone insufficiency and hippocampal physiology (Gilbert and Sui 2006; Sui and Gilbert 2003; Gilbert 2004; Gilbert and Paczkowski 2003).

RESULTS

General Estimates of Toxicity. No evidence of maternal toxicity was present in dams exposed to perchlorate (Figure 1A). Animals gained weight during pregnancy at the same rate and body weights after parturition were not different among the groups. A significant main effect of Day [$F(13,1326)=706.89$, $p<0.0001$] was observed but no significant effects of Dose [$F(3, 102)=2.31$, $p>0.08$] or Dose X Day interaction [$F(39, 1326)=0.54$, $p>0.93$] were detected.

Analysis of pup body weights (Figure 1B, C) also revealed significant main effects of Day [$F(5,475)=3429$, $p<0.0001$) and Sex [$F(1,95)=11.44$, $p<0.0010$), but no overall main effect of Dose [$F(3,95)=2.0$, $p>0.12$] , Dose X Sex [$F(3, 95)=0.92$, $p>0.43$], or Dose X Day X Sex interactions [$F(15, 475)=0.34$, $p>.99$] were detected. Although a significant Dose X Day interaction was revealed [$F(15, 475)=2.22$, $p<0.005$], mean

contrasts tests failed to identify any significant reduction in pup body weight at any age in either gender.

Eye opening was not delayed, nor did brain or hippocampal weights differ across dose groups (Table 1, all p values >0.50). Water intake of the dam was comparable across dose groups and intake of perchlorate estimated from water consumption values is summarized in Table 2.

Thyroid Hormone Analyses. Dams were sacrificed on the day pups were weaned (PN30) and blood collected for hormone analysis. No changes in serum levels T3 were detected in the dam [$F(3,91)=1.07$, $p>0.36$] (Figure 2A). Serum T4 levels in dams were reduced [$F(3, 90)=55.44$, $p<0.0001$] in a dose-dependent manner by 16%, 28% and 60 %, for 30, 300, 1000 ppm dose groups, respectively (Figure 2B). Dam TSH serum levels were increased three-fold and this increase was restricted to the highest dose level as shown in Figure 2C [$F(3,94)=45.41$, $p<0.0001$].

Serum levels of thyroid hormone were determined in trunk blood sampled from pups on PN4, PN14 and PN21. As minimal blood is available in young animals, samples were pooled from all culls within a litter, regardless of gender on PN4. Individual serum samples from males and females at PN14 and PN21 were evaluated and no differences were seen between genders (all p's>0.05), so a mean hormone value was calculated per litter. As depicted in Figure 3A, no differences in serum T3 concentrations were detected in pups on PN4 [$F(3,68)=0.39$, $p>0.76$] or PN14 [$F(3,89)=2.19$, $p>0.094$], but a 10-14% reduction in T3 relative to control levels was observed on PN21 [$F(3,90)=3.22$, $p<0.0263$]. Mean contrast tests indicated this reduction in T3 was evident at the 300 and 1000ppm dose groups (Dunnett's T, $p<0.05$). No significant changes in serum T4 levels

were detected in pups on PN4 [$F(3,81)=1.40$, $p>0.24$] or PN14 [$F(3,90)=1.94$, $p>0.129$]. A modest but significant reduction in T4 was seen in PN21 pups [$F(3,90)=9.88$, $p<0.0001$] in the 300 and 1000ppm dose groups (~9-20% reduction relative to control levels, Dunnetts T, $p<0.05$). Serum TSH data from pups are summarized in Figure 3C. No change in TSH was detected in serum from PN4 animals [$F(3,58)=0.58$, $p>0.62$]. TSH was elevated marginally in pups on PN14 [$F(3,82)=4.82$, $p<0.0039$] and seen only at the intermediate dose levels (Figure 3C). Very modest increases in TSH were detected on PN21, but were more variable and failed to reach statistically significant levels [$F(3,87)=1.23$, $P>0.30$]. All serum hormone concentrations had returned to control levels in adulthood.

Behavioral Assessments. No group differences were detected in any of the behavioral studies performed in perchlorate-treated animals. No difference between treatment groups were seen in either horizontal or vertical motor activity (Supplementary Material, Figure 1). Latency to find the hidden platform in the Morris water maze was reduced over days in all groups indicating learning of the task, but no differential rate of acquisition was seen as a function of perchlorate treatment (Supplementary Material, Figure 2). Similarly in probe trials, the percentage of time animals spent in the correct quadrant increased on successive trials in control and perchlorate-treated animals. Trace fear conditioning using a robust training paradigm of 6 CS-US pairings showed clear evidence of conditioning to cue in the 24-h after postraining test session, but no differences among treatment groups were evident (Supplementary Material, Figure 3A). Similarly, conditioning to context,

evaluated by placing the animal back in the original test box 24 h after training did not differ among the groups (Supplementary Material, Figure 3B).

Electrophysiological Assessments. Examination of histological material resulted in elimination of total of 12 animals due to inaccurate electrode placement, and these deletions were equally distributed across dose groups. See supplemental material, Figure 4 for depiction of electrode placements in the animals included in the analyses below.

Tests of Excitatory Synaptic Transmission. Input-output functions describe the relationship between intensity of the applied current and magnitude of the resulting evoked synaptic response. Changes in slope of the curve describing this relationship are indicative of alterations in excitatory synaptic transmission at the perforant path to dentate granule cell synapse. Persistent reductions in the efficacy of excitatory synaptic transmission were induced by developmental exposure to perchlorate in adult male offspring. Figure 4 displays the group mean absolute values for PS (mV of negative component of the field potential depicted in inset of Figure 4A) and EPSP slope (mV/ms, slope of line between two points depicted in inset of Figure 4B) amplitude recorded in the dentate gyrus of control and perchlorate-exposed animals. Clear dose-dependent reductions in amplitude of both measures can be seen. The overall ANOVA revealed a significant main effect of Dose [$F(3,60)=4.38$, $p<0.0075$] and a significant Dose X Intensity interaction [$F(72,1440)=3.56$, $p<0.0001$] for the PS measure. Planned contrasts of the intensity response function using Holm-Bonferroni correction to control for Type I error rate confirmed a significant reduction in PS amplitude at all dose levels tested - 0 vs 30ppm [$F(24,1440)=1.51$, $p<0.03$], 0 vs 300ppm [$F(24,1440)=3.83$, $p<0.0001$], and the 0 vs 1000ppm dose groups [$F(24,1440)=9.82$, $p<0.0001$]. EPSP slope IO functions

followed a similar pattern but the dynamic range of this measure is much smaller than for PS and the data were more variable. The overall ANOVA failed to detect a statistically significant effect of Dose [$F(3,60)=1.74$, $p>0.16$) or a Dose X Intensity interaction [$F(72,1440)=1.14$, $p>0.20$), despite a clear reduction in amplitude evident at the high dose level (Figure 4B). Although no significant interaction was seen in the main analysis, planned contrasts of the response intensity function confirmed a significant Dose X Intensity interaction limited to the 0 vs 1000 ppm dose group [$F(24,1440)=4.10$, $p<0.0001$].

Tests of Inhibitory Synaptic Transmission. Paired pulse functions provide an index of the relative balance between excitatory and inhibitory influences on hippocampal output. Previous work in animals with diminished thyroid capacity induced by PTU has revealed evidence of a shift in this balance towards a diminished inhibitory tone (Gilbert et al. 2006). A similar pattern was seen as a result of developmental perchlorate exposure. Results presented in Figure 5 demonstrated the time- (IPI) and intensity- dependent triphasic pattern of depression, facilitation, and depression in control animals. Strong inhibition produced by delivery of stimuli at maximal stimulus strength (100% max) revealed little difference among the groups (Figure 5A). A progressively increasing degree of dispersion is evident as conditioning pulse intensity is reduced to yield PS response of 50 and 20% of maximal (Figure 5B and 5C). An upward shift in the entire paired pulse curve occurs as conditioning pulse intensity is reduced, and this shift is most dramatic in perchlorate-exposed animals at the lowest stimulus strength (Figure 5C). An overall three-way ANOVA yielded significant main effects of Dose [$F(3,57)=5.67$, $p<0.0018$], Intensity [$F(2,114)=21.51$, $p<0.0001$] and IPI [$F(4,228)=130.39$, $p<0.0001$].

Significant Dose X Intensity [$F(6,114)=4.46$, $p<0.0004$] and Dose X IPI [$F(12,228)=3.30$, $p<0.0002$] interactions were also detected. Step down ANOVAs to further explore the effects of Intensity and IPI revealed that the alteration in synaptic inhibition occurred at the 20% [$F(3,301)=10.17$, $p<0.0001$] and 50% [$F(3,311)=5.59$, $p<0.0010$] stimulus intensities. Significant differences at the 300 and 1000 ppm dose levels were detected at both stimulus intensity levels (Dunnetts T, $p<0.05$). Stepdown analysis of the main effect of IPI indicated that significant dose-related differences were evident at several IPIs. No differences were seen at the shortest IPI of 10ms ($p>0.18$). A significant effect was detected at the 20ms IPI [$F(3,183)=3.30$, $p<0.0215$], and Dunnett's T indicated that this difference was limited to the difference between controls and the 30ppm dose group. At IPIs of 30 ms [$F(3,183)=4.22$), $p<0.0065$); 70ms [$F(3,183)=15.77$, $p<0.0001$] and 250ms [$F(3,183)=6.72$, $p<0.0003$], significant differences were seen at both the 300 and 1000 ppm dose groups (Dunnetts T, $p<0.05$).

Tests of Synaptic Plasticity. Long term potentiation (LTP) is a well-established model of synaptic plasticity believed to embody the cellular substrate of learning and memory. Application of high intensity trains produced significant increases in EPSP slope amplitude (~20% increase above pretrain baseline amplitudes), and the magnitude of this increase was comparable between control and perchlorate-treated animals [Figure 6A, $F(3,54=0.20$ $p>0.85$]. LTP-induced increases in PS amplitudes are of larger magnitude (~80% in controls) and greater increases were seen in the high dose of perchlorate than in controls (Figure 6B). ANOVA revealed a significant main effect of Dose [$F(3,51)=3.12$, $p<0.0337$] and mean contrast tests supported the conclusion of an augmented PS LTP in the 1000ppm perchlorate group (Dunnetts T, $p<0.05$).

DISCUSSION

Developmental exposure to perchlorate produced modest reductions in circulating thyroid hormones in dams and pups and no signs of overt toxicity. No evidence of behavioral alterations was detected in tests of motor activity, spatial learning, or fear conditioning. However, reductions in excitatory and inhibitory synaptic transmission were observed in the dentate gyrus of adult offspring of perchlorate-treated dams. These findings are the first to demonstrate mild degrees of hormone insufficiency induced by perchlorate early in life lead to persistent deficits in synaptic function in adulthood. The absence of effect on behavioral assessments of learning and memory may be a function of dose, degree of hormonal disruption, duration of prenatal exposure, or the cognitive demands and sensitivity of the behavioral tasks.

Perchlorate Impairs Hippocampal Excitatory Synaptic Transmission. Perchlorate produced dose-dependent reductions in excitatory synaptic transmission in the dentate gyrus. Both EPSP and PS components of the compound field potential were decreased in amplitude by developmental exposure to perchlorate. These observations are consistent with findings of graded levels of thyroid hormone insufficiency induced by PTU (Gilbert and Sui, 2006). The EPSP slope measure is the summed synaptic activation at the level of the dendrites where the bulk of synaptic connections are made between cortical afferents and hippocampal neurons. Reductions in field potential estimates of EPSP slope are indicative of reductions in afferent input, transmitter release or postsynaptic responsiveness. Diminution of the somatic response, the PS, reflects a reduction in

excitability of the cell. This could result from reduced cortical innervation, reduced synaptic responsiveness, or alterations in the firing properties of the granule cells. Collectively, these observations indicate a dose-dependent decline in the efficiency of synaptic transmission at this first synaptic junction into the hippocampal formation. Importantly reductions in PS measures were evident at the lowest dose of perchlorate tested (30ppm, 4.5 mg/kg-day).

Perchlorate Alters Excitatory/Inhibitory Balance in Dentate Gyrus. In hippocampal circuits, activation of inhibitory interneurons dampens the firing of granule cells (PS) without affecting the synaptic component of the field response (EPSP slope). A standard test of inhibitory function delivers pairs of stimulus pulses close together in time - the degree of inhibition is reflected as a reduction in the amplitude of the PS of the second response relative to the first, and the magnitude of the suppression is stimulus dependent (Burdette and Gilbert 1995). A triphasic pattern of depression, facilitation, and depression is characteristic of paired pulse responses in the dentate gyrus, and results from the influence of temporally successive and overlapping phenomena including recurrent inhibition, presynaptic facilitation, and feedforward inhibition.

Perchlorate produced a shift towards reduced depression/enhanced facilitation in paired pulse tests. Depression of the PS is most robust at very short intervals (early paired pulse depression). It is mediated by GABAergic interneurons synapsing on the soma of granule cells that serve to limit the degree of granule cell firing through feedback circuits (Fudaka et al. 1996; Papatheodoropoulos and Kostopoulos 1998). Feedback inhibition at the 10 ms IPI was not impacted by perinatal perchlorate exposure at any dose or any

intensity. However, a slight and significant reduction was evident at the 20 ms IPI at low to moderate intensities. As the interval between pulses is increased, this strong inhibition wanes and paired pulse facilitation predominates (IPIs of 50-70 ms). This interval corresponds to a time when increases in presynaptic transmitter release contribute maximally to the amplitude of the field potential amplitude generated by the second pulse of the pair. Facilitation is the summed effect of presynaptic and postsynaptic factors at the granule cell synapse and at the interneuron (Papatheodoropoulos and Kostopoulos 1998). The magnitude of facilitation was significantly enhanced in perchlorate exposed animals at the lower stimulus intensities. A second period of depression follows facilitation and is induced with longer IPIs (250 ms). It is smaller in amplitude than early paired pulse depression and its mechanism less well understood. Late paired pulse depression involves feedforward inhibitory circuits and can be modulated by antagonists of n-methyl-d-aspartate glutamate receptor (Gilbert and Burdette 1996; Joy and Albertson 1993). This form of paired pulse depression was also reduced by perchlorate at the 300 and 1000ppm dose levels.

The overall pattern of effects seen in tests of paired pulse inhibition is similar to that observed in the PTU model of hypothyroidism (Gilbert et al. 2006; Sui and Gilbert 2003). Reduced paired pulse inhibition may reflect a direct impact of hypothyroxenemia on phenotypic expression of inhibitory neurons as previously described for PTU and methimazole (Berbel et al. 1996; Gilbert et al. 2006). In these studies, a reduction in expression of parvalbumin-containing interneurons was seen in hippocampus and cortex of hormone-deficient animals and was coupled with impairments in inhibitory synaptic transmission. Alternatively, reduced paired pulse inhibition may be a further

manifestation of impaired glutamatergic activation at inhibitory synapses, or of reduced release of GABA from interneuronal populations.

Whatever the mechanisms, interneuronal networks play a central role in generating behaviorally relevant network driven patterns of activity in the adult brain. In the developing brain, even minor disturbances in this population of neurons alters the balance of excitation and inhibition and disrupts the fine-tuning of networks that determine the quality of information processing across cortical domains. Childhood epilepsy, schizophrenia and autism have all been linked to a disruption in the development of populations of interneurons (Ben-Ari et al. 2004; Levitt et al. 2004). A relative state of disinhibition may manifest as increased susceptibility to seizures, a speculation consistent with recent reports in animals experiencing brief and transient episodes of thyroid hormone insufficiency *in utero* (Auso et al. 2004). A reduction in inhibitory function may also contribute to the augmentation of PS LTP in the dentate gyrus of thyroid hormone-compromised animals previously described for PTU (Gilbert 2004; Gilbert and Packzowski 2003; Gilbert and Sui 2006) and now for perchlorate (see below).

Perchlorate Produces Modest Alterations in Long-Term Synaptic Plasticity. LTP of the EPSP slope was not altered in perchlorate-treated animals, a finding distinct from previous work with PTU. These data indicate that the mechanisms controlling synaptic plasticity are intact and distinct from those subserving baseline synaptic transmission. Paradoxically, yet consistent with the effects of PTU, a significant augmentation of PS LTP over control levels was observed (Gilbert 2004; Gilbert and Paczkowski 2003; Sui et

al. 2005). Reductions in the tonic level of inhibition as described above in paired pulse tests may contribute to an augmentation of the PS amplitude after LTP induction. Alternatively, augmented PS LTP may derive from an increase in the synchrony of cell firing. Subtle changes in the structure of dendritic spines have been reported in hypothyroidism (Madiera et al. 1992) and can influence the efficiency of synaptic transmission and augment cell excitability (Carlisle and Kennedy 2005). Restructuring of spines triggered by LTP-like synaptic activation may represent a means whereby granule cells compensate for reduced synaptic input.

Perchlorate and Thyroid Hormones: Relationship to Developmental Neurotoxicity.

Thyroid hormones were decreased in dams and pups as a function of perchlorate exposure, and reductions in synaptic transmission were observed at a dose that only marginally reduced (~15%) circulating levels of thyroid hormone in dams (30ppm, 4.5mg/kg-day). We are unaware of any non-thyroidal actions of perchlorate that could readily account the present findings on hippocampal physiology. Previous published reports of the effects of perchlorate on neurodevelopment are limited to those of York and colleagues who summarize the results of findings from Regulatory Guideline Studies (York et al 2004; 2005a; 2005b). There was some degree of overlap in dose levels used in these studies and the present report (30 mg/kg-day ~ 300ppm, see Table 1) and considerable overlap in serum thyroid hormone reductions. In the present study 300 and 1000ppm reduced T4 in pups by ~11% and ~27%, respectively. The high dose of 30 mg/kg-day (~300ppm) in the York et al. (2005a) studies produced maximal effects on

thyroid hormone reductions on PN21 (~19% decreases from control values) and fell between our two high dose groups.

At the time of adult testing, euthyroid conditions had returned yet deficits in neurophysiological measures persisted indicating a permanent change in brain function was induced as a consequence of altered thyroid status during development. Although modest reductions in serum hormones were seen in pups, no hormone estimates are available for the prenatal period. At weaning, serum T4 and TSH levels were more dramatically altered in dams than in pups. It is possible that prenatal hormone insufficiencies are critical for the observed changes in hippocampal circuitry. Although the granule cells of the dentate gyrus are of postnatal origin, the perforant path input to dentate gyrus is derived from axons of neurons in the entorhinal cortex, an area with a primarily prenatal developmental ontogeny. On the other hand, severe hypothyroidism beginning just prior to birth is sufficient to induce alterations in hippocampal structure and dentate physiology and a recent report of selective transport and accumulation of perchlorate in the mammary gland suggest an increased vulnerability of the neonate to perchlorate (Anderson et al. 2003; Dohan et al. 2007; Gilbert and Paczkowski 2003; Gilbert 2004). Caution must be applied in attempts to directly link serum hormone concentrations and resulting brain dysfunction. Thyroid homeostasis is a dynamic, cyclical condition and the relationship of maternal, fetal and neonatal serum hormone concentrations, windows of vulnerability, and differential tissue sensitivities during development are complex. Significant regulatory control over local intrahippocampal concentrations of thyroid hormones occurs (e.g., brain deiodinases and transport proteins) such that brain hormone concentrations at the critical site or during the critical

developmental window may not be reflected in serum hormone measures (Guadano-Ferraz et al. 1999; Visser et al. 2007). The pharmacokinetics and dosimetry of perchlorate, its relationship to iodine inhibition and thyroid hormone reductions during pregnancy and infancy, and the subsequent impact on brain development requires further study.

Developmental Exposure to Perchlorate – Impact on Adult Behavior. The absence of effect of perchlorate on motor activity in the adult is consistent with previous reports (York et al. 2004; 2005a). There were no effects of perchlorate observed in trace fear conditioning. We had anticipated deficits as this form of fear conditioning has been shown to rely upon the activation and integrity of the hippocampus (Chowdhury et al. 2005). However, Wiltgen et al. (2006) recently demonstrated impaired trace fear conditioning in animals with hippocampal lesions with a single CS-US pairing that were overcome with repeated CS-US pairings. Robust conditioning induced by the multiple CS-US pairings in the present study may have masked subtle impairments in trace fear conditioning. York et al. (2004) did not detect learning deficits in adult offspring of perchlorate-treated dams using passive avoidance conditioning and a simple position discrimination task. In the present study, we used a more complex test of spatial learning, the Morris water maze, but also failed to uncover deficits in perchlorate-treated animals. The lack of effect on spatial learning was surprising given previous observations of altered hippocampal synaptic transmission coupled with spatial learning impairments following thyroid hormone disruption induced by PTU or methimazole (Akaike et al. 1991; Gilbert and Sui 2006). However, relative to perchlorate, the degree of hormone

suppression induced by PTU and methimazole was more severe (see Akaike et al. 1991; Gilbert and Sui 2006) and significant differences among these treatments exist in mechanism of toxicity, kinetics, and dosimetry.

The lack of effect of developmental perchlorate exposure on learning was also surprising in the context of such severe reductions in hippocampal synaptic transmission. However, to the extent that 'learning' is reflected in tests of 'synaptic plasticity', the failure of perchlorate to detrimentally impact hippocampal LTP is consistent with a lack of effect on 'behavioral plasticity'. It is possible that the augmentation of PS LTP is a reflection of an adaptive or compensatory response in cell physiology which aids in the reversal of learning deficits. Alternatively, many other brain regions are engaged in the performance of even simple learning tasks and as such the sluggish physiology of one region may not be mimicked in other brain regions, or be directly reflected in global downstream measures. In addition, significant behavioral compensation may mask underlying behavioral deficits that are apparent earlier in development or may be revealed with more demanding cognitive tasks. Despite the lack of behavioral effects in the specific tasks used in the present study, impairment of synaptic transmission in adult offspring clearly indicates a permanent detriment in brain function remains as a consequence of developmental perchlorate exposure.

Conclusions. In summary, hypothyroxenemia induced in dams and pups by developmental exposure to perchlorate was associated with deficits in excitatory and inhibitory synaptic function in hippocampus. Physiological endpoints provide an integrated measure of the functional consequences of early disruption of the thyroid axis

and its impact on brain structure and development. Effects were dose-dependent, evident at mild levels of hormone insufficiency, and persisted despite return to normal thyroid status at the time of testing. Decrements in hippocampal synaptic LTP were not detected, nor were deficits in hippocampal-based learning tasks, perhaps related to the relative sparing of synaptic plasticity. Recent reports from the CDC demonstrating a strong association between perchlorate exposure and serum levels of thyroid hormone in women with marginal iodine deficiencies raise considerable public concern about the impact of this contaminant on the developing fetus (Blount et al. 2006). The present data provide evidence in a rodent model that modest degrees of thyroid hormone reduction induced by perchlorate result in persistent decrements in brain function.

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Table 1. Brain and Hippocampal Weights. Mean (+/- SE) brain weights by dose group at postnatal day (PN) 4, PN14 and PN21 in male and female offspring. Brain weights were taken from pups at culling on PN4 and a mean calculated per litter, pooling across gender due to insufficient sample for each sex. Brain and hippocampal weights increased with age and no treatment related effects of perchlorate exposure were noted ($p>0.05$).

Male					Female			
	Brain Weight (gm)				Brain Weight (gm)			
Dose (ppm)	0	30	300	1000	0	30	300	1000
PN4 (M+F)	0.324 (±0.003)	0.321 (±0.005)	0.328 (±0.003)	0.311 (±0.003)				
PN14	1.139 (±0.0138)	1.126 (±0.0268)	1.149 (±0.0129)	1.100 (±0.0233)	1.114 (±0.0146)	1.096 (±0.0221)	1.104 (±0.0218)	1.106 (±0.0158)
PN21	1.404 (±0.0171)	1.374 (±0.0311)	1.394 (±0.0129)	1.383 (±0.0276)	1.350 (±0.0146)	1.369 (±0.0171)	1.353 (±0.0196)	1.352 (±0.0242)
	Hippocampus Weight (mg)				Hippocampus Weight (mg)			
PN14	35.0 (±1.70)	37.0 (±1.61)	36.5 (±1.83)	34.7 (±2.27)	34.9 (±1.69)	35.5 (±1.72)	35.7 (±1.30)	36.1 (±1.09)
PN21	50.2 (±1.66)	49.8 (±2.15)	47.8 (±1.45)	46.9 (±1.97)	46.6 (±1.16)	45.8 (±2.37)	46.6 (±1.06)	47.4 (±1.37)

Table 2. Water and Perchlorate Intake. Water consumption (ml/kg-day) and dam body weights between GD6-PN9 were used to calculate mean (± SE) intake of perchlorate and are expressed as mg/kg-day.

Drinking Water Concentration ppm	Water Consumption Mean (±SE) ml/kg-day	Perchlorate Intake Mean (±SE) mg/kg-day	n Dams/ Litters
0	138.3 (±3.47)	0	28
30	142.0 (±4.43)	4.5 (±0.11)	26
300	135.1 (±2.91)	44.2 (±0.70)	29
1000	132.2 (±3.40)	140.3 (±2.95)	23

Figure Legends

Figure 1. Body Weights in Dams and Pups. (A) Mean ± SE body weight of dams during gestation and lactation did not differ across dose groups. Body weights of male (B) and female (C) offspring did not differ across dose groups. Significant main effects of Day and Gender were observed. Although a significant Dose X Day interaction was revealed, mean contrasts tests failed to identify any significant reduction in pup body weight at any age in either gender.

Figure 2. Thyroid Hormones in Dams. Mean ± SE T3 (A), T4 (B) and TSH (C) from dams treated with perchlorate from GD6 and sacrificed on PN30 at the termination of exposure and weaning of the pups. T3 was not altered, T4 showed a dose-dependent reduction with maximal declines observed at 1000ppm. TSH was increased but only at the highest dose (* Dunnett's T, $p<0.05$). Numbers within histogram bars represent the sample sizes.

Figure 3. Thyroid Hormones in Pups. Mean ± SE T3 (A), T4 (B) and TSH (C) from perchlorate-treated offspring on postnatal day (PN) 4, PN14 and PN21. Data from males and females were taken from pups at culling on PN4 and pooled at the time of sample to provide sufficient serum for hormone assays. No differences in serum hormones were detected between genders on PN14 and PN21 ($p>0.05$) so data were collapsed across and mean value per litter at each age was analyzed. T3 and T4 were significantly reduced on PN21 in the 300 and 1000ppm dose groups (* Dunnetts T, $p<0.05$). Marginal but statistically significant increases in TSH were detected on PN14 at the two midrange

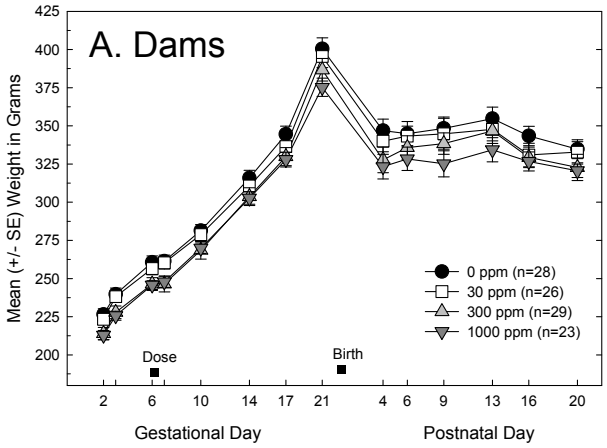
doses. Somewhat larger changes in TSH were seen on PN21 but increases in variability negated statistical significance. Numbers within the histogram bars represent sample sizes.

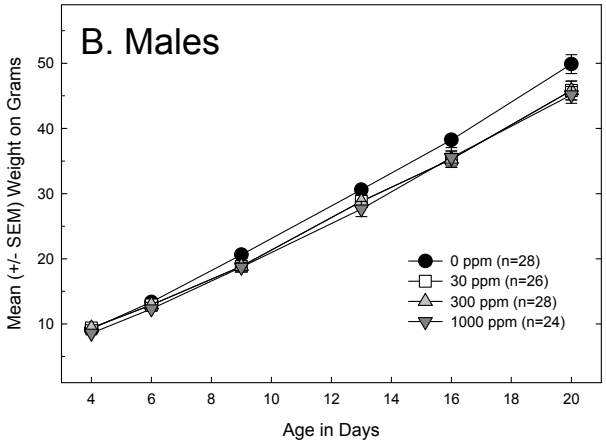
Figure 4. Baseline Synaptic Transmission is Impaired in Adult Male Offspring. Mean (± SE) PS (A, mV) and EPSP slope (B, mV/mS) amplitudes in the dentate gyrus were reduced in a dose-dependent manner in adult offspring of perchlorate-treated dams. PS amplitudes were significantly reduced by all doses of perchlorate. EPSP slope amplitude was reduced by 1000ppm perchlorate. Insets in A depict a typical response to a high intensity pulse and how the PS was scored on each averaged waveform at each intensity for each subject. Inset in B depicts a response to a high intensity pulse and the points on the rising phase of the waveform between which the slope was calculated as an estimate of EPSP amplitude.

Figure 5. Paired Pulse Depression/Facilitation Is Altered In Adult Male Offspring.

Mean (± SE) paired pulse ratio across 5 interpulse intervals (IPI) in response to maximal stimulus intensities (A), intensities producing population spikes (PS) equivalent to 50% maximal (B), and intensities producing PS equivalent to 20% of maximal. Perchlorate shifted paired pulse functions upward to a more disinhibited state that was most prevalent at the low stimulus intensity (C, 20% Max). Significant differences were detected between 0ppm and the 300ppm (*) and 0 and 1000ppm (#) dose groups (Dunnett's T, $p<0.05$).

Figure 6. Long-Term Potentiation (LTP) Is Enhanced in Adult Male Offspring. Probe stimuli recorded before and after LTP-inducing trains of maximal stimulus strength (1500μA) increased EPSP slope amplitude (A, mean ± SE), indicating LTP was successfully induced in all groups to the same degree ($p>0.05$). (B) LTP was also induced in the PS, but the magnitude of increase was greater in perchlorate-treated animals from the high dose group. Note difference in scale between A and B.


A. Dams
Mean (+/- SE) Weight in Grams
425
400
375
350
325
300
275
250
225
200
Dose
Birth
2 6 10 14 17 21 4 6 9 13 16 20
Gestational Day
Postnatal Day
0 ppm (n=28)
30 ppm (n=26)
300 ppm (n=29)
1000 ppm (n=23)


B. Males
Mean (+/- SEM) Weight on Grams
50
40
30
20
10
4 6 8 10 12 14 16 18 20
Age in Days
0 ppm (n=28)
30 ppm (n=26)
300 ppm (n=28)
1000 ppm (n=24)

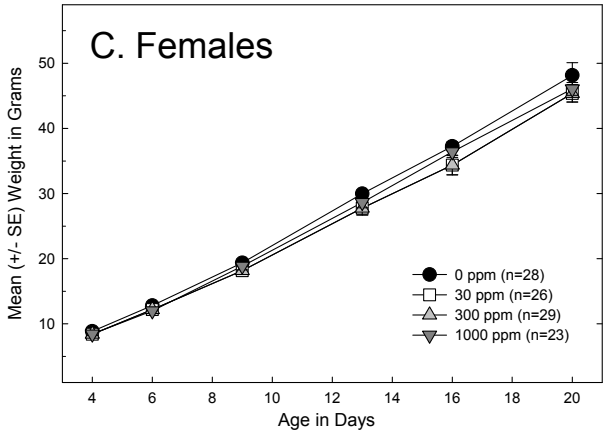

C. Females
Mean (+/- SE) Weight in Grams
50
40
30
20
10
4 6 8 10 12 14 16 18 20
Age in Days
0 ppm (n=28)
30 ppm (n=26)
300 ppm (n=29)
1000 ppm (n=23)

Figure 1

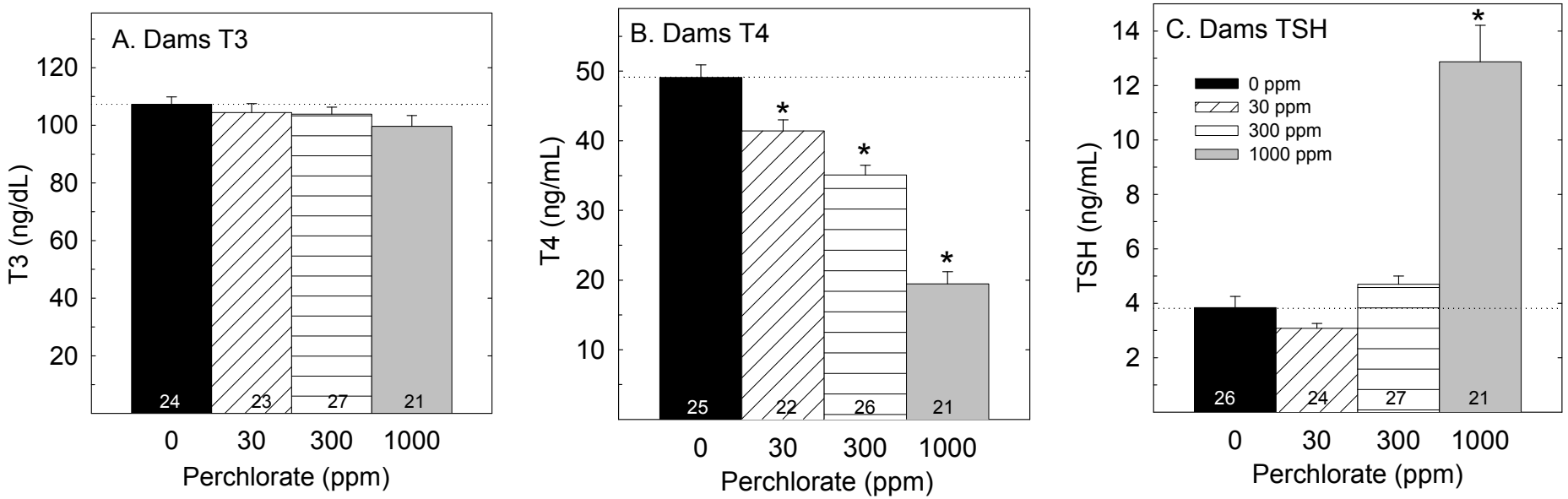

A. Dams T3
T3 (ng/dL)
120
100
80
60
40
20
24
23
27
21
0
30
300
1000
Perchlorate (ppm)
B. Dams T4
T4 (ng/mL)
50
40
30
20
10
*
*
*
25
22
26
21
0
30
300
1000
Perchlorate (ppm)
C. Dams TSH
TSH (ng/mL)
14
12
10
8
6
4
2
0 ppm
30 ppm
300 ppm
1000 ppm
*
26
24
27
21
0
30
300
1000
Perchlorate (ppm)

Figure 2

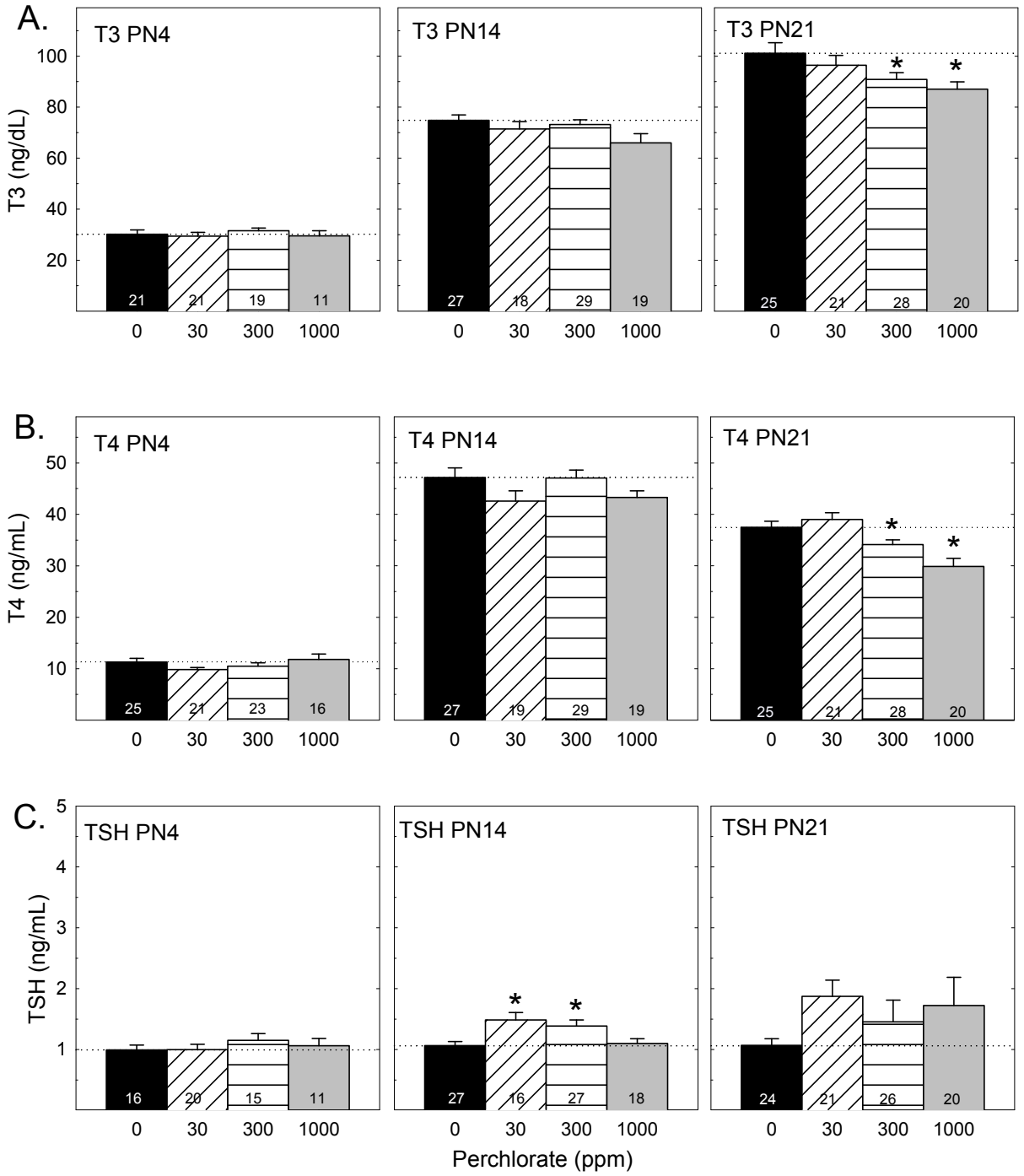

A.
T3 PN4
T3 PN14
T3 PN21
T3 (ng/dL)
100
80
60
40
20
21
21
19
11
27
18
29
19
25
21
28
20
*
*
0
30
300
1000
B.
T4 PN4
T4 PN14
T4 PN21
T4 (ng/mL)
50
40
30
20
10
25
21
23
16
27
19
29
19
25
21
28
20
*
*
C.
TSH PN4
TSH PN14
TSH PN21
TSH (ng/mL)
5
4
3
2
1
16
20
15
11
27
16
27
18
24
21
26
20
*
*
Perchlorate (ppm)

Figure 3

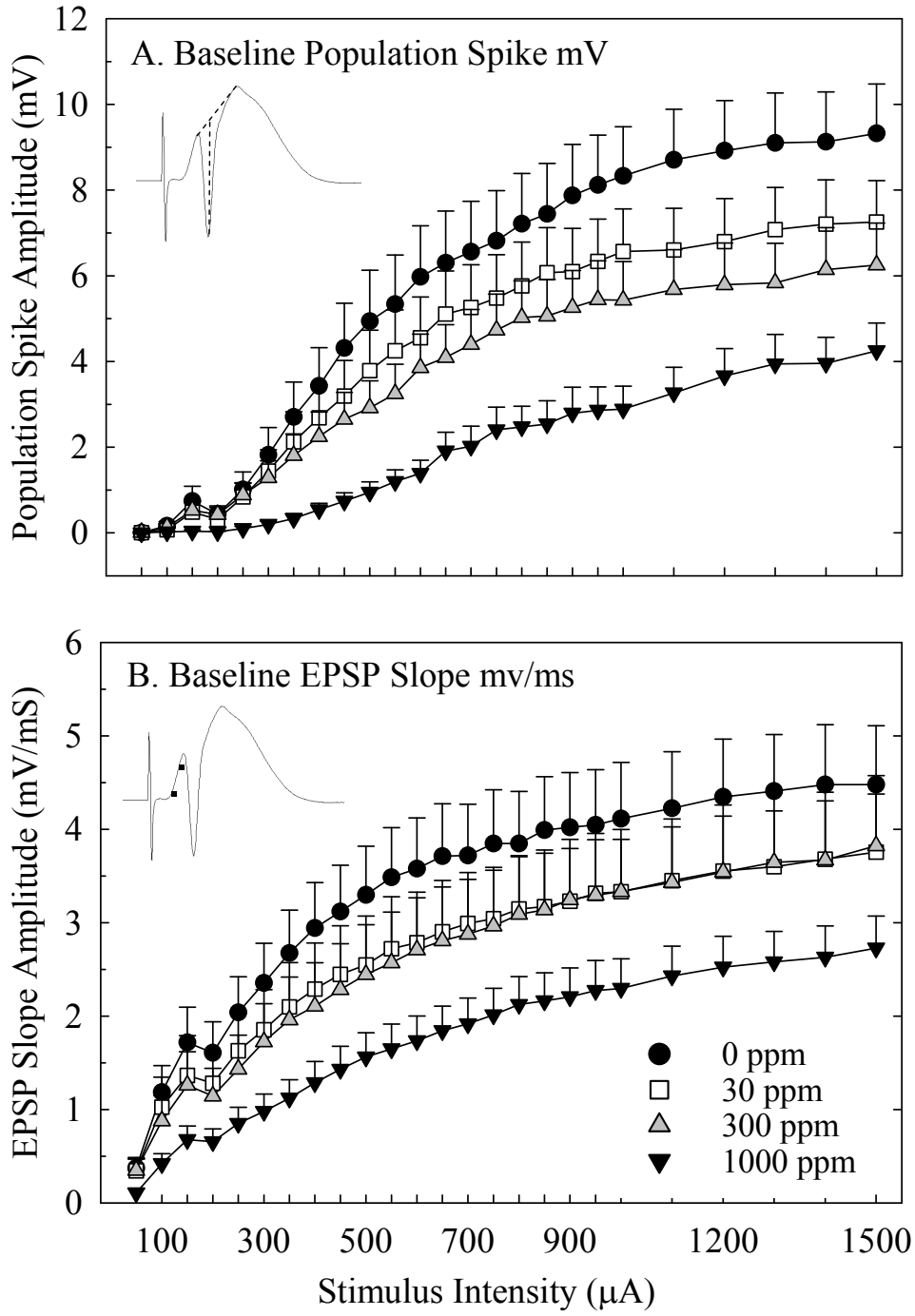

A. Baseline Population Spike mV
Population Spike Amplitude (mV)
0
2
4
6
8
10
12
B. Baseline EPSP Slope mv/ms
EPSP Slope Amplitude (mV/mS)
0
1
2
3
4
5
6
0 ppm
30 ppm
300 ppm
1000 ppm
100
300
500
700
900
1200
1500
Stimulus Intensity (μA)

Figure 4

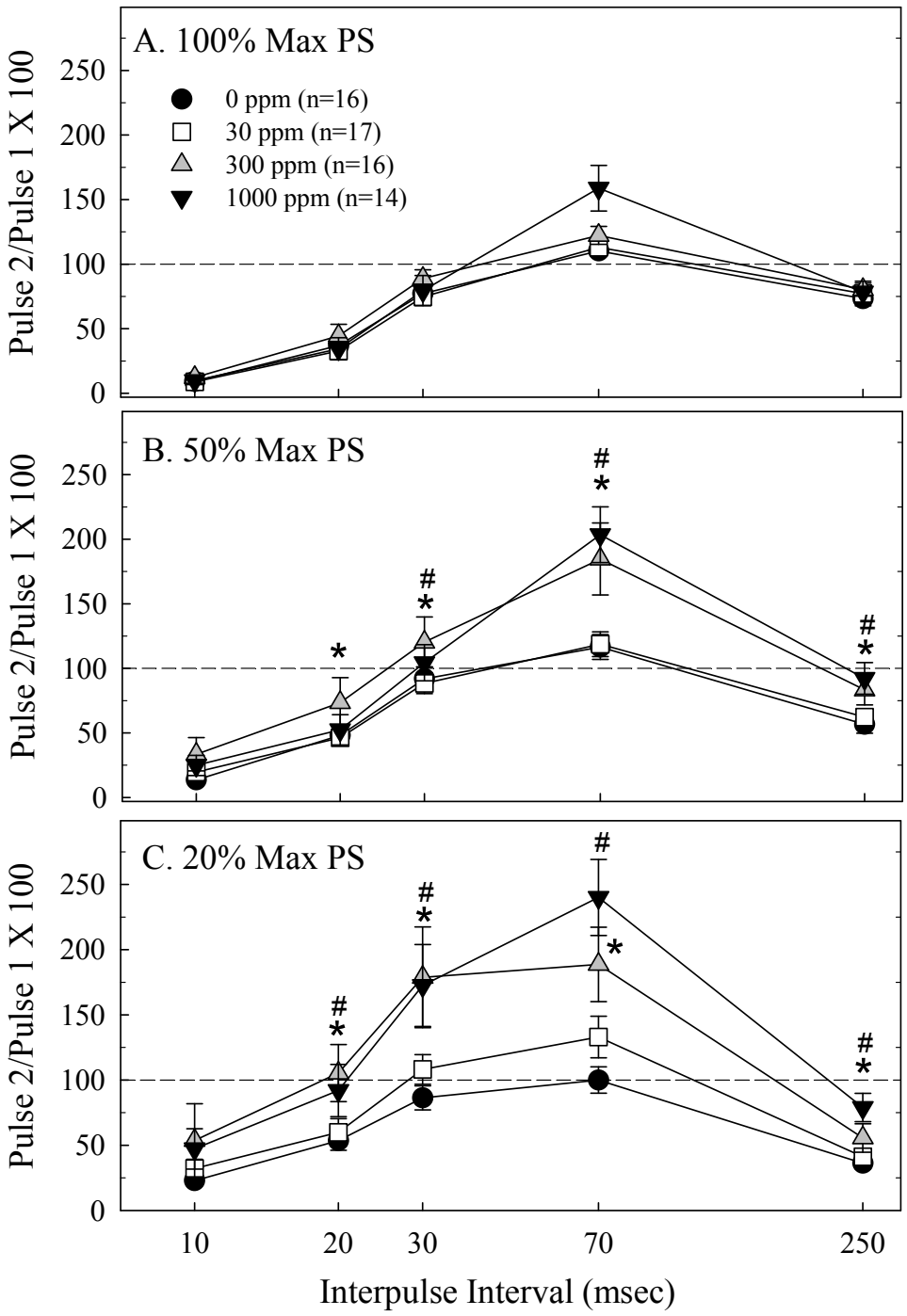

A. 100% Max PS
0 ppm (n=16)
30 ppm (n=17)
300 ppm (n=16)
1000 ppm (n=14)
B. 50% Max PS
C. 20% Max PS
Pulse 2/Pulse 1 X 100
0
50
100
150
200
250
10
20
30
70
250
Interpulse Interval (msec)
*
#

Figure 5

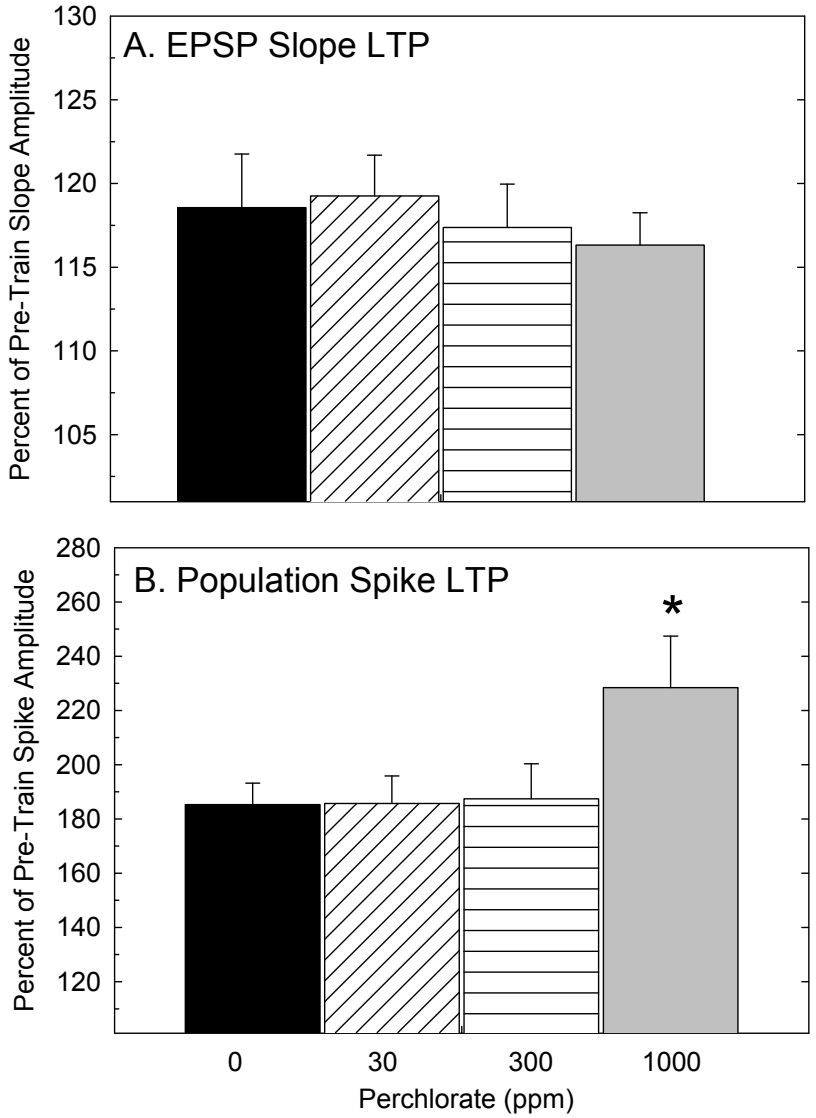

A. EPSP Slope LTP
Percent of Pre-Train Slope Amplitude
130
125
120
115
110
105
B. Population Spike LTP
Percent of Pre-Train Spike Amplitude
280
260
240
220
200
180
160
140
120
*
0
30
300
1000
Perchlorate (ppm)

Figure 6