TRAINING-INDUCED ELEVATIONS IN EXTRACELLULAR LACTATE IN
HIPPOCAMPUS AND STRIATUM: DISSOCIATIONS BY COGNITIVE STRATEGY AND
TYPE OF REWARD

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ABSTRACT

Recent evidence suggests that astrocytes convert glucose to lactate, which is released from the astrocytes and supports learning and memory. This report takes a multiple memory perspective to test the role of astrocytes in cognition using real-time lactate measurements during learning and memory. Extracellular lactate levels in the hippocampus or striatum were determined with lactate biosensors while rats were learning place (hippocampus-sensitive) or response (striatum-sensitive) versions of T-mazes. In the first experiment, rats were trained on the place and response tasks to locate a food reward. Extracellular lactate levels in the hippocampus increased beyond those of feeding controls during place training but not during response training. However, striatal lactate levels did not increase beyond those of controls when rats were trained on either the place or the response version of the maze. Because food ingestion itself increased blood glucose and brain lactate levels, the contribution of feeding may have confounded the brain lactate measures. Therefore, we conducted a second similar experiment using water as the reward. A very different pattern of lactate responses to training emerged when water was used as the task reward. First, provision of water itself did not result in large increases in either brain or blood lactate levels. Moreover, extracellular lactate levels increased in the striatum during response but not place learning, whereas extracellular lactate levels in the hippocampus did not differ across tasks. The findings from the two experiments suggest that the relative engagement of the hippocampus and striatum dissociates not only by task but also by reward type. The divergent lactate responses of the hippocampus and striatum in place

and response tasks under different reward conditions may reflect ethological constraints tied to foraging for food and water.

KEYWORDS:

Multiple memory systems; place learning; response learning; lactate; astrocytes

1. INTRODUCTION

Neuroendocrine responses to an experience can regulate brain processes involved in learning and remembering that experience (Gold, 2014; Gold and Korol, 2014). In particular, release of the hormone epinephrine into blood from the adrenal medulla enhances learning and memory across many tasks and species (Gold, 1995; Gold and Korol, 2012). Although **circulating epinephrine does not readily cross the blood-brain barrier to enter the brain (Axelrod et al., 1959), the hormone's peripheral** actions, largely at the liver, increase blood glucose levels. The increase in blood glucose levels is both necessary and sufficient for the enhancement of learning and memory by epinephrine (Gold, 2014; Gold and Korol, 2014). Glucose itself enhances learning and memory when administered by systemic administration or by direct brain injections (Gold, 2001; Gold and Korol, 2012; Korol, 2002; Korol and Gold, 2007; Messier, 2004; Messier et al., 1999; Smith et al., 2011; Morris and Gold, 2013; van der Zwaluw et al., 2015).

Of particular interest here, brain lactate may function downstream from glucose to modulate learning and memory. According to this view, glucose enters astrocytes where it can be converted to lactate, which is subsequently used under conditions of heightened activation such as during cognitive processing (Newman et al., 2011). Like glucose, direct intrahippocampal injections of lactate enhance working memory (Newman et al., 2011) and memory for inhibitory avoidance training (Suzuki et al., 2011). Interfering with lactate transport into neurons by pharmacological or gene expression manipulations impairs memory and attenuates the ability of lactate or glucose to enhance memory (Newman et al., 2011; Suzuki et al., 2011), suggesting that

glucose may enhance memory by conversion to lactate in astrocytes for delivery to neurons.

Past findings indicate that extracellular glucose levels in the hippocampus are diminished by spatial working memory testing (McNay et al., 2000, 2001; McNay and Gold, 2001; Newman et al., 2011), with the magnitude of reduction corresponding to the cognitive load of the task (McNay et al., 2000, 2001). The decrease in extracellular glucose levels in the hippocampus during working memory testing is mirrored by an increase in extracellular lactate levels during testing (Newman et al., 2011). The reciprocal changes in hippocampal extracellular glucose and lactate levels are consistent with the idea that lactate may serve as a supplementary energy substrate to neurons during a time of heightened energy utilization (Brown and Ransom, 2015; Magistretti et al., 1999; Pellerin, 2003; Pellerin and Magistretti, 2012). The use of lactate as an energy source is one of several roles lactate may perform to support cognitive functioning (Fryer and Brown, 2015), such as contributions to astrocytic energy needs, particularly to support glutamate and potassium clearance (Dienel and McKenna, 2014; Sonnewald, 2014), glia-neuronal signaling (Tang et al. 2014; Barros, 2013; Bergersen and Gjedde, 2012; Bozzo et al., 2013), and regulation of neurovascular coupling (Lauritzen et al., 2013; Gordon et al., 2008), that in turn may regulate delivery of energy substrates and nutrients to the brain during demanding tasks.

Extensive evidence indicates that different cognitive attributes are subserved by the activity of multiple memory systems. In particular, place (spatial) and response (habit) learning are particularly sensitive to perturbations of functions in the hippocampus and striatum, respectively (White et al., 2013; White and McDonald, 2002;

Packard and Goodman, 2013; Korol, 2004; Gold et al., 2013; Poldrack and Packard, 2003; Packard and McGaugh, 1992; Chang and Gold, 2003a, 2004; Kathirvelu and Colombo, 2013; Korol and Pisani, 2015). Support for participation of the hippocampus and striatum in these different cognitive attributes comes from demonstrations of double dissociations of task by brain area using lesions or pharmacological interference (Kosaki et al., 2015; McDonald and White, 1994; Soares et al., 2013; Dagnas et al., 2013), direct injections of glutamate (Packard, 1999), glucose (Pych et al., 2006; Stefani and Gold, 2001; Canal et al., 2005), and estradiol (Korol and Pisani, 2015; Zurkovsky et al., 2007, 2011) in these brain areas.

Several neurochemical and neurophysiological measures of activity in the hippocampus and striatum also exhibit task-specific differences (Pych et al., 2005; Gold, 2004; Mizumori and Jo, 2013; Colombo, 2004; McIntyre et al., 2003; Chang and Gold, 2003b; Pleil et al., 2011; Rubio et al., 2012). In particular, contrasting the response in the hippocampus, extracellular glucose in the striatum does not decline, and may actually rise, during working memory testing (McNay et al., 2001). Thus, the striatum may have metabolic requirements and responses to experiences that differ from those in the hippocampus. Regional differences in the glucose response to memory testing may also reflect the varying contributions of different brain areas to different types of cognition. These regional differences in physiological responses to experience together with the important role of lactate provisions from astrocytes in modulating hippocampus-sensitive learning and memory (Newman et al., 2011; Suzuki et al., 2011), suggest that, when compared to the hippocampus, the striatum may demonstrate a very different pattern of lactate responses to training on tasks that have selective cognitive attributes.

To test the task and regional specificity of metabolic responses to learning, the present experiments examined fluxes in extracellular lactate levels in the hippocampus and striatum while rats were trained on place and response versions of mazes designed to tap the function of each of these brain regions. The first experiment measured extracellular lactate concentrations in the hippocampus and striatum while rats learned to find food in mazes that rely on those neural systems. Because food intake *per se* increased lactate levels in the brain, perhaps obscuring training-related changes, we also examined extracellular lactate concentrations in a parallel second experiment in which rats were trained using water as the reward to solve the same mazes. The lactate responses to training in hippocampus and striatum dissociated not only by learning strategy, but, unexpectedly, also by the reward used during training.

2. METHODS

All procedures described in this report were approved by the University of Illinois Urbana-Champaign and the Syracuse University Institutional Animal Care and Use Committees, and were consistent with the Guide for Care and Use of Laboratory Animals. The animal facilities at both universities are accredited by the Association for Assessment and Accreditation of Laboratory Care.

2.1. Experiment 1: Hippocampal and striatal lactate responses to maze training with a food reward

This experiment monitored lactate levels throughout training on place and response versions of a food-motivated plus-shaped maze. These versions of the maze are sensitive to hippocampus and striatum manipulations, respectively (Chang and

Gold, 2003a, 2004; [Korol et al., 2013](#); Zurkovsky et al., 2006, 2007, 2011).

2.1.1 Experimental Design

Three-month-old male Sprague-Dawley rats (Harlan Laboratories) were housed in individual cages and were maintained on a 12:12 hr light-dark cycle. The rats had free access to food and water until food restriction procedures were started. All rats received unilateral implantation of guide cannulae for lactate biosensor probes (Pinnacle Technology Inc., Lawrence, KS) under stereotaxic control and were allowed at least one week to recover after surgery. Seven days before training, rats were placed on a food restriction regimen, which reduced body weights and maintained the rats' weights at 80-85% of baseline. During food restriction, rats received a small allotment of food reward (Frosted Cheerios®) in their home cages for several days prior to training to familiarize the rats with the reward used during training. Rats were handled for 3 minutes each day for 5 consecutive days prior to training.

Rats with lactate biosensor probes placed into the dorsal hippocampus or dorsolateral striatum were trained on either a place or response learning task. Extracellular hippocampal and striatal lactate levels were also obtained in a separate control group of food-restricted rats that were not trained but received the food reward every 90 sec while kept in a holding cage, i.e. on a schedule that approximated the reward schedule of trained rats. This feeding group controlled for eating- vs. training-related effects on brain neurochemical measures. Thus, there were six experimental groups: hippocampus-place (N=5), hippocampus-response (N=5), hippocampus-untrained-fed (N=4), striatum-place (N=5), striatum-response (N=5), striatum-untrained-

fed (N=5), reflecting a 2 (brain site) X 3 (training conditions) experimental design.

2.1.2. Implantation of bioprobe guide cannulae

Rats were anesthetized with isoflurane for surgery. A guide cannula was stereotaxically implanted unilaterally into the dorsal hippocampus (coordinates in mm: -3.8 AP, 2.5 ML, 0.9 ventral to dura) or dorsolateral striatum (coordinates in mm: AP $\pm$ 0.2, ML $\pm$ 2.0, -1.5 mm ventral to dura) of each rat (Paxinos and Watson, 2006). For striatum, the cannula was positioned at a 15° angle with the ventral tip away from midline to avoid contact with the headstage. The guide cannula was affixed to the skull using four screws as anchors for dental cement. The housing for the headstage (Pinnacle Technology, Inc.), which holds a potentiostat, circuitry and battery for measuring lactate via wireless telemetry, was cemented around the guide cannula.

2.1.3. Behavioral training

To avoid the need for repeated insertions of the biosensors into the brain, all training was conducted with a single session. To acclimate rats to the training environment, rats were placed in a clean holding cage in the testing room with limited access to visual cues for 15 min prior to maze training. Rats were trained within one session on a single day to find food in a plus-shaped 4-arm maze, configured into a T, with the stem serving as the start arm and the other two arms as the choice arms. The arms of the maze (45 cm long x 14 cm wide x 7.5 cm wall height) radiated from a square center area of 14 x 14 cm. The reward was placed at the end of the goal arm in a perforated food receptacle affixed to the floor of the maze. Rats were required to turn

at the choice point to find the reward in the goal arm. To eliminate the use of food odor cues to solve the maze, inaccessible food was placed under receptacles at the ends of all arms. Also to eliminate the use of odor and other intramaze cues to find food, the maze was rotated 90° between trials.

For each trial, rats were allowed a maximum of two minutes to make a choice. A choice was indicated when all four paws crossed into the choice arm. The trial ended when the rats had consumed the reward, after 10 seconds in the choice arm, or when the 2-min maximum was reached. At that time, the rat was returned to its holding cage for a 30-sec intertrial interval. Rats were trained to a criterion of 9/10 correct, including at least 6 consecutive correct choices or to a maximum of 100 trials.

For hippocampus-sensitive place training, the maze was located in the center of the testing room containing a rich assortment of extra-maze cues. For each trial, the start arm was either the north or south arm of the maze, assigned in semi-random manner, with no more than three consecutive starts from the same arm. For a given rat, food was located in either the east or west arm position, randomly assigned and counterbalanced across rats. This task is solved most effectively by learning to turn into the arm positioned in **the same particular location of the room on all trials** to find the reward.

For striatum-sensitive response training, the same T-shaped maze was surrounded by curtains to produce a cue-poor extramaze training environment. Rats were trained to turn in one direction to find food, starting from the north or south arm of the maze. As above, start arms were assigned in a semi-random manner with no more than three consecutive starts from the same arm. Turning direction, either right or left,

was counterbalanced across rats. In contrast to place training, the response task is solved most effectively by learning to use the same body turn, either right or left, on all trials, to find the reward. The reward on both mazes was 1/3 of a Frosted Cheerio®.

A final group of rats, the untrained-fed control group, was given 1/3 of a Frosted Cheerio® every 90 seconds for 40 minutes. This was done to assess any changes in brain lactate values as a result of eating Frosted Cheerios® on the maze in both the place and response conditions.

2.1.4. Biosensor measurements of lactate

The biosensor probes project 3 mm beyond the end of the guide cannulae (see <http://www.pinnaclet.com/pinnacle-guide-cannulas.html> and <http://www.pinnaclet.com/lactate.html> for more information). The probes end at an inactive epoxy tip of 0.2 mm. Above the tip is a 1 mm sensing cavity (176 µm diameter) housing a Pt-Ir sensing electrode coated with lactate oxidase that lies below a reference electrode. A voltage (+0.6 V) is applied to the sensing electrode from a potentiostat in the headstage. The oxidation of lactate leads to the production of H₂O₂ and pyruvate. Further oxidation of H₂O₂ at the Pt-Ir wire produces a current measured between the sensing and reference electrodes. Readings of the current were transmitted wirelessly to a computer that recorded values in 1-sec bins using Pinnacle Technology Laboratory v. 1.6.7 software. The biosensors for lactate have a range of 0 - 8 mM with an *in vitro* sensitivity of 4.7 nA/mM (Hu and Wilson, 1997).

Probes were calibrated for lactate before and after training by placement in phosphate-buffered saline with addition of 20 µM lactate increments, generating a

regression line used to calculate concentration. The probes respond specifically to lactate but are also sensitive to ascorbate. To preclude interference from ascorbate with lactate measures, the probes are coated with a membrane containing ascorbate oxidase to metabolize ascorbate before it can reach the biosensor. Sufficiency of this membrane was confirmed twice, after training and after calibration for lactate. On these tests, 250 μ M of ascorbic acid was added to a beaker containing phosphate-buffered saline to ensure no response was generated. One rat was excluded because of poor probe performance.

The biosensors were inserted via the guide cannulae one day before behavioral testing while the rat was under brief isoflurane anesthesia. At this time the biosensors were connected to the potentiostat and the top of the headstage was put in place. Lactate measures during the 5-min period before testing were averaged and used as baselines for each rat. Collection of lactate measures continued through 40 minutes of training, a time when the fastest learners reached criterion performance. Data after that time were collected for rats until they reached criterion or completed 75 trials. The neurochemical data from time points beyond 40 minutes, not shown because of decreasing *N*s over time as rats reached criterion, did not differ substantially from the values at 40 minutes.

2.1.5. Histology

Immediately after behavioral testing, rats were overdosed with Fatal Plus (45 mg/kg) and decapitated, and brains removed for histological evaluation of biosensor placements. The hemisphere of the brain that contained the biosensor was placed into

phosphate-buffered formalin for at least 48 hours followed by 20% glycerol for at least 2 days until ready for sectioning. Sections (40 μm) were collected through the hippocampus or striatum, mounted on gel-subbed slides, and stained with cresyl violet prior to light microscopic determination of cannula location.

2.1.6. Data analysis and statistics

The number of trials to reach a criterion of 9/10 correct choices including at least 6 consecutive correct choices was used as a measure of learning. This measure of learning was compared across tasks (place vs. response) with a *t*-test. For lactate, baseline values were calculated from the regression equation generated by the standard curve. Change in concentration (μM) from baseline was plotted against time to generate lactate response curves for graphical purposes. A single lactate response value for each rat was determined by calculating the average change from baseline in the 10-minute epoch from 15-25 minutes, a time when values plateaued in trained rats.

Baseline lactate levels were each compared across tasks using *t*-tests. Two-way ANOVAs of lactate responses, averaged from 15-25 minutes after the start of training, were conducted for brain structure (hippocampus, striatum), training task (place, response) and their interactions. One-way ANOVAs were applied to the lactate values to identify effects across the three training conditions (untrained, response, place) within brain structure.

Two additional main questions drove paired statistical comparisons: 1) whether the changes in lactate during training were significantly different from the lactate responses to reward alone and 2) whether extracellular lactate within a brain region

differed during place versus response training. Finally, we used paired *t*-tests to determine whether lactate responses were significantly different from baseline responses within each group. Alpha = 0.05 for all tests.

2.2. Experiment 2: Hippocampal and striatal lactate responses to maze training with a water reward

General methods were as described in Experiment 1 except for the reward used during training. In this experiment, rats were placed on a water restriction schedule where they had access to water in a petri dish placed in their home cages for 1 hour per day for 1 week prior to training. Rats with hippocampal or striatal biosensor insertions were tested on either place or response learning tasks to find water. The water reward was placed in an open polystyrene petri dish (60 mm diameter, BD Falcon, Tewksbury, MA) at the end of the goal arm during training. After reaching the water, rats were allowed to drink for 2-3 seconds on each trial. Each arm contained a petri dish to avoid the use of the container as an intra-maze cue to guide learning.

As in Experiment 1, lactate biosensors were inserted through the guide cannulae into the hippocampus or striatum twenty-four hours before training. In a subset of animals, lactate recordings were taken on this day while rats received water in the petri dish in their home cages. This untrained, water-rewarded group serves as an important control for lactate responses to drinking without training on either place or response tasks (as shown in Fig. 5 and 6). Together with maze-trained rats, there were six experimental groups: hippocampus-place (N = 4), hippocampus-response (N=5), hippocampus-untrained-water (N= 5), striatum-place (N = 4), striatum-response (N = 5),

striatum-untrained-water (N = 5). All other training, neurochemical, histological, and statistical procedures were conducted as described in Experiment 1.

2.3. Experiment 3: Blood glucose and lactate responses to eating and drinking

An additional experiment examined blood glucose and lactate levels during food (Cheerios®) or water consumption. As in the untrained groups in Experiments 1 and 2, food or water was presented every 90 seconds for 50 minutes. Blood glucose and lactate levels were sampled every 10 minutes from tail nicks, with blood collected directly onto commercial glucose (OneTouch Ultra, Milpitas, CA) and lactate (Lactate Plus, Waltham, MA) strips accompanying the respective meters. This procedure allowed both glucose and lactate measures from each rat before and during provision of either food (N = 4) or water (N = 4).

Baseline blood values were compared in rats receiving different rewards using unpaired *t*-tests. Responses to eating or drinking were assessed by comparing blood glucose and lactate concentrations in baseline samples taken prior to reward with concentrations at the plateau using paired *t*-tests. Plateau values were derived from the average of samples at 20 and 30 minutes after food or water presentation. For all analyses, $\alpha = 0.05$.

3. RESULTS and DISCUSSION

3. 1. Experiment 1: Lactate responses to training for a food reward

Training-related increases in lactate levels differed across learning strategies when evaluated within brain region and differed across brain regions when evaluated within task. The results obtained with probes placed in the hippocampus are shown in Figure 1, with changes in hippocampal extracellular lactate concentrations over time before and during training in Figure 1A and the averaged values from 15-25 minutes after the start of training in Figure 1B. Hippocampal lactate levels increased substantially during learning in all three groups (place learning, response learning, untrained-food) but with a clear difference across groups based on behavioral condition ($F_{2,11} = 4.32$; $p < 0.05$). In particular, extracellular lactate in the hippocampus increased from baseline by approximately 550 μM during place training. In contrast, hippocampal lactate increased by approximately 350 μM above baseline during response training, an increase only slightly higher than was seen in control rats not trained on either version of the maze but that received food rewards on a schedule similar to that during training. The hippocampus lactate response to place training was significantly higher than that of untrained-food rats ($p < 0.02$) and was higher, but not significantly higher, than the rise in response-trained rats ($p < 0.08$); the lactate rise in response-trained rats was not significantly different from that seen during feeding alone ($p > 0.3$).

The pattern of results seen with striatal probes was different than that seen with hippocampal probes (Figures 2A and 2B). While lactate rose significantly above baseline in all conditions, the differences in striatal lactate values across behavioral conditions during minutes 15-25 were not statistically significant ($F_{2,12}=0.86$, $p>0.4$). Moreover, the increases in striatal lactate during training on either task did not exceed those evident after provision of food reward ($p>0.2$ place, $p>0.5$ response). Therefore, the results obtained with striatal probes did not reveal an effect of training condition beyond the increases seen in untrained but fed rats.

Baseline lactate values in the hippocampus, averaged for the 5 min before training and combined across training conditions, were slightly higher than those of striatum, but this difference was not statistically significant ($t_{18} = 0.70$; $p>0.5$, Figure 3A). Rats trained on the response version of the task reached criterion performance slightly faster than did those trained on the place version of the task, but this difference was also not statistically significant ($t_{18}= 1.21$; $p>0.2$, Figure 3B).

The significant difference in hippocampal lactate increases in place- vs. response-trained rats was apparently the result of the use of different cognitive rules to solve the mazes. Note that the maze itself, locomotor activity, and number of rewards during the first 20 minutes of training, were very similar across rats trained to find food by choosing a particular place or by turning in a specific direction. The differences in lactate responses across brain areas suggest that the engagement of the hippocampus was greater during place training than during response training. This interpretation is consistent with past evidence showing that place learning on this maze is a

hippocampus-sensitive task (Chang and Gold, 2003a; Packard et al. 1989; Pynch et al. 2006; Korol and Pisani, 2015; Gold et al., 2013; Zurkovsky et al., 2007).

The results from the hippocampus lactate measures fit readily into a multiple memory systems perspective of the relative hippocampal engagement in place and response learning. According to this view, the hippocampus would be preferentially activated to solve the spatial learning version of the maze and would be activated less to solve the response version. In contrast, however, extracellular lactate levels in the striatum increased similarly throughout place training, response training, or feeding in untrained rats. Thus, the results obtained with striatum measures did not show the task-specific increases in lactate levels during response training that would be expected from a multiple memory systems perspective.

Because neither the hippocampal nor striatal rises in lactate levels during response training differed from the lactate change during eating, it is possible that the lactate responses to food ingestion may have masked responses to response training itself. Eating may contribute importantly to the findings either as an arousal-producing stimulus or by food-initiated increases in blood glucose levels and subsequent increases in lactate levels resulting from the metabolism of the ingested food.

3.2. Experiment 2: Lactate responses to training for a water reward

In this experiment, rats were trained to find water instead of food and the lactate responses were measured while rats were trained on the same place and response versions of the mazes as in Experiment 1. When using water reward, a different pattern

emerged: the striatum and not the hippocampus exhibited training-related increases in lactate levels that dissociated by task.

For the hippocampus, extracellular lactate levels increased significantly in rats trained on either the place ($p < 0.05$) or response tasks ($p < 0.05$), but did not increase significantly above baseline in untrained rats allowed to drink ($p > 0.3$; Figure 4A and 4B). The hippocampal lactate response was not statistically different across training conditions when water was the reward ($F_{2,11} = 0.90$; $p > 0.4$). Training-related increases ranged from ~155 to 180 μM above baseline for place- and response-trained rats, respectively, and were not different between groups ($p > 0.7$).

The striatum results, shown in Figures 5A and 5B, revealed striking differences in the magnitude of lactate increases across training conditions for water reward during minutes 15-25 of training ($F_{2,11} = 8.90$, $p < 0.005$). During this 10-min period, response-trained rats had significantly higher lactate responses than those of either the place-trained ($p < 0.05$) or reward-untrained ($p < 0.002$) groups. Placed-trained rats also demonstrated training-related elevations in lactate, but these increases from baseline were not statistically significant and were not statistically higher than those of the untrained water-rewarded group ($p > 0.2$).

Baseline lactate values, i.e. averages for the 5 min before training, were comparable across brain areas ($t_{16} = 1.64$, $p > 0.1$; Figure 6A). As shown in Figure 6B, the trials to reach criterion in the place vs. response tasks for water reward were not significantly different ($t_{16} = 0.31$, $p > 0.7$).

Thus, using water as a reward produced a qualitatively different lactate response in the brain than what was seen during the maze training for food. When trained for

water, the striatal lactate response, but not the hippocampal response, distinguished place and response learning strategies in a manner concordant with a multiple memory systems view.

3.3 Histology

Histological placements are shown in Figure 7. All rats in Experiments 1 and 2 had probe tips placed correctly into the dorsal hippocampus or dorsolateral striatum. The placements were overlapping across and within groups; individual placements did not appear to account for or modify the pattern of results. Very few placements were outside the clustered placements: one rat had a striatal placement that was medial to the others and three rats had probe placements that were largely within the hippocampus but with approximately 25% protruding ventrally, some portion of which reflects the nonsensory epoxy tip. For each of these four rats, the lactate responses were not outliers within their groups (Grubb test, $P > 0.05$). Moreover, omission of their data did not significantly affect the lactate results described in this report.

3.4. Blood glucose and lactate levels after eating or drinking

Food- or water-restricted untrained rats received the respective rewards on schedules to match those in the trained groups included in Experiments 1 and 2. Blood glucose levels increased from a baseline of 4.9 ± 0.3 mM to 6.7 ± 0.4 mM 50 minutes after the start of food presentation; the increase reached asymptote at ~20 minutes after eating onset and was stable thereafter. The change in blood glucose concentrations during the plateau, i.e. 20-30 min after food presentation, was significantly elevated

above baseline ($t_3=9.41$, $p< 0.005$, Figure 8). Blood glucose levels were stable during drinking, starting at 5.2 ± 0.1 and ending at 50 min at 5.1 ± 0.1 mM, with no change from baseline detected during the plateau ($t_3=0.95$, $p> 0.4$). No significant differences in baseline blood glucose were detected between food- and water-restricted rats ($t_6=1.13$, $p> 0.2$). Baseline blood lactate concentrations were also similar in food- (1.8 ± 0.06 mM) and water- (2.0 ± 0.16 mM) restricted rats ($t_6=0.97$, $p> 0.3$). Blood lactate did not increase from the beginning to end of feeding (from 1.8 ± 0.2 mM to 2.1 ± 0.2 mM at the final 50-min sample) and was not different from baseline during the 20-30 minutes after eating onset ($t_3=1.3$, $p>0.2$; Figure 8). During water provision, blood lactate levels showed a transient increase across the 50 minutes of drinking. However, concentrations were not different from baseline at the end of sampling ($t_3 = 0.05$, $p> 0.05$) or during the plateau period (20-30 minute epoch; $t_3 = 2.62$, $p> 0.05$).

The absence of significant increases in blood glucose or lactate levels after receipt of water suggests that ingestion of water alone was not sufficient to elicit an arousal response in the periphery. Because blood glucose increased after feeding but not drinking, the rise in blood glucose after food reward is likely based more on the energy gained from the food than an arousal response to reward *per se*. Moreover, the rise in blood glucose with food was not accompanied by a similar rise in lactate with food. Thus, it is unlikely that the training-related brain lactate responses in food-motivated tasks result solely from peripheral changes in lactate.

Extracellular brain lactate levels predominately reflect lactate production and transport out of astrocytes together with uptake by neurons, though there is evidence for lesser contributions of lactate from blood and from neurons that may contribute to

extracellular lactate levels (Walz and Mukerji, 1988). The findings that regional extracellular lactate levels in brain vary with training conditions even as blood lactate levels do not increase substantially after either food or water reward suggest that delivery of lactate from blood to brain is not a significant contributor to the training-related fluxes in hippocampal and striatal lactate levels seen here. The findings therefore support the idea that extracellular lactate is mainly derived locally from astrocytes.

3.5. Comparisons of hippocampal and striatal lactate response across reward types

In the untrained groups, food reward ($p < 0.05$) but not water reward ($p > 0.2$) resulted in increases in lactate levels above baseline in both the hippocampus and striatum (Figures 1, 2, 4, 5). A trend for larger lactate responses in untrained rats to food compared to water was also evident when comparisons were made within each brain region across reward type; however, these food vs water differences were not statistically significant (hippocampus, Fig. 1 vs 4: $p < 0.1$; striatum, Fig 2 vs 5: $p < 0.2$). The largest responses to training were seen in the hippocampus for food reward on the place task and in the striatum for water reward on the response task. Of interest, the magnitudes of the increases in these conditions were comparable; the lactate response plateaued at $551 \pm 72 \mu\text{M}$ in the hippocampus of rats place-trained for food and at $478 \pm 69 \mu\text{M}$ in the striatum of rats response-trained for water ($t_8 = 0.73$, $p > 0.5$). These data suggest that although the changes in extracellular lactate to food and water were different, the potential for a training-related increase in brain lactate levels was apparently not different. The results also highlight the unique responses of each

memory system to, and perhaps their unique role in, selective attributes of cognition including the learning strategy required by the task and now the reward type used during specific types learning.

4. GENERAL DISCUSSION

Using a multiple memory system perspective, these experiments extended tests of the relationships between hippocampal lactate and cognitive functions to both the hippocampus and striatum while rats learned tasks that are solved using either place or response strategies. In the hippocampus of rats trained to find food, there was clear differentiation of the magnitude of increase in lactate during place vs. response training; place learning led to a larger increase in lactate levels than did response training or feeding in untrained rats. This pattern of results is consistent with a multiple memory system view in which the hippocampus, and not striatum, is particularly important for place learning.

However, a multiple memory system view also predicts that the striatum engagement should be more evident during response training than during place learning. Surprisingly, this was not the pattern of results seen in rats trained to find food, where striatal lactate failed to increase during either response or place training beyond the increases seen during feeding alone.

When rats were trained to find water instead of food in the identical place and response mazes, the pattern of results was very different from that seen during training with food reward. In rats trained to find water, striatal levels increased more in response-trained rats than in place-trained or untrained-water rats. However, the

increases in hippocampal lactate levels with water reward were comparable across the place and response training conditions, therefore failing to differentiate across tasks. These results are consistent with expectations from a multiple memory system perspective for the striatum but not the hippocampus. Thus, in rats trained for water reward, the results obtained in the striatum but not hippocampus fit well into a multiple memory systems framework while the converse profile of results was seen with food reward.

The novel pattern of results described here suggests that the attributes associated with the hippocampus and striatum may vary not only by task but may also interact with the reward used in training. It is important to note that most rodent studies of multiple memory systems have used food rewards when assessing the relative contributions of the hippocampus and striatum to different classes of learning tasks. For example, in food-motivated tasks, lesions or pharmacological inactivation of the hippocampus impair spatial learning but leave response learning intact, or sometimes enhanced. Conversely, similar impairments of striatal function impair response learning but do not impair and sometimes enhance spatial learning (cf.: White et al., 2013; White and McDonald, 2002; Packard and Goodman, 2013; Gold et al., 2013; Poldrack and Packard, 2003; McDonald et al., 2004; Gold, 2004; Pyle et al., 2005; McElroy and Korol, 2005; Soares et al., 2013; Hunsaker and Kesner, 2013; Gold, 2004; Gold et al., 2013; Korol and Pisani, 2015). A smaller set of experiments has examined learning motivated by escape from water, revealing patterns of results similar to those seen with food-rewarded tasks (e.g., Dagnas et al., 2013; Hawley et al., 2015; Leong et al., 2015; Kosaki et al., 2015).

Many experimental parameters can shift the balance of control between memory systems including stress, cue availability, and massed vs. spaced trials (Restle, 1957; Packard and Goodman, 2013; Wingard et al., 2015). In studies performed within a reward class, stress and stress hormones impair place learning but leave response learning intact or enhanced (Packard and Goodman, 2012; Leong et al., 2015; Schwabe, 2013; Sadowski et al., 2009). Similarly, systemic estradiol enhances place learning but impairs response learning for food reward, results apparently reflecting independent actions of direct application of estradiol into the hippocampus or striatum (Korol, 2004; Zurkovsky et al., 2007, 2011; Korol and Pisani, 2015; Pleil et al., 2011). Differential release of neurotransmitters, activation of transcription factors, and other biological factors in the hippocampus and striatum during and after training show correlations between these measures and the brain area used for learning and memory across tasks (Pych et al., 2005; Gold, 2004; Colombo, 2004; McIntyre et al., 2003; Chang and Gold, 2003a,b; Rubio et al., 2012; Mizumori et al., 2004; Mizumori and Jo, 2013). These correlational studies testing multiple memory systems were also mainly performed using training to find food rewards. To our knowledge, the impact of the interactions between factors like these and different motivational states and rewards has not been explicitly tested to characterize the interactions of task attributes and memory systems. The different lactate responses in the hippocampus and striatum while rats learn place and response tasks for food vs. water rewards points to a need for a broader assessment of brain area by cognitive attribute to incorporate differences in the roles of multiple memory systems based on reward. In particular, the results suggest that the engagement of the hippocampus and striatum during learning may

dissociate not only according to the attributes and strategies needed to solve the mazes but may also dissociate in interaction with reward class. Studies assessing effects of drugs that impair lactate production and delivery to neurons will be needed to identify more fully the differences across memory systems imposed by motivation stimuli used during training.

The influence of reward class on the relative participation of multiple memory systems may relate to different physiological responses to food and water restriction or ethological requirements of learning to find food or water or to avoid predators. Searches for food or escapes from a predator are both tasks that may involve increased demands for behavioral flexibility and spatial navigation. For example, expanding territorypresumably increases the chances of finding foodand novel escape routes, while increasing the time and distance required for safe return to the nest(e.g., Maaswinkel and Whishaw 1999; Sherry, 2006; Healey, 2006).In contrast, searching for water is more likely to represent response, or habit, learning because unlike food sites that can be depleted and may shift with availability, water tends to be located in a relatively constant position in the environment, whether in a river, lake, or puddle. This line of reasoning is similar to that presented by Petrinovich and Bolles (1954) in contrasting learning based on food or water rewards, who noted that "different deprivation states . . . call forth different *kinds* of behavior." Petrinovich and Bolles related this view to similar comments by Krech (1951) who suggested that varying "...the nature of motivation will vary all the attributes of behavior and experience."

In their experiment, Petrinovich and Bolles (1954) showed that food deprivation produces more variability in behavior while water deprivation produces stereotypic

responses, terms that meld well with contemporary views of flexible (hippocampus-sensitive) and habit (striatum-sensitive) strategies to solve maze problems (White and McDonald, 2002; White et al., 2013; Packard and Goodman, 2012, 2013; Poldrack and Packard, 2003; Yin and Knowlton, 2006). The present findings support these early views and suggest that descriptions of the functions of multiple memory systems should incorporate the motivational attributes as well as the cognitive attributes of tasks used to identify structure-function relationships. It should be noted, however, that the specific tasks used in the present experiments revealed faster learning for water overall and with no bias toward place learning to find food and toward response learning to find water.

Considerable evidence indicates that glucose is an important regulator of cognitive processing (Gold and Korol, 2012; Gold, 2014). In a non-rewarded spontaneous alternation test of working memory, extracellular glucose levels in the hippocampus are depleted by memory testing and memory is enhanced by direct hippocampal infusions of glucose, suggesting that glucose depletion in the brain may limit optimal processing (McNay et al., 2000, 2001; McNay and Gold, 2001). Astrocytes can produce lactate from glycogen stores, and the lactate can be released into the extracellular space to supplement neuronal metabolic needs. Consistent with this view, the decrease in glucose levels seen while rats are tested on a working memory task is matched by a reciprocal increase in lactate levels (Kurita et al., 2002; Newman et al., 2011). These findings suggest that lactate may serve as a supplementary energy substrate to neurons during a time of heightened activity or energy utilization (Brown and Ransom, 2015; Magistretti et al., 1999; Pellerin, 2003; Pellerin and Magistretti,

2012; Li and Freeman, 2015), thereby blunting limitations imposed on processing during times of glucose depletion.

Additional evidence suggests that glucose effects on learning and memory may be mediated by uptake of glucose into astrocytes with subsequent production and provision of lactate. Interference with the breakdown of glycogen stores in astrocytes impairs memory (Hertz et al., 2013; Gibbs and Hutchinson, 2012; Newman et al., 2011; Suzuki et al., 2011). Also, enhancement of memory by glucose is blocked by α -cyano-4-hydroxycinnamate, a drug that blocks lactate uptake into neurons via the monocarboxylate-2 transporter (Newman et al., 2011). These findings suggest that glucose enhancement of memory may be mediated by astrocytic conversion of glucose to lactate, with subsequent provision of lactate to neurons to support learning and memory processing. For example, infusions of lactate, like those of glucose, directly into the hippocampus enhance memory (Newman et al., 2011; Morris and Gold, 2013). Moreover, upon stimulation of whiskers in rats, a manipulation that results in glycogenolysis in astrocytes (Swanson, 1992), there is a selective increase of glucose uptake into astrocytes but not into neurons in the whisker region of somatosensory cortex (Chuquet et al., 2010). The astrocytes might then produce and deliver lactate to extracellular fluid to support the heightened energy requirements of neurons during activation (Li and Freeman, 2015).

In summary, there are two main findings reported here. First, the present results support a role for astrocytic lactate in processing information needed for learning and memory (Magistretti et al., 1999; Newman et al., 2011; Suzuki et al., 2011). Though not directly tested, according to this view, brain systems that are engaged in learning show

larger increases in extracellular lactate that is used to help fuel neurons, i.e., lactate contributions to neuronal energy needs on demand (Magistretti et al., 1999; Pellerin, 2003; Pellerin and Magistretti, 2012) via the astrocyte-neuron-lactate-shuttle mechanism. Because direct measures of the fluxes in metabolites of glycogen and glucose have not been conducted here or before in the context of multiple memory systems and food vs water rewards, the role of lactate in brain energy balance is speculative.

However, it is important to note that there are alternative views suggesting that elevations in extracellular lactate such as those seen here provide for heightened lactate signaling, not its metabolism, that is critical for learning and memory modulation (Dienel and Cruz, 2016). There are other functions that may also contribute to the lactate's actions on brain processing (e.g., Chih et al., 2001; Mangia et al., 2009; Dienel, 2012; Dienel and McKenna, 2014; Sonnewald, 2014; Fryer and Brown, 2015), such as acting as a neuronal signal (Tang et al. 2014; Barros, 2013; Bergersen and Gjedde, 2012; Bozzo et al., 2013), contributing to energy needs to support extracellular clearance and glutamate degradation within astrocytes (Dienel and McKenna, 2014; Sonnewald, 2014), and regulating neurovascular coupling (Lauritzen et al., 2013; Gordon et al., 2008).

Second, the results reported here also suggest that the type of reward used for training on the place and response versions of the mazes modulates the relative contributions of the hippocampus and striatum during learning these tasks. Specifically, the hippocampus is more likely to be engaged in spatial learning tasks for food reward, but not water reward, while the opposite is true for the striatum, where habit learning

increases striatal engagement when water, but not food, reward is used. Therefore, in addition to the cognitive attributes of tasks, the nature of the reward used for learning is an important factor in identifying functions of multiple memory systems.

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FIGURE LEGENDS:

Figure 1: Changes in hippocampal extracellular lactate concentrations in food-rewarded rats that were untrained, place-trained, and response-trained. A. Training-related changes in hippocampal lactate concentrations over time. B. Changes in lactate levels above baseline during 15-25 min after the start of training. Extracellular lactate levels in the hippocampus increased significantly from baseline in both trained and untrained food-rewarded rats, but were highest during place training. Note that the training-related rise in hippocampal lactate in place-trained rats was significantly potentiated compared to increases seen in untrained-fed rats and marginally above those trained on the response task. + = $p < 0.05$; * = $p < 0.05$.

Figure 2: Changes in striatal extracellular lactate concentrations in untrained, place-trained, and response-trained food-rewarded rats. A. Changes in striatal extracellular lactate concentrations over time during training for food reward. B. Changes in lactate levels during 15-25 min after the start of training. Lactate levels increased in striatum similarly under all treatment conditions and, of particular note, did not differ by task. + = $p < 0.05$.

Figure 3: A. Baseline extracellular lactate concentrations in hippocampus and striatum. No significant differences were evident across these brain areas. B. Trials to reach criterion of 9/10 correct. The place task required more trials to reach the learning criterion than did the response task, but this difference was not statistically significant.

Figure 4: Changes in hippocampal extracellular lactate concentrations in untrained-water, place-trained, and response-trained rats. A. Training-related changes in hippocampal extracellular lactate concentration over time during training for water reward. B. Changes in extracellular lactate during 15-25 min after the start of training for water reward. There was a small, non-significant, increase in lactate in untrained-water rats. Training on either place or response tasks resulted in significant increases in lactate levels at 15-25 min after the start of training but these increases did not differ significantly by task. + = $p < 0.05$.

Figure 5: Changes in striatal extracellular lactate concentrations in untrained, place-trained, and response-trained water-rewarded rats. A. Training-related changes in striatal extracellular lactate concentrations over time. B. Changes in lactate concentrations above baseline 15-25 min after the start of training. Response training produced significantly higher extracellular lactate levels in the striatum as compared to place training or untrained-water rats. + = $p < 0.05$; * = $p < 0.05$; ♦ = $p < 0.05$.

Figure 6: A. Baseline extracellular lactate levels in hippocampus and striatum. No significant differences were evident across these brain areas. B. Trials to reach criterion of 9/10 correct in the maze tasks. Learning to find water in the place and response task required approximately the same number of trials to reach criterion.

Figure 7. *Left*: Sample placements for biosensor cannulae in the hippocampus and striatum. For the striatum, cannulae were implanted at a 15° angle away from midline to accommodate the large headstage. 40 µm sections were stained with

cresyl violet and images were taken at 12.5x magnification. *Right.* Illustration of probe placements for all rats color-coded by group (see legend). In the food reward condition, a separate control group received food without training. In the water reward condition, control lactate responses were obtained while rats received water in their home cages 24 hr before training. Therefore, there is no separate histology group for the water controls. Atlas figures from Paxinos and Watson, 1986.

Figure 8: Changes in blood glucose and lactate concentrations in untrained control rats during provision of Cheerios (left, dark bars) or water (right, light bars) in a holding cage. Data reflect averages of samples drawn at 20 and 30 minutes after the onset of food or water access. Note the large change from baseline in circulating glucose during eating ($p < .005$) but no other significant changes. + = $p < 0.05$.

FIGURE 1

FIGURE 2

Food Reward
Striatum

A

B

FIGURE 3

Food Reward

A

B

FIGURE 4

Water Reward Hippocampus

A

B

FIGURE 5

FIGURE 6

Water Reward

FIGURE 7

FIGURE 8