ARTICLE
A Low-Cost Morris Water Maze for Undergraduate Research: Construction and Demonstration in a Rat Model of Obesity-Induced Diabetes

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The Morris Water Maze (MWM) is a standard task for assessing hippocampal-dependent learning and memory, but the cost of commercial versions of the task may be prohibitive for some undergraduate research projects. We describe the construction of a low-cost MWM for use with rodents and demonstrate the effectiveness of the MWM in a study of the effect of diet-induced obesity on cognitive function in rats. Previous studies have described an impairment in MWM performance in rats fed a high-fat diet combined with streptozotocin injection (to model Type 2 diabetes). We attempted to replicate this finding with our water maze design, and to test the ability of a novel anti-inflammatory treatment to reduce cognitive deficits in the diabetic model. Across five days of hidden-platform training,

rats in all groups (normal pellet diet vs. high-fat diet, vehicle vs. treatment) improved on the water maze at similar rates. On a 30-second probe trial, each group showed a preference for the target quadrant used during training. These results did not replicate previous findings that a high-fat diet combined with streptozotocin injections produces deficits in the water maze. However, the results validate the effectiveness of a low-cost water maze ($400 USD) constructed from commonly available materials for hidden platform water maze training. When combined with a low-cost video tracking solution (less than $1,000), we expect this apparatus will be of use to other undergraduate researchers interested in learning and memory.
    Key words: Morris Water Maze (MWM)

Since the original description of Morris Water Maze (MWM) by Morris (Morris, 1981), the task has become a standard tool for assessing learning and memory (D'Hooge and De Deyn, 2001). A recent search of PubMed (www.ncbi.nlm.nih.gov) using the term "morris water maze" resulted in over 8,000 citations, with over 1,500 citations in 2015-16 alone. In typical versions of the task, rats or mice learn to escape from a pool of water by swimming to find a submerged platform. Solving this "hidden platform" version of the task requires locating the position of the platform relative to extramaze cues. Performance on the hidden-platform version of the task is impaired by damage to the hippocampus, and the MWM has been a valuable tool for understanding how a larger system of brain structures supports memory (reviewed in D'Hooge and De Deyn, 2001). In light of the importance of navigation in rodent studies of learning and memory, and the variety of navigation tasks that can be conducted with the MWM, the maze is of great use in studies of the brain systems and pharmacology of learning and memory, assessment of rodent models of neurocognitive disorders, and testing potential treatments for neurocognitive disorders (D'Hooge and De Deyn, 2001).

    Given the prominence of the MWM in studies of hippocampal-dependent memory in rodents, the task is a valuable addition for undergraduate neuroscience laboratories. Our laboratory is interested in learning and memory, but most of our research is done using operant tasks (using standard operant chambers) or using custom-built mazes for assessing different types of navigation strategies in rats. [Recently, one of the authors (LMG) was pursuing a research project as part of his undergraduate senior capstone research project, which focused on the ability of a cocktail of anti-inflammatory compounds to treat

cognitive impairments in rats. The focus of the project was on recent studies which had demonstrated impaired MWM performance in a rat model of Type 2 diabetes (Datusalia and Sharma, 2014). For the research project, we wished to replicate the MWM impairment observed in rats fed a high-fat diet, and to then test the ability of an anti-inflammatory cocktail to remediate this impairment. However, as our lab was not equipped with a water maze, we explored commercial systems. Unfortunately, for our needs (a single project conducted as an undergraduate senior research study), the commercial systems we explored were prohibitively expensive (costing more than $5,000 USD for a complete package, and an additional $9,000 USD for a video tracking and control package for the water maze), so we developed and tested a less expensive water maze and video tracking solution for use in an undergraduate research project.

    Our research study was motivated by an interest in neuroinflammation as a common mechanism underlying cognitive deficits in several disease states. Our project specifically focused on diabetes, which the CDC states that 9.3% of the US population currently suffer from, and which costs the United States over $176 billion in annual direct medical costs alone (American Diabetes Association, http://www.diabetes.org/advocacy/news-events/cost-of-diabetes.html). While many of the health risks associated with diabetes are well-known, diabetes has also been linked to cognitive deficits, which have been linked to an increase in neuroinflammation in the hippocampus, impacting memory and other cognitive processes (Datusalia and Sharma, 2014). Slowing this damage by reducing inflammation could produce better outcomes for patients with diabetes and improve their cognitive functioning.

Several studies have demonstrated an increase in inflammation in animal models of diabetes and obesity, along with deficits in tasks which depend on the integrity of the hippocampus (Farr et al., 2008; Kodl and Seaquist, 2008; Boitard et al., 2014). Boitard and colleagues (2014) examined the impact of a high fat diet on juvenile and adult rats. Although there were no significant effects found in adults, juvenile rats that were given a high fat diet had much higher levels of cytokines, indicating inflammation. Juvenile rats on a high fat diet had normal acquisition in learning the location of a hidden platform in the MWM, but showed impaired performance on a MWM probe trial (in which the platform was removed) conducted 4 days after training (Boitard et al., 2014). Boitard and colleagues (2014) did not observe MWM deficits in adult rats (placed on a high fat diet at approximately 12 weeks of age). While not focusing on a rat model of diabetes (rats showed significant increases in weight, but only adult rats showed an increase in glucose levels), these data suggest that some inflammatory effects of obesity may be related to MWM impairments.

Another study conducted by Datusalia and Sharma (2014) induced diabetes in rats using a high fat diet combined with a low dose of streptozotocin, which attacks the insulin-producing beta cells of the pancreas. Diabetic rats demonstrated a significant increase in neuroinflammatory enzyme levels and almost double the amount of cytokines compared to controls. This increase in neuroinflammation was accompanied by impaired acquisition and probe trial performance in the MWM (Datusalia and Sharma, 2014).

While several studies have shown improvements in memory performance in diabetic rats after treatment targeting inflammation, our interest was in testing a recently proposed drug combination to treat neuroinflammation (Anastasio, 2015). Using a model of microglial inflammation, which has previously been shown to be the mechanism of neuroinflammation in Type 2 diabetes (Hwang et al., 2014), Anastasio (2015) used a computational approach to propose that a combination of glimepiride, ibuprofen and low doses of nicotine would provide optimal and long-lasting protective effect against inflammation (Anastasio, 2015).

Our goal in this study was to validate our water maze apparatus, by training rats to find a hidden platform across several days of acquisition, and then testing memory for the platform location using a probe trial, in which the platform is removed, and rats are allowed to search for the platform for 30 seconds. We expected that rats would show acquisition of the platform location across several days of training, and would concentrate their search during the probe trial on the location at which the hidden platform was placed during training.

Our second goal in the study was to replicate a previous study (Datusalia and Sharma, 2014), which found that rats on a high fat diet given streptozotocin (to model Type 2 diabetes) showed impaired MWM acquisition and probe trial performance compared to rats maintained on a normal pellet diet. We also attempted to extend this research finding, by testing the efficacy of a drug combination (including glimepiride, ibuprofen, and nicotine) suggested by Anastasio (2015) to slow or reverse the MWM impairments expected in diabetic rats. We predicted that rats given the high fat diet + streptozotocin would show impairments in acquisition and probe trial performance on the MWM, and that these impairments would be reduced with anti-inflammatory drug treatment.

MATERIALS AND METHODS

Animals
Forty-two male Sprague Dawley rats (100-124 g of initial body weight) were obtained from Envigo (Indianapolis, IN). Rats were housed in pairs or groups of three, and placed on a 12 hour light/dark cycle. All testing was conducted during the light phase. Twenty-two rats were placed on a high fat diet (60% kcal from fat, D12492 Research Diets, New Brunswick, NJ) ad libitum, while the other rats were placed on a normal pellet diet (Teklad 8604, Envigo, Indianapolis, IN) ad libitum. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Wabash College.

STZ and Induction of Diabetes
Three weeks after being placed on the high-fat diet (HFD), rats were given a low dose treatment of streptozotocin (STZ, 35 mg/kg body weight, i.p.), dissolved in citrate buffer (pH 4.5) or vehicle (citrate buffer), at a volume of 1.5 mL/kg. Fifteen weeks after injection, fasting blood glucose levels were assayed using standard blood glucose meters for confirmation of diabetes. Blood was collected 15 mm from end of the tail, after making a shallow diagonal incision using a scalpel. The HFD + STZ treatment was judged to successfully induce diabetes if the fasting blood glucose levels were significantly higher in the HFD + STZ group compared to the NPD group (Furman, 2001). Rats with blood glucose of ≥150 mg/dl were classified as diabetic (Furman, 2001), but all rats carried on with the study regardless of blood glucose levels. All rats were screened for cognitive impairment 21 weeks after the start of their respective diets (Datusalia and Sharma, 2014).

Neuroinflammatory Treatment
On the 19th week after the start of their respective diets, rats were placed into four groups: HFD + Treatment (HFD+, N = 11), HFD + Vehicle (HFD-, N = 11), Normal Diet + Treatment (NPD+, N = 10), and Normal Diet + Vehicle (NPD-, N = 10). Treatment groups were to receive daily injections of an anti-inflammatory cocktail for the duration of the experiment. The anti-inflammatory treatment was comprised of glimepiride (0.09 mg/kg), nicotine (2.5 mg/kg), and ibuprofen (60 mg/kg) dissolved in 5:1 phosphate buffer to methanol solution. Nicotine was removed after initial injection due to adverse effects from the rat injected. After three days of injections were administered, five rats in the treatment group died (three from the HFD+ group, and two from the NPD+ group), and all injections were discontinued
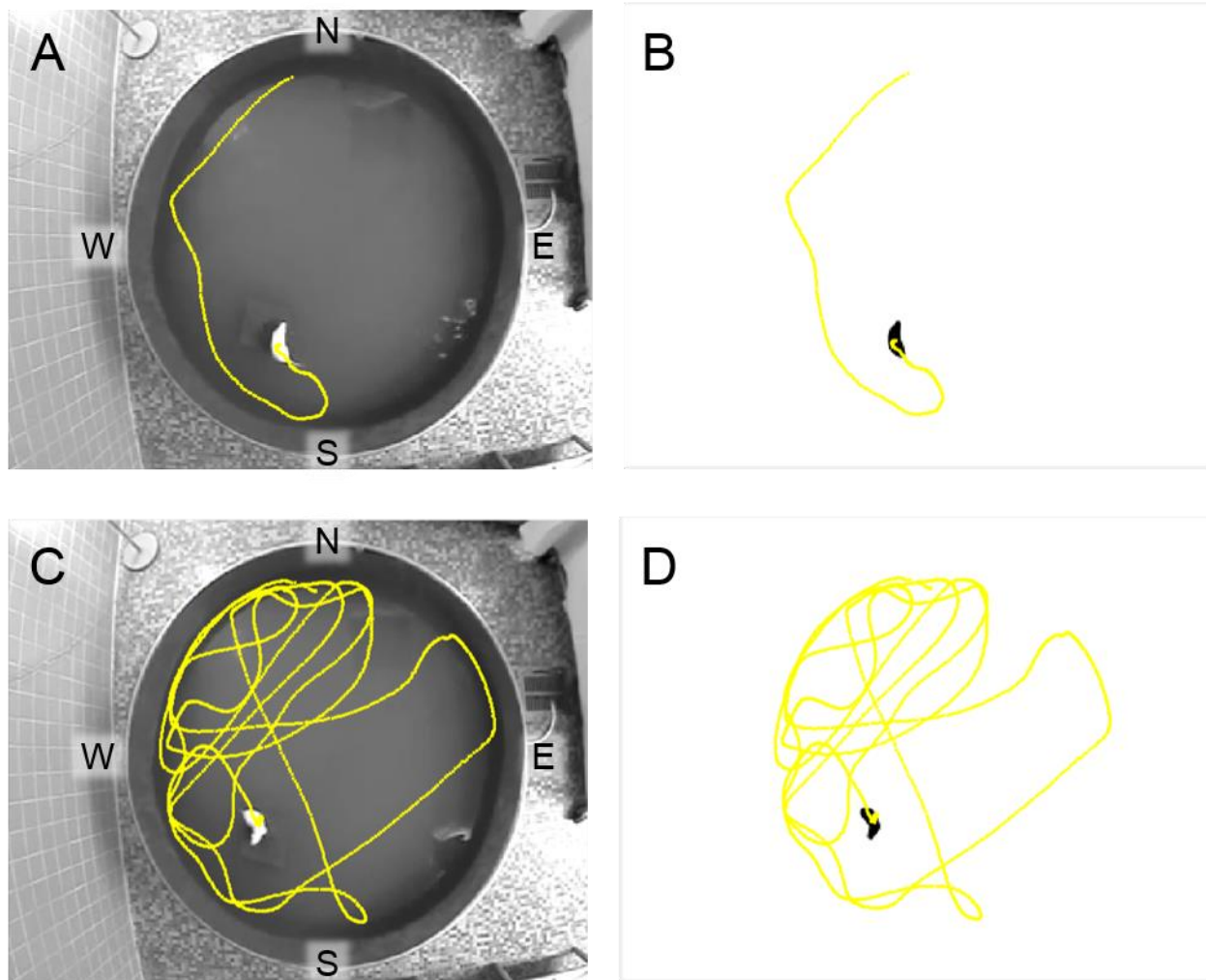
Figure 1. Morris Water Maze and position tracking examples. Separate trials and rats are shown in *A-B*, and in *C-D*. *A, C*: Frames from the training video, taken as the rat reached the hidden platform. Relative to the camera's orientation, up was arbitrarily designated as "North" (see N, W, S, E labels in *A, C*). *B, D*: Example of the subtraction image, after removing the background, smoothing the resulting image, and applying a threshold. Black pixels indicate the position of the rat. Overlaid on each image (*A-D*) is the path of the rat (yellow dots) taken from the starting location (North) to the hidden platform (SW quadrant). In *A-B*, the rat reached the platform in 8.5 seconds, travelling 326 pixels. In *C-D*, the rat reached the platform in 66.6 seconds, travelling 2,434 pixels.

at this point. While the anti-inflammatory treatment was discontinued early, and before training on the MWM, we included the treatment condition as a between-subjects factor in our analyses below, to determine if the acute treatment with the anti-inflammatory cocktail affected performance on MWM. Final group sizes for each condition were: HFD + Treatment (HFD+, N = 8), HFD + Vehicle (HFD-, N = 11), Normal Diet + Treatment (NPD+, N = 8), and Normal Diet + Vehicle (NPD-, N = 10).

Apparatus
Water maze. A list of materials used to construct the maze and platform is available as Supplementary Material. A galvanized circular stock tank (1.83 m x 0.61 m, Tartar, Dunnville, KY, http://tarterusa.com) was used as the water maze (see Figure 1). A spigot was first attached to the galvanized tank, along with a short segment of garden hose,

to allow the tank to be easily drained. In general, we would recommend using a room for the MWM that is a minimum of 4 meters square (so that experimenters can easily pass around each side of the pool), with easy access to a sink.

We positioned the tank in a larger room, equipped with a floor drain which we used to empty the tank: if no drain had been available that was below the tank, an additional pump would have been required, to drain the tank into a sink in the lab. The tank was filled by connecting a short length of garden hose to a faucet in the lab space. The tank was filled at least 24 hours before training, to allow the water in the tank to reach room temperature. To hide the position of the platform, tempura paint was added to the water, using combinations of blue and white, adjusted until the platform was difficult for humans to see, which is generally sufficient to hide the platform from rats (Morris, 1981).

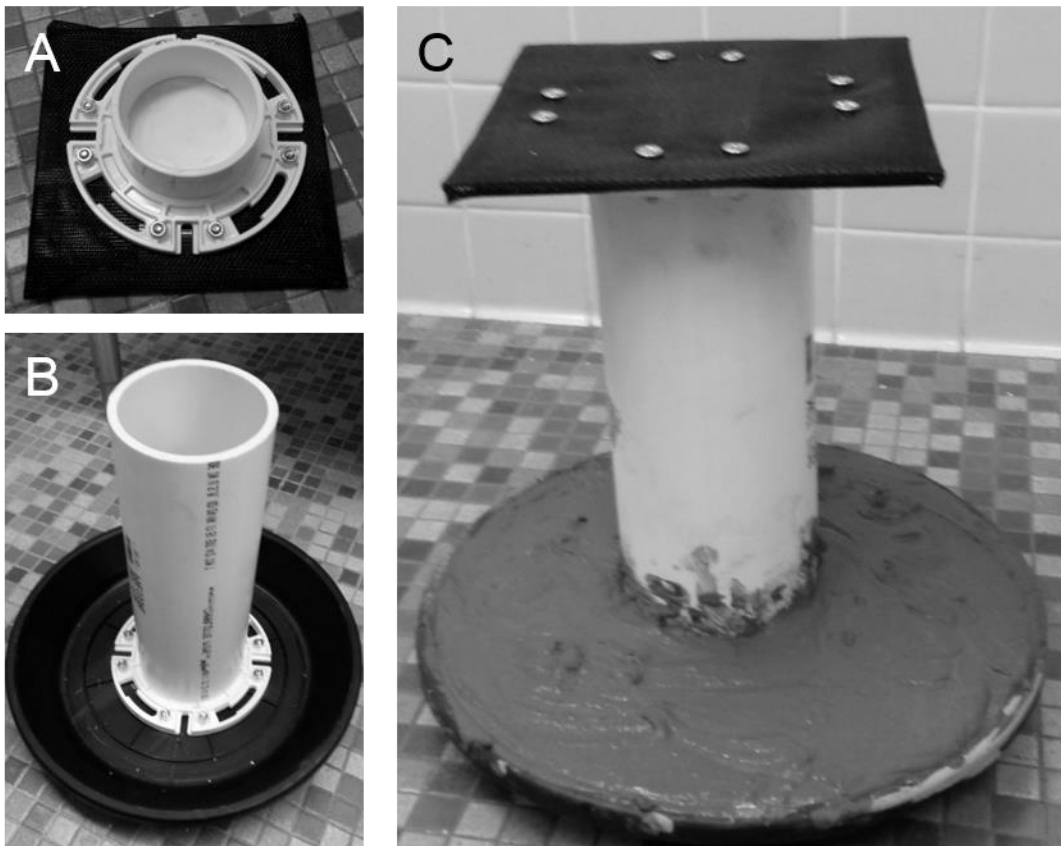Galvanized stock tanks typically have one or more

Figure 2. Construction of the water maze platform. *A:* Underside of the hidden platform. *B:* Construction of the base for the hidden platform. *C:* Assembled platform, with concrete added to the plastic saucer forming the bottom of the base. The platform was painted blue before use.

prominent seams running vertically down the wall of the task, where the metal forming the tank has been welded. These seams can serve as prominent landmarks, which we did not want rats to be able to use for navigation. To create a set of walls to hide the tank seams, four wall panels (61cm x 244cm) were created by cutting two larger plastic wall panels (Plas-Tex R Waterproof Wall Panel, Bright White, 8 feet by 4 feet by 0.060 feet, Parkland Plastics, Middlebury, IN). The bottom of the galvanized tank and one side of each panel was painted blue with spray paint (Painter's Touch 2X Ultra cover – Brilliant Blue, Rust-oleum Corporation, Vernon Hills, IL) in a well ventilated area, and allowed to dry for at least two days.

Adhesive velcro strips were attached to each wall panel, and to the top rim of the galvanized tank, and between the wall panels where they overlapped, to allow wall panels to be secured in place during training, but still be easy to remove. This design introduced a visual landmark to the maze (formed by the vertical seam between the wall panels), and so the four seams were arranged to fall on the cardinal axes of the pools (directions arbitrarily assigned N, S, E and W), so as to not fall directly behind the hidden platform location.

Platform. The hidden platform was constructed from a square polycarbonate sheet (Lexan, 11 inch x 14 inch x 0.093 inch, SABIC Innovative Plastics, Pittsfield, MA), cut to 21.6 cm by 21.6 cm. The platform was wrapped in a black mesh screen (Pet Resistant Charcoal Screen, Adfors,

Grand Island, NY) to allow rats to find purchase when climbing out of the pool and attached to a plumbing flange (3 inch Hub x 4 inch Inside, Total Knockout Closet Flange, Sioux Chief, Peculiar, MO) using eight bolts (see Figure 2A). This platform could then be attached to the base, which was a short piece of plastic plumbing pipe, which was glued into a second flange with all-purpose PVC cement. The bottom flange was bolted to a plastic planting saucer (Newbury 14 inch Black Poly Saucer, Southern Patio, http://www.southernpatio.com) to form the bottom of the base of the platform (see Figure 2B). The saucer was then filled with cement (Vinyl Concrete Patcher, Quikrete, Atlanta, GA) and allowed to dry (see Figure 2C).   The platform was not glued to the base, so that it could be removed, and different versions of the platform (such as round platforms, or platforms of different sizes, to vary the difficulty of the maze, Vorhees and Williams, 2006) could be used with the same base. Between trials, the platform could be moved manually, or removed (for probe trials). Final weight of the platform was approximately 9kg. Water was added to the pool until the top of the hidden platform was covered by approximately 2 cm of water.

Behavioral Task
Rats were trained to find a hidden platform using the apparatus described above. Training was modeled on the procedure used by Datusalia and Sharma (2014), and using recommendations made by Vorhees and Williams (2006).

For five consecutive days, rats completed five training trials per day, in which they were allowed a maximum of 120 seconds to find the hidden platform (Datusalia and Sharma, 2014). If a rat failed to find the platform within 120 seconds (which happened on 12 trials in the first day of training), the animal was removed from the water and placed on the hidden platform for approximately 15 seconds. Rats were trained in groups of 8-10 animals, and each rat was placed into an individual glass box lined with towels during the intertrial interval (ITI), which was an average of 9.7 minutes (interquartile range: 8.4 to 11 minutes) from the time the rat reached the platform to the start of the rat's next trial. In some cases (32 trials, predominantly on the first day of training), after initially reaching the platform, rats would jump off the platform and continue swimming. In these cases, latency and distance were calculated using the first time that rats reached the platform, but the trial was allowed to continue until rats returned to the platform, or until 120 seconds had elapsed.

The order of the starting locations (using the North, East, Northwest, and Southeast starting locations) were presented in the same pseudorandom order for each rat. During these trial runs, the rat was placed in the water facing the wall in one quadrant of the pool.

Position of the rat was recorded using an overhead camera (Panasonic WV-BP330, black and white CCTV camera, fitted with a Computar camera lens [3.5-8mm 1 1.4 1/3" CS mount]). Video from each session was recorded to DVD, to be analyzed offline with custom rat tracking software (ratTracker4Matlab, described below) to measure the time and distance traveled to the platform location for each trial. After five days of hidden platform training (in which the hidden platform was placed in the Southwest quadrant), a probe trial was conducted by removing the platform from the pool and recording the rat's swim path for 30 seconds from a novel starting location (Northeast). Performance on the probe trial was assessed by the time spent in the target quadrant, distance travelled in the target quadrant, distance travelled before reaching the original platform location and the number of times they crossed the platform area during the 30 second trial. Behavioral trials were completed on the 21st week after starting the diet, and 18 weeks after the streptozotocin injection (Datusalia and Sharma, 2014).

Video processing
Position tracking was done offline, using the finalized DVDs from each session. First, each session was converted to an .mp4 file using HandBrake (https://handbrake.fr/). Tracking was then done for each session using custom position tracking software (ratTracker4Matlab.m, N. C. Schmitzer-Torbert, 2017, available at http://virtualnavigation tools.com/rattracker4matlab/), written in Matlab. The ratTracker4Matlab.m program is designed to track a single small target (such as a rat) against a static background. After loading a video, users identify the location of the rat at the start of each trial, and identify the end of each trial. During each trial, the position of the target is determined by subtracting the current frame from a reference frame (and the program assumes that there is a static background). All of the pixels whose absolute value exceeds a threshold (set by the user), and fall within a maximum radius (set by the user) from the last calculated position of the target are used to calculate the updated position, by simply taking the average in the x- and y-dimension. Examples of position tracking from a hidden platform training session are shown in Figure 1.

Statistical Analysis
Differences in weight between NPD and HFD groups were determined with a *t*-test. Differences between the various treatment groups were analyzed with a 2-Way ANOVA for all behavioral measures during training and the probe trial. Statistical tests with a $p < 0.05$ were considered statistically significant for this study.

RESULTS

The data set used for the analyses described below is available online (https://osf.io/z9fym/files/).

Fifteen weeks after the injection of streptozotocin (STZ) injection, the High Fat Diet (HFD) group had significantly higher blood glucose measurements (M = 273 [SD = 159] mg/dL) than the Normal Pellet Diet (NPD) group (M = 95 [8.5] mg/dL, $t(35)= 5.001$, $p < 0.001$). Only one rat in the HFD group had a blood glucose measurement (107 mg/dL) that was within the range of blood glucose measurements of the NPD group (82-108 mg/dL). A total of 14 of 19 (74%) HFD rats were classified as diabetic (glucose $\geq$ 150 mg/dL). In analyses below, all rats are included, but the pattern of the results is not changed by excluding rats in the HFD group with fasting blood glucose measurements < 150 mg/dL.

Weights of the rats at the time of the streptozotocin injection (week 3, relative to the start of their diets) and on the day of glucose (week 18, relative to the start of the diets) were also examined using a two-factor ANOVA with Time (week 3, week 18) as a within-subjects factor and Diet (NPD, HFD) as a between subjects factor. The main effect of Diet was significant (F(1, 35) = 18.8, p < 0.001, $\eta^2 = 0.32$), with rats in the HFD group weighing significantly more than rats in the NPD group. There was also a significant increase in weight between the two measurements (Time: F(1, 35) = 236, p < 0.001, $\eta^2 = 0.87$), while the interaction of Time × Diet approached significance (F(1, 35) = 4.0, p = 0.053, $\eta^2 = 0.10$). Overall, these results indicate that rats in the HFD group were heavier than the rats in the NPD group (in week 3, HFD: M = 286g, SD = 20g, NPD: M = 241g, SD = 15g), and both groups tended to show similar increases in weight after the STZ injection (mean weight change: HFD = +136.8g, SD = 69.4g, NPD = +152.6g, SD = 30.0g) though there was a trend for rats in the NPD group to gain more weight between weeks 3 and 18. It was also observed that weight at week 3 (STZ injection) was positively correlated with week 18 fasting blood glucose levels (r(35) = 0.66, p < 0.001), while weight at week 18 (when blood glucose was collected) was negatively correlated with blood glucose ($r(35)$ = -0.37, p =0.024). Rats with the highest fasting blood glucose levels in week 18 were those with high weights at the time of STZ injection, and which gained less weight over
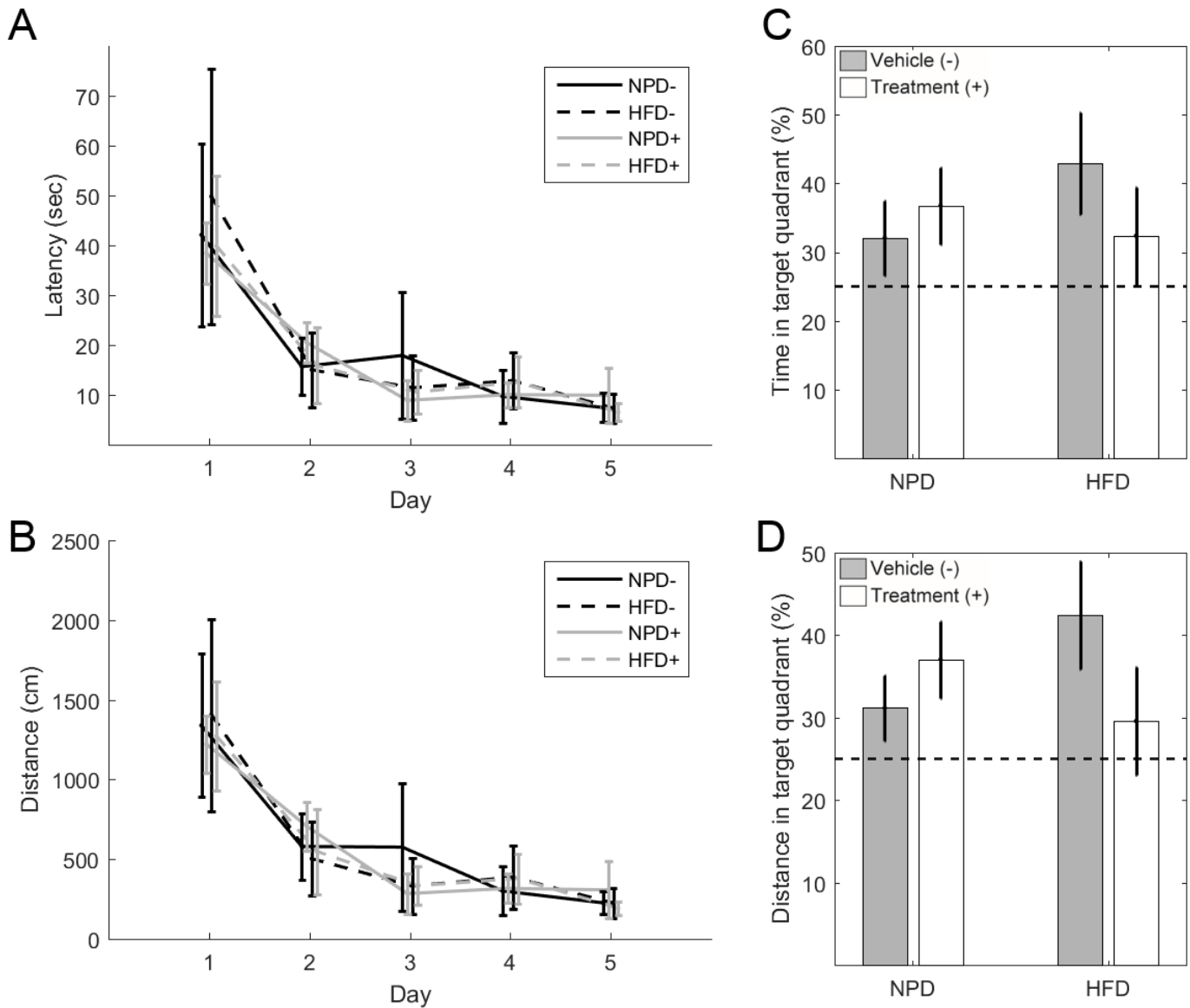
Figure 3. Behavioral performance during water maze training and probe trial. *A:* Latency to reach the hidden platform during training. *B:* Distance travelled to the hidden platform during training. During training, latency and distance travelled to the hidden platform improved across days, at similar rates for all groups. *C:* Percent of time spent in the target (SW) quadrant during the probe trial. *D:* Percent of distance travelled in the target (SW) quadrant during the probe trial. On the probe trial, the HFD- spent more time and travelled farther in the target quadrant than the HFD+ and NPD- groups, while the NPD+ group did not differ significantly from the HFD+ or NPD- groups. NPD: normal pellet diet, HFD: high fat diet, +/-: treatment/vehicle. Dashed line in *C-D* indicates chance performance (spending equal percentages of time/travelling equal amounts of distance in each quadrant).

the 15 weeks after STZ injections. Note: due to a record-keeping error, initial weights at the start of treatment for each rat were lost, but the two groups (HFD and NPD) did not differ at the start of their respective diets.

Training Data
Across the five days of hidden platform water maze training, each group of rats learned the location of the hidden platform at a similar rate (see Figure 3A-B). Latency and

distance travelled to reach the hidden platform were analyzed using an ANOVA with Day (1-5) as a within-subjects factor, and Diet (normal vs high-fat) and Injection (anti-inflammatory vs vehicle) as between-subjects factors. While analysis revealed a main effect of Day over the training period (Latency: $F(4, 132) = 55.9$, $p < 0.001$, $\hat{\eta}^2 = 0.63$, Distance: $F(4, 132) = 70.8$, $p < 0.001$, $\hat{\eta}^2 = 0.68$), there were no significant main effects or interactions (All Fs < 1, n.s.).
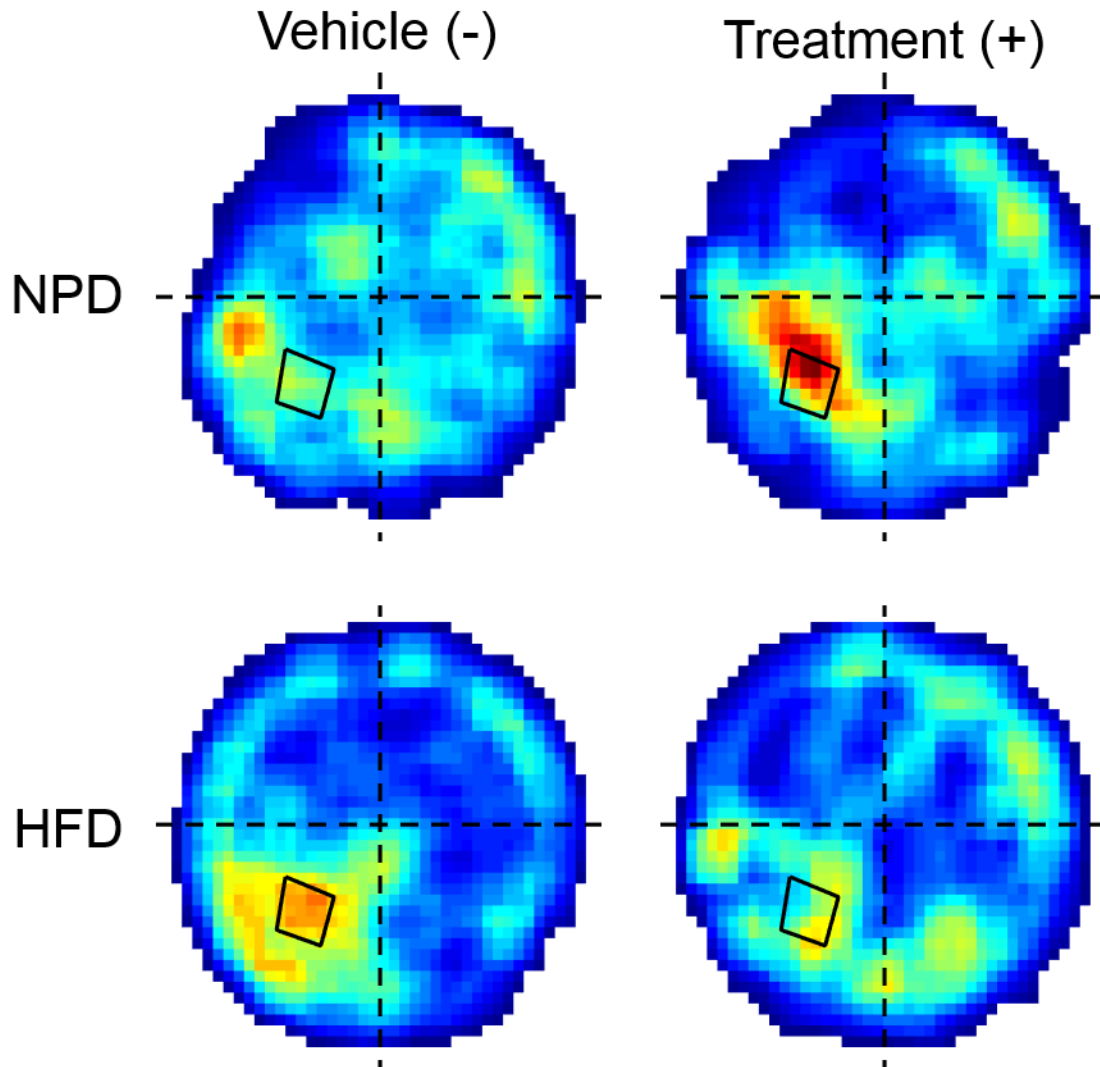
Figure 4. Search behavior during the water maze probe trial. Blue to red indicates low to high occupancy during the probe trial. Solid black line: outline of the hidden platform. The platform does not appear to be square due to wide-angle distortion by the camera. Dashed lines: boundaries between the quadrants. Each plot uses the same color scale. Bin size = 5 cm, raw occupancy maps were smoothed by convolution with a 25 cm x 25 cm uniform filter. The NPD+ and HFD- groups focused more of their search behavior in the target quadrant (SW), though all groups searched more in SW than in other quadrants. NPD: normal pellet diet, HFD: high fat diet, +/-: treatment/vehicle.

Probe Trial Performance

After five days of training, a single probe trial was conducted on Day 6, in which the platform was removed and each rat was allowed to swim for 30 seconds after being released from a novel starting location. The percent of time spent (Figure 3C) and distance travelled (Figure 3D) in the target quadrant (SW), which had contained the hidden platform, were analyzed using two separate two-way ANOVAs, with Diet (HFD, NPD) and Injection (Vehicle, Treatment) as between-subjects factors. There were no significant main effects of Diet or Injection (All Fs < 1.3, all ps > 0.28, all $\hat{\eta}^2$ < 0.036, except that the main effect of Diet for Distance was F(1,33) = 3.1, p = 0.088, η^2 = 0.086), due to a non-significant trend for the HFD rats to travel farther in the target quadrant than the NPD rats.

There was, however, a Diet × Injection interaction (Latency: F(1)=6.9, p = 0.012, η^2= .17, Distance: F(1) = 10.9, p = 0.002, $\hat{\eta}^2$ = 0.25) for both measures. Post-hoc comparisons (α = 0.05) revealed that the HFD- group performed significantly better than the NPD- and HFD+ group on percent time spent in the target quadrant and the distance travelled in the target quadrant. Occupancy maps (Figure 4) showing search behavior on the probe trial indicated that the HFD- and NPD+ rats focused more around the platform location than any other group. Overall, these data indicate that for the vehicle-treated rats, those on the high-fat diet + STZ treatment performed better on the probe trial compared to rats on the control diet, rather than the impairment which was expected among diabetic rats on the MWM probe trial.

DISCUSSION

Using a water maze built from commonly available materials, our results with rats on the control diet (fed standard laboratory pellets) replicate those of Datusalia and Sharma (2014), demonstrating similar acquisition rates and probe trial performance in rats trained to find a hidden platform. These data validate our version of the MWM and support the use a low-cost (less than $400 USD) option for undergraduate research.

We were also unable to replicate a finding by Datusalia and Sharma (2014) that diabetic rats were impaired in MWM acquisition and probe trial performance. Overall, rats in the diabetic group receiving vehicle treatment outperformed other groups on the probe trial, which is inconsistent with previous research showing a cognitive deficit in diabetic rats. While we failed to replicate the MWM impairments expected for diabetic rats, it is possible that differences in our apparatus or training may account for the discrepancy between our results and those of Datusalia and Sharma (2014). However, our results do fit to a degree with a larger pattern in which the cognitive deficits associated with a high fat diet (and not necessarily diabetes) assessed using the MWM are variable. Among five studies that have used the MWM to assess cognitive deficits produced by a high fat diet or the diabetic model, deficits in acquisition are typically produced by the obesity and diabetes (Farr et al., 2008; Datusalia and Sharma, 2014; Sharma et al., 2015; Xu et al., 2015; Datusalia and Sharma, 2016), but one report, by Farr and colleagues (2008), failed to show deficits on the probe trial even after showing impairments in MWM acquisition in obese mice. Thus, our failure to replicated earlier results on the effect of diabetes on MWM performance may be due to variability between studies.

We also attempted to test the efficacy of a drug combination of glimepiride, ibuprofen, and nicotine to reduce inflammation and to reduce cognitive deficits associated with the diabetic model. However, our anti-inflammatory treatment produced acute toxicity, forcing us to discontinue treatment after several injections. Rats receiving acute anti-inflammatory treatment did not show any differences in acquisition or probe trial performance compared to rats fed a normal pellet diet, and among diabetic rats, those given the acute anti-inflammatory treatment showed worse probe trial performance than those given vehicle treatment. Whether these differences in probe trial performance are due to effects of the acute anti-inflammatory treatment on MWM performance, or a selection bias (in which rats that remained in the treatment group differed from those that died in their potential to learn the MWM) are unclear, but any effects of the treatment should be interpreted with caution. While we were not able to replicate the effect of diabetes in this rat model on MWM performance, we do believe that our results in our control group (the rats fed a normal pellet diet) support the use of our water maze for undergraduate research projects on learning and memory. For such studies, especially those of hippocampal function, the Morris Water Maze is a standard technique for assessing learning and memory. As such, access to a water maze is an important issue for undergraduate neuroscience labs, but cost of commercial water mazes may be prohibitive for some undergraduate labs. While commercial water mazes offer high quality options for the MWM (using pools that have no seams, options for platforms that can be automatically lowered and raised, and which are standardized across different laboratories using the same vendor), at the time we conducted our study we were unable to find a commercial vendor to supply a water maze for a cost that was reasonable for our project. For example, one commercial option that we explored would have cost more than $1,300 USD for a pool and platform, without shipping, and more than $5,000 USD for a basic package including a stand for the pool and an insert for the floor of the water maze pool. These costs seemed reasonable to us, but exceeded our budget for a single project (motivated by student interest) in a lab that did not expect to continue to use the water maze in the future. Our water maze was fashioned from readily available materials, and cost us approximately $400 USD to complete (not including costs for our video tracking solution).

For our video tracking setup, the camera and lens used in our lab no longer appear to be available, but comparable options would likely cost several hundred dollars. For recording video, we used a VCR/DVD reorder (Sanyo, http://sanyo-av.com), which also does not appear to be easily available today. But, any solution (such as a DVR) which allows for recording video which can be converted into a digital format (.mp4, .mpeg, .avi, etc.) would be compatible with the ratTracker4Matlab program. One simple solution would be to use a digital camcorder, mounted over the water maze, and connected to a television to serve as a monitor, or even a webcam with a high resolution. The tracking program that we used (ratTracker4Matlab) is available for free, but does require a Matlab license (approximately $500 for an academic license) to run.

Studies of learning and memory offer excellent opportunities for undergraduates who are interested in neuroscience, ranging from basic studies of how memory operates to clinical studies relevant to human disorders and pathology. We hope that our description of a low-cost version of the Morris Water Maze helps to make standard technique for assessing learning and memory accessible to a wider range of undergraduate researchers.

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