

Supplementary Material – Experimental Procedures

Motor Activity. Motor activity was assessed using six photocell devices (Motron Electronic Motility Meter, Motron Produkter, Stockholm, Sweden). Each device had a 5 x 8 matrix of photodetectors in the platform that were illuminated by a single overhead incandescent (30W) lamp; movements that occluded these detectors were recorded as horizontal activity. An array of six photoemitters and detectors were also placed 16.5 cm above the platform to record vertical activity (rearing). Each device was housed in a sound- and light-attenuating ventilated cubicle in a dedicated test room. A clear plastic chamber (33 x 21 x 26 cm) with removable lid was placed over each platform to contain the rat during testing. Motor activity was recorded in five successive 6-min intervals during a single 30-m session. All testing occurred between 7:00 am and 12:00 noon on the same day. Order of testing was counterbalanced across dose groups.

Morris Water Maze Acquisition. Spatial learning was assessed in the Morris water maze as optimal performance of this task requires the integrity of the hippocampus (Morris, 1989). One male offspring (PN94-114) was selected from each of a subset of litters to evaluate spatial learning using the Morris water maze. The water maze consisted of a white circular galvanized tank with a diameter of 1.7 meters, filled with tap water adjusted to 24°C and made opaque by the addition of white powdered paint. Four locations around the edge of the pool were defined as start points, and divided the pool into 4 equally sized quadrants. A circular acrylic escape platform, 10 cm in diameter, was placed 2 cm below the surface of the water in the middle of one of the 4 quadrants of the

pool. The pool was placed in a 4.1 X 3.1 meter room containing invariant spatial stimuli. A video camera suspended from the ceiling above the middle of the tank permitted the observer to follow the animal's behavior on a video-monitor placed in one corner of the room. Salient visual cues were present in the room and included a door, a computer and VCR on a cart in the corner, four posters of different shapes and colors mounted on each of four walls. On the day prior to beginning of formal acquisition testing, all animals were acclimated to the task by individually placing them in the pool and allowing a 60-s free swim with no platform present. Thereafter, animals were tested for 2 daily trials, each trial separated by approximately 3-5 m, for 15 consecutive days. Animals were placed into the tank, facing the wall of the pool, and allowed to circumnavigate the pool in search of the escape platform for a maximum of 60 s. On each day the start points used for each trial varied in a pseudo-random sequence such that no two trials on the same day commenced from the same starting quadrant. Latency to reach the escape platform was recorded and the animals permitted 15 s to rest on the platform before removal from the tank. If an animal failed to locate the platform within 60 s, it was guided there by the experimenter, placed upon it for 15 s, and assigned a latency score of 60 s for that trial.

Probe Trials. 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 platform was reinserted at the end of this period and animals were permitted to rest on it for 15 s to avoid extinction of the search behavior. The percentage of time animals spent in each quadrant of the pool was recorded for each probe trial. Escape latencies on probe trial days were based on data from the 2nd trial only.

Cue Learning and Swim Speed. Visual and motor competence were assessed to determine if perchlorate-induced alterations of these abilities influenced performance in the water maze task, independent of cognitive function. Cue testing was conducted the day after completion of acquisition learning. Under this condition, the platform was visible resting above the water level with a glove identical to that worn by the experimenter was suspended above it. Animals were placed in the pool as previously described and latency to reach the visible platform was recorded on two consecutive trials. Swim speed was determined using a videotracking system of pathlength (HVS Image, Buckingham, UK) of the first probe trial where animals swam freely for 60 s in the absence of the escape platform.

Fear Conditioning. Delay fear conditioning primarily probes amygdala function, whereas trace conditioning is believed to require information from the hippocampus (Bangasser et al. 2006; Rodrigues et al. 2004). Trace fear conditioning to both cue and context were assessed in the present study to probe hippocampal function. Two different chambers were used, one for training and context testing, and a separate chamber in different room for cue testing. The conditioning chamber (Habitest, Coulbourn Instruments, Allentown, PA) had aluminum side walls with Plexiglas front and back walls, an aluminum ceiling, and a standard grid floor that consisting of parallel steel rods (5 mm diameter and 15mm spacing). The dimensions of the chamber were (31 X 25 X 29 cm, length X width X height). A small animal shock generator (H13-16, Coulbourn Instruments) was used to generate a scrambled footshock (1mA, 0.5 s duration) that served as the unconditioned stimulus (US). Conditioning boxes were enclosed in a sound attenuating chamber. The conditioning chamber was sprayed with Windex™ cleaner to

provide a distinct olfactory cue and were wiped down with this cleaner between each animal. On day 1, animals were placed the conditioning chamber dimly illuminated by a single house light. The training procedure consisted of a 2-m baseline period followed by onset of a 15 s compound light/tone conditioning stimulus (CS). The light component of the CS (1.5 cm round disk, 18 lux) was centered on the left wall of the test box. The tone component of the CS was elicited from a Sonalert, located 5 cm from the top and 2.5 cm from the right side of the back wall of the chamber, generated a broadband tone at 86dB.

In trace fear conditioning, CS offset and US onset were separated by a 30 s trace interval. Training consisted of 6 CS-US pairings with a 3 min intertrial interval. The following day animals were placed into the training chamber and activity monitored by an infrared motion detector (Colbourn Instruments) mounted on the ceiling of each test chamber. Total activity counts within a 5-m test period were recorded and taken as a measure of conditioning to context. Animals were returned to holding cages in a dimly-lit room for 1 h. Conditioning to cue was assessed by placing animals in a different test box in a different brightly lit room with the sound attenuating enclosure chamber doors left open. The chamber was of similar dimension as the training box but was equipped with black and white vertical stripes on the back and two side walls and a smooth white plexiglass floor. This chamber was sprayed with apple scented disinfectant to provide an olfactory cue distinct from that of the training chamber. The chamber was cleaned with this disinfectant between animals. Activity was monitored by a ceiling-mounted infrared motion detector identical to that used in the training box. A 2-m baseline activity level was assessed prior to 6 CS presentations separated by a 2-m intertrial interval.

Results of Behavioral Studies

Prior perchlorate treatment did not affect motor activity in adulthood (Supplementary Figure 1). Results of ANOVAs for session-total counts yielded a non-significant difference between treatment groups for both horizontal and vertical motor activity. Habituation was evident as decreases in motor activity across intervals within the test session, and was more pronounced for vertical than horizontal activity. Repeated-measures ANOVAs resulted in a significant effect of interval for both horizontal [$F(4,140)=64.1, p<0.0001$] and vertical [$F(4,140)=63.7, p<0.0001$] activity, but no significant effect for either perchlorate treatment or the treatment-by-interval interaction.

Acquisition of spatial learning in the Morris water maze was not impacted by perchlorate exposure. Latency to find the hidden platform was reduced over days in all groups indicating learning of the task, but no differential rate was observed as a function of perchlorate treatment (Supplementary Figure 2A). Standard performance controls for motoric and visual function were similar across dose groups (Supplementary Figure 2A, Cue). A series of five probe trials was interjected throughout the acquisition period such that on every 5th trial (1st trial of Days 3, 6, 9, 12 and 15), the platform was removed and animals allowed to swim freely for 60 s before its reinsertion. The percentage of time animals spent in the correct quadrant was also calculated as another index of learning. Supplementary Figure 2B clearly shows an acquisition curve for probe trials over days of training, but no differential effect was evident in control vs perchlorate-treated animals ($p>0.10$). The number of platform crossings was also recorded during probe trials and found to increase over successive probe trials, but did not discriminate between performance of perchlorate-treated and control animals (Supplementary Figure 2C).

Trace fear conditioning was assessed in adult offspring using a robust training paradigm of 6 CS-US pairings. Evidence of conditioning to cue was apparent by a suppression of ongoing activity when assessed 24-hours after training when the CS was presented in a novel environment. No differences were evident in conditioning to cue as a function of developmental exposure to perchlorate (Supplementary Figure 3A). Similarly, conditioning to context, evaluated by placing the animal back in the original test box 24 hours after training did not differ among the groups (Supplementary Figure 3B).

Results of Histological Analysis. Supplementary Figure 4 depicts electrode placements for dentate gyrus recording electrodes (Supplementary Figure 4A) and stimulating electrodes in the perforant path (Supplementary Figure 4B) for animals included in the neurophysiological assessments.

References for Supplementary Material

- Bangasser DA, Waxler DE, Santollo J, Shors TJ. 2006. Trace conditioning and the hippocampus: the importance of contiguity. *J Neurosci*. 26:8702-8706.
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Rodrigues SM, Schafe GE, LeDoux JE. 2004. Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. *Neuron*. 44:75-91.

Swanson LW. 1998. BrainMaps: Structure of the Rat Brain, 2nd Edition, Elsevier:Amsterdam.

Supplementary Figure Legends

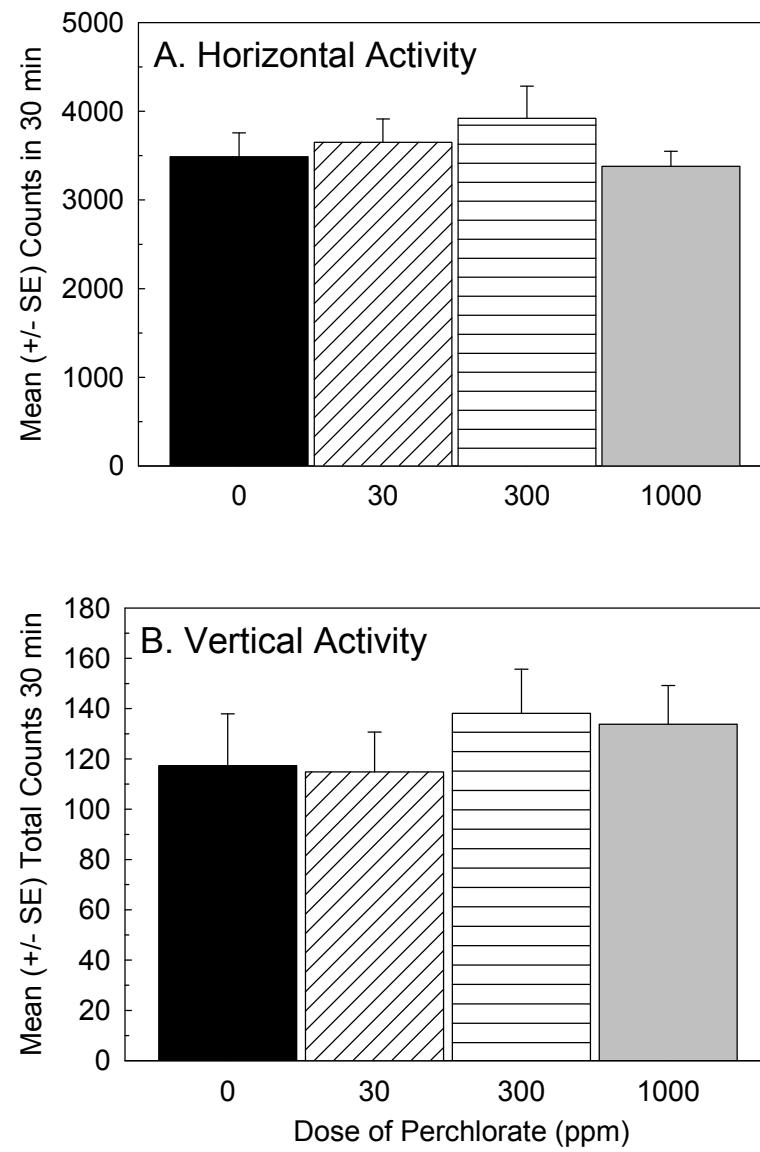
Supplementary Figure 1. Motor activity assessed in adult male offspring at 13 months of age was not altered by developmental exposure to perchlorate. Mean (\pm SE) activity counts over a 30 minute testing period for (A) horizontal and (B) vertical (i.e. rearing) activity were similar across dose groups.

Supplementary Figure 2. Spatial Learning Assessed in the Morris Water Maze Was Not Impaired in Adult Male Offspring (3 months of age). (A) Mean \pm SE latency to find the hidden platform was reduced over days in control animals and was used as an index of learning. The rate of decline was comparable across all dose levels. In a cued version of the task, all animals demonstrated competence indicating sensory or motor impairments were not likely responsible for learning deficits. (B) Probe trials were interspersed throughout the training period (first trial on days 3,6,9,12,15) assessed the percent of time (mean \pm SE) animals spent searching in the correct quadrant over a 60 second free-swim period when the platform was removed. The percent time values increase over time as a function of learning. The mean number of platform crossings also increased with

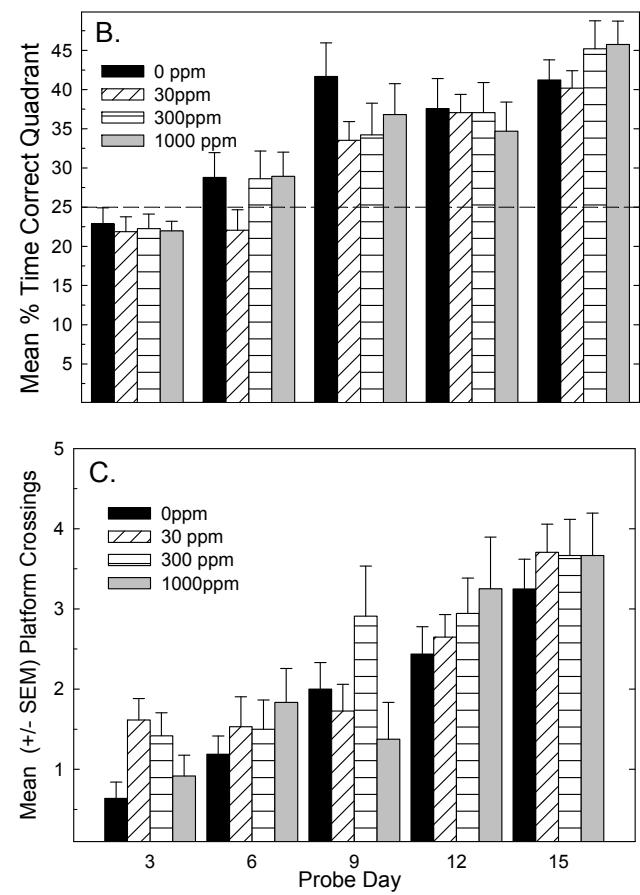
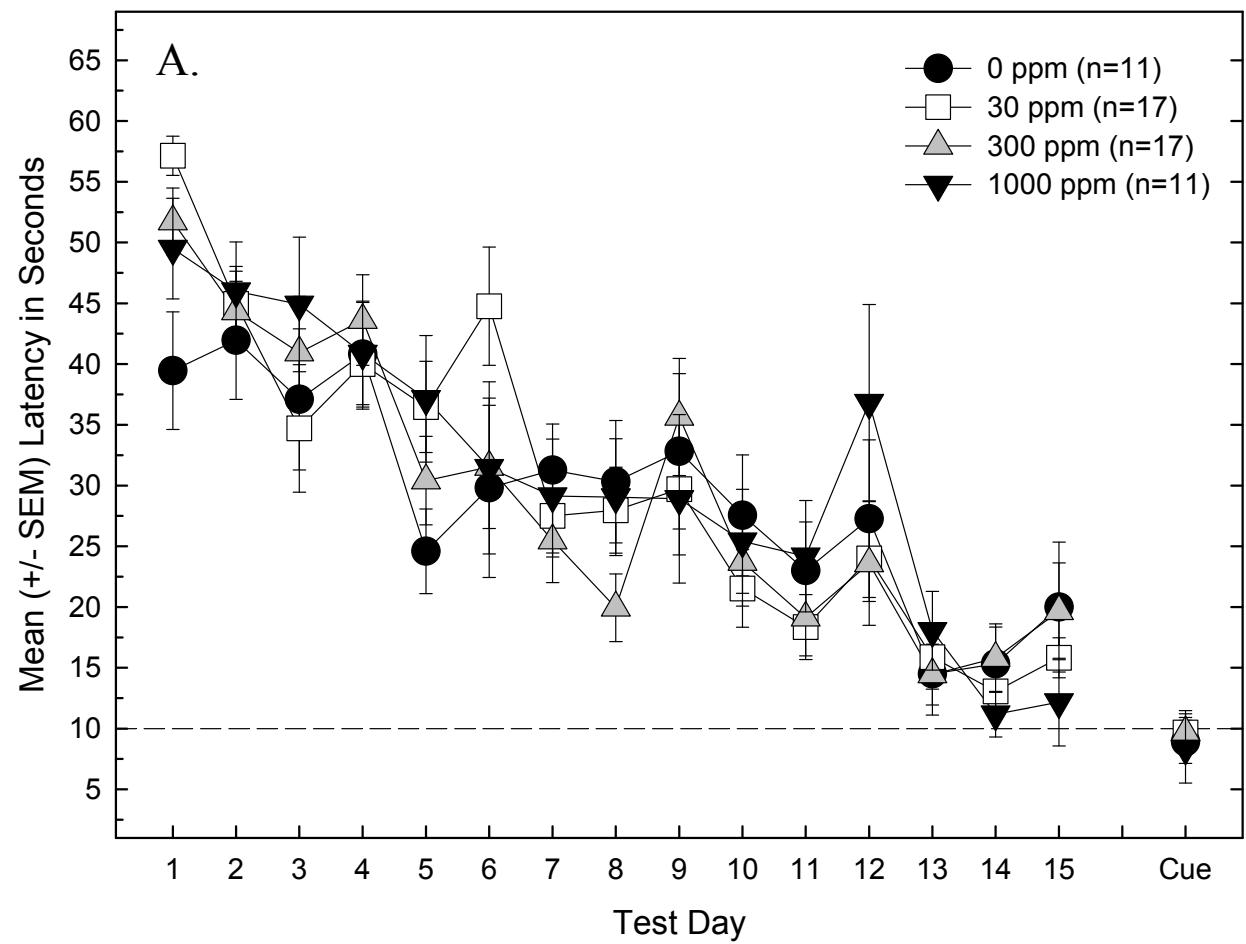
successive probe trials (B). There were no group differences detected in either of these additional measures of learning.

Supplementary Figure 3. Trace Fear Conditioning was Not Impaired in Adult Male Offspring (4-5 months of age). (A) Activity counts/minute (mean \pm SE) in a novel chamber were markedly suppressed relative to baseline during the first minute following presentation of the conditioned stimulus (CS) when assessed 24 hours after training. Suppression of activity upon CS presentation is indicative of conditioned fear responding. No differences in the degree of learning was seen between control and perchlorate-treated animals. (B) Activity counts/minute (mean \pm SE) in the training box in the absence of CS presentation is taken as a measure of conditioning to contextual cues. No differences were detected among groups.

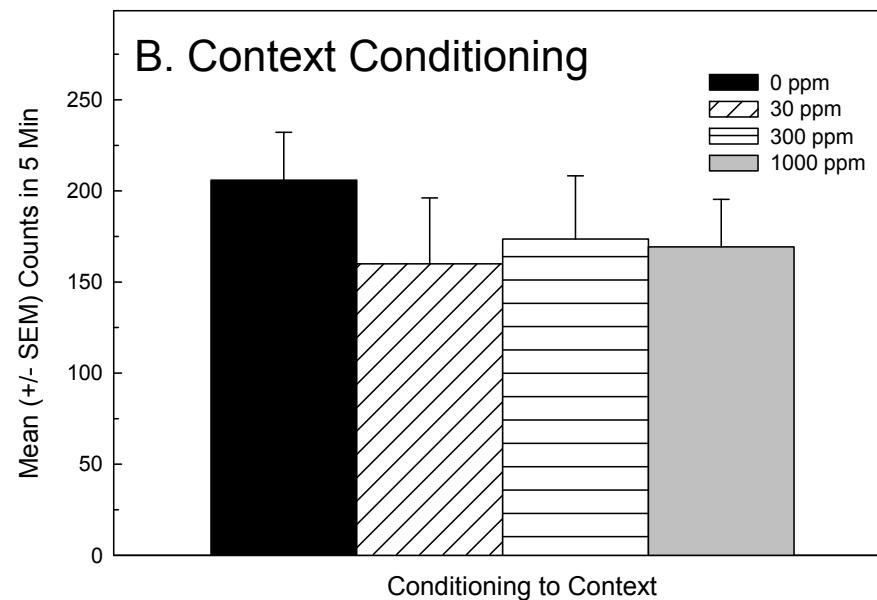
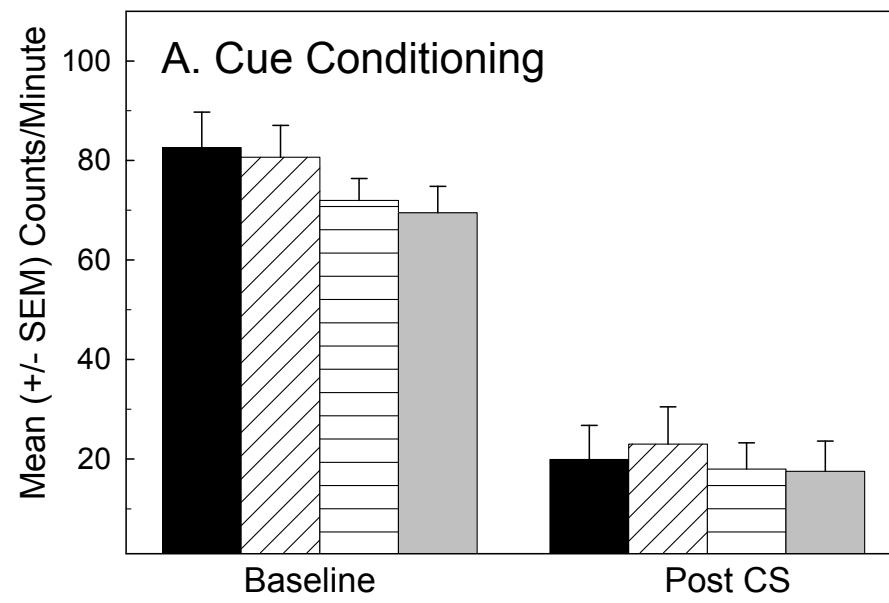
Supplementary Figure 4. Approximate electrode positions in the dentate gyrus (A) and perforant path (B) are indicated on a nissl stained section taken from Swanson (1998). For simplicity, representative placements from control and all 3 dose groups are marked on a single image. Actual placements appeared in these approximate positions but on plates from Paxinos and Watson (2005) atlas ranging from 56 to 62 for the dentate gyrus and 86 to 90 for the perforant path.



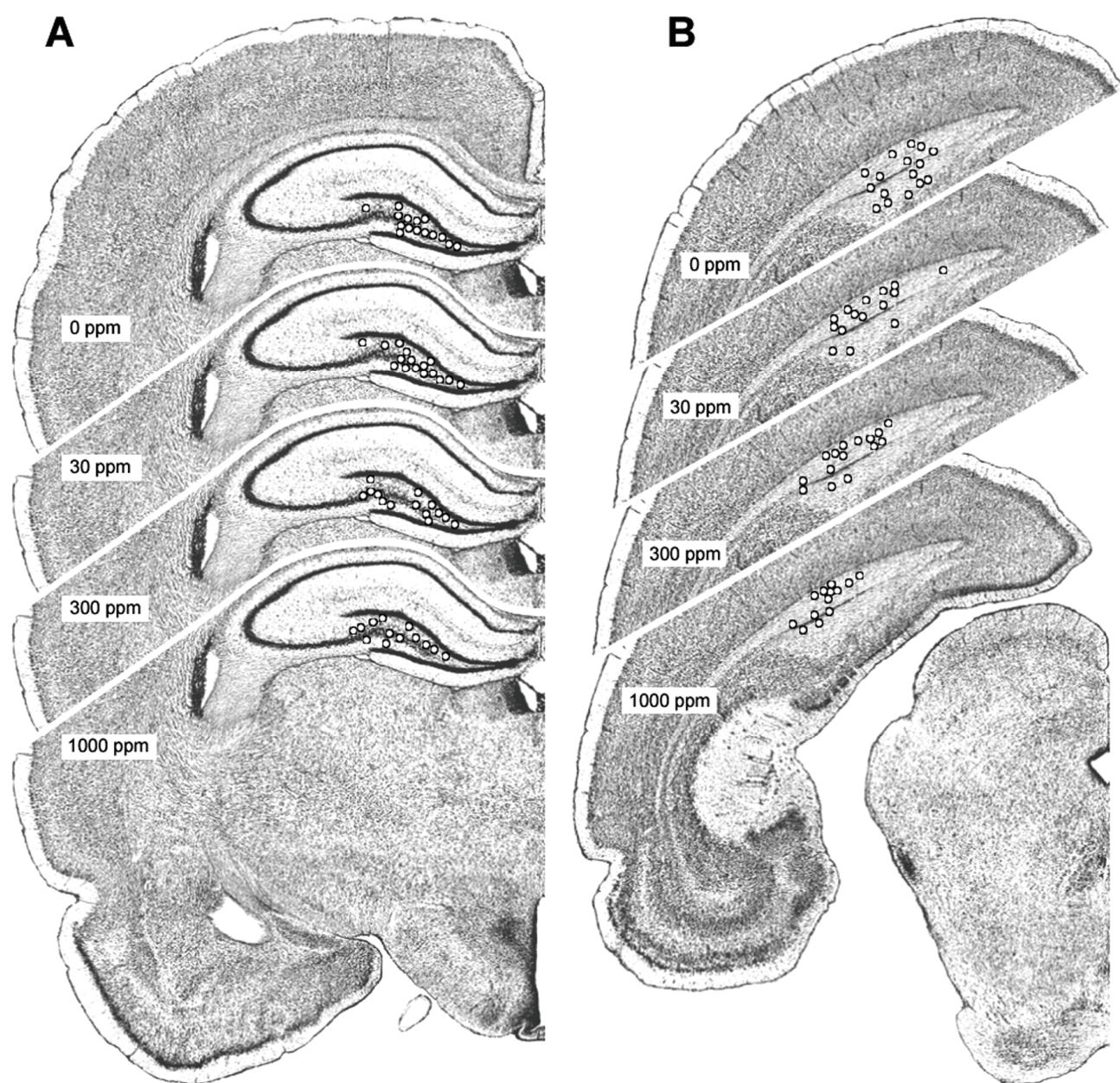
Supplementary
Figure 1



Supplementary
Figure 2



Supplementary
Figure 3



Supplementary
Figure 4