Transitional versus surgical menopause in a rodent model: etiology of ovarian hormone loss impacts memory and the acetylcholine system

Supplemental Methods

Water radial-arm maze

The 8-arm WRAM had platforms hidden at four arm ends. A subject was placed in the start alley, and had 3m to find a platform. Once found, the animal remained on it for 15s, and was returned to its heated cage for a 30s ITI. The just-chosen platform was removed from the maze. These events were repeated until all four platforms were located. Consequently, there were 4 trials/day, with platformed arms reduced by one on each subsequent trial. Hence, WM was increasingly taxed as trials progressed. Testing was for twelve days, with platform locations constant at the beginning of each session. Errors were quantified using orthogonal measures of WM and RM errors, with the last four days used to evaluate WM and RM (1-7). Working memory correct (WMC) errors were first and repeat entries into arms from which a platform had been removed during that day.

Morris water maze

For the MM, the rat was dropped off at either North, South, East, or West, and had 60s to locate the platform. Rats were given 4 trials/day for 5 days. The ITI was 5-8m (8-10). To evaluate whether rats localized the platform to the spatial location, after all test trials a 60s probe trial was given, removing the platform. A video camera/tracking system tracked and analyzed each rat's path (Ethovision, Noldus Instruments).

Delayed-match-to-sample plus maze

The plus-maze had a hidden platform at the end of one arm (each arm=38.1cm x 12.7cm). The platform location was the same within a day, but changed across days. Rats received six trials/day, 90s trial time, 15s platform time, 30s ITI. Trial one was the information trial, two was the WM test trial, and three-six were recent memory test trials (11). After 6 days at a 30s ITI, a 6-hr delay was instilled after the information trial. The day after delay testing, rats received 0.2 mg/kg IP scopolamine (Sigma-Aldrich Inc., St Louis, MO), 20m prior to the information trial (12-15). The following day rats received 0.4 mg/kg, IP scopolamine following the same procedure. To ensure drug clearance, sacrifice occurred 48 hrs after the last scopolamine challenge, well after reported transient post-scopolamine dose-dependent fluctuations in ACh levels have stabilized (16-19).

Brain acetylcholinesterase assays

From the left hemisphere, the frontal cortex and hippocampal CA1/CA2 regions were dissected, placed in pre-weighed tubes, immediately placed on dry ice and then a –70ºC freezer until analysis. Frontal cortex was taken from the dorsal aspect, with the most medial 1.5-2mm portion of the frontal cortex taken. Next, the brain was cut in the coronal plane and the CA1/CA2 region was excised, with dentate gyrus and alveus excluded.

AChE was analyzed using an acetylcholinesterase assay kit (Invitrogen, Carlsbad, CA), and Tris-HCl and NaCl from Sigma (St. Louis, MO). Tissues were homogenized at 0°C. In a pilot study (unpublished), we measured AChE levels at multiple time points (0-60m), and losses across time were negligible. Thus, we infer that AChE does not change activity at 0°C in obtained brain tissues. We added 500ul ice-cold homogenization buffer (20mM Tris-HCl, pH 8.0, 137mM NaCl) to frozen tissues and homogenized (PowerGen 125) and sonicated (Fisher Dismembrator 100), keeping tubes in an ice-water bath. Lysates were centrifuged at 16,000*x*g for 15m at 4˚C and stored on ice. A 1:10 dilution of supernatant in homogenization buffer was prepared, and 25ul aliqots were assayed per kit instructions. A standard curve was determined for each plate. Each

plate was incubated in the dark at 25˚C, and fluorescence read at 30m (excitation 560±10nm, emission 590±10nm). Values were interpolated off a standard curve and normalized to tissue weight (U AChE/gram wet tissue weight).

Statistical analyses

For WRAM, two testing blocks (block 1=days 2-8, block 2=days 9-12) were analyzed using 1-between (Treatment) 3-within (Block, Day and Trial) repeated-measures ANOVAs (1-7). Post-delay trials were analyzed using a 1-between (Treatment), 1-within (Trial) repeated-measures ANOVA.

MM analyses tested Distance (cm) to the platform using a 1-between (Treatment), 2-within (Days and Trials) repeated-measures ANOVA. For probe trial data, percent distance in the previously platformed (target) quadrant was compared to the diagonally opposite quadrant (20). Rats that learned the platform location should spend the greatest percent distance in the target vs. opposite quadrant. An ANOVA with Treatment as the between variable, and Quadrant as repeated-measures, was used.

For DMS, initial learning days (days 1-6) were blocked (block1=days 1-3, block 2=days 4-6), and trials 2-6 were averaged across blocked days (21). Each block was analyzed separately using a 1-between (Treatment) and 1-within (Trial). Performance on day 6 was assessed using a 1-between (Treatment), 1-within (Trial) repeated-measures ANOVA. Since all groups performed similarly on this day, this was considered baseline for the delay and scopolamine challenge trials. For delay and scopolamine challenges, performance on the test trial after the challenge was compared to that same trial of the baseline scores from day 6 (21). Repeated-measures ANOVA with Treatment was the between variable and Day (test trial on manipulation day vs. test trial at baseline) as repeated-measures was used.

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