EXTRACELLULAR LEVELS OF GLUCOSE IN THE HIPPOCAMPUS AND STRIATUM DURING MAZE TRAINING FOR FOOD OR WATER REWARD IN RATS

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Running title: Hippocampal and striatal glucose and learning

Key words: Multiple memory systems; place learning; response learning; glucose; hippocampus; striatum

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[a] This work was supported by NSF IOS 13-18490, NIDA DA038798, NIA AG057947, and by P30 AG034464 through the Center on Aging and Policy Studies at Syracuse University.

Abstract

Peripheral and central administration of glucose potently enhance cognitive functions. The present experiments examined changes in brain extracellular glucose levels while rats were trained to solve hippocampus-sensitive place or striatum-sensitive response learning tasks for food or water reward. During the first minutes of either place or response training, extracellular glucose levels declined in both the hippocampus and striatum, an effect not seen in untrained, rewarded rats. Subsequently, glucose increased in both brain areas under all training conditions, approaching asymptotic levels ~15-25 min into training. Compared to untrained-food controls, training with food reward resulted in significant increases in the hippocampus but not striatum; striatal levels exhibited large increases to food intake in both trained and untrained groups. In the rats trained to find water, glucose levels increased significantly above the values seen in untrained rats in both hippocampus and striatum. The present findings suggest that decreases in glucose early in training might reflect an increase in brain glucose consumption, perhaps triggering increased brain uptake of glucose from blood, as evident in the increases in glucose late in training. Together with past findings measuring lactate levels under the same conditions, the initial decreases in glucose may also stimulate increased production of lactate from astrocytes to support neural metabolism directly and/or to act as a signal to increase blood flow and glucose uptake into the brain.

Introduction

Glucose enhances cognitive functions in rodents as well as in several human populations including healthy young adult and elderly individuals and those with Alzheimer's disease (Gold, 2001, 2014; Gold and Korol, 2012, 2014; Mantantzis et al., 2018; Messier, 2004; Messier et al., 1999, 2010; Smith et al., 2011; van der Zwaluw et al., 2015). In past experiments with rats tested on a spatial working memory task, extracellular glucose levels in the hippocampus decreased early and recovered later in testing, the recovery possibly secondary to the onset of testing-related increases in peripheral blood glucose levels (McNay et al., 2000, 2001; McNay and Gold, 2001; Newman et al., 2011). In addition to revealing a decrease in extracellular glucose levels in the hippocampus during memory testing, the magnitude of the reduction in extracellular glucose levels was a function of task difficulty, or cognitive load, and also brain structure (Korol, 2002; McNay et al., 2000, 2001). Interestingly, striatal extracellular glucose levels failed to decline during spatial working memory and indeed appeard to increase over the course of testing (McNay et al., 2001). The significance for learning and memory of these fluxes in extracellular glucose levels is supported by evidence that glucose also enhances learning and memory when microinjected directly into brain targets selected because of their involvement in specific tasks (McNay and Gold, 1998; Morris and Gold, 2013; Ragozzino et al., 1998; Schroeder and Packard, 2003; Stefani et al., 1999; Stefani and Gold, 2001).

The hippocampus and striatum are two neural systems known to be important participants in selective learning of mazes based on spatial (place) or response (habit) learning, respectively (Colombo, 2004; Gold et al., 2013; Korol, 2004; Korol and Pisani,

2015; Packard and Goodman, 2013; Poldrack and Packard, 2003; White and McDonald, 2002; White et al., 2013). Evidence showing particular importance of the hippocampus and striatum for place and response learning includes double dissociations using lesions or drug disruption of the brain areas (Dagnas et al., 2013; Kosaki et al., 2015; McDonald and White, 1994; Packard and McGaugh, 1992; Soares et al., 2013), as well as regulation of the brain areas by direct injections of glutamate, dopamine agonists, glucose and estradiol (Canal et al., 2005; Korol and Wang, 2018; Packard, 1999; Packard and Teather, 1999; Packard and White, 1991; Pych et al., 2006; Stefani and Gold, 2001; Zurkovsky et al., 2007, 2011). Additionally, the hippocampus and striatum also exhibit place and response learning-specific neurophysiological and neurochemical responses during learning that are associated with these cognitive functions of the hippocampus and striatum (Chang and Gold, 2003a,b; Dagnas et al., 2013; Kathirvelu and Colombo, 2013; McIntyre et al., 2003; Mizumori and Jo, 2013; Pleil et al., 2011; Pych et al., 2005; Rubio et al., 2012; Teather et al., 2005).

One of the mechanisms by which brain glucose might regulate learning and memory is by contributing to astrocytic production and release of lactate into extracellular fluid (Adamsky and Goshen, 2018; Alberini et al., 2018; Gao et al., 2016; Gold, 2014; Gold & Korol, 2012; Newman et al., 2011; Steinman et al., 2016; Suzuki et al., 2011; Tadi et al., 2015). Recently, we studied the responses of extracellular lactate while rats were trained on place and response mazes (Newman et al., 2017) that used either food or water as reward. With food reward, extracellular lactate levels increased beyond those of feeding controls during place but not response training, but striatal

levels did not exceed the increases in controls for either maze. In contrast, with water reward, lactate did not increase above control levels in the hippocampus during training on either task but increased in the striatum during response but not place learning. Thus, the responses of extracellular lactate to training differed by task but did so with a striking interaction with reward.

Because lactate is derived from glucose, largely in astrocytes, the present study examined changes in glucose in the hippocampus and striatum under training and reward conditions matching those of the previous study with lactate (Newman et al., 2017) to assess similarities and differences in the responses of the two energy substrates to training. In particular, one question was whether changes in extracellular glucose levels during training would also vary in a task by reward manner similar to the results seen with lactate, thereby supporting the view that glucose and lactate are sequentially linked to effects on learning and memory. A second question was whether decreases and increases in glucose during training would be the inverse of lactate responses, thereby maintaining relatively constant combined substrate availability during training.

Methods

Subjects

Male Sprague-Dawley rats (3-4 months old; Harlan Laboratories, Indianapolis, IN) were housed individually with free access to food and water under a 12-hour light/dark cycle. All procedures reported here were approved by the University of Illinois Urbana-Champaign and the Syracuse University Institutional Animal Care and Use

Committees and are consistent with guidelines detailed in *Guide for Care* and *Use of Laboratory Animals.* The vivarium facilities at both universities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Cannula Implantation Surgery

Rats were anesthetized using 2-4% isoflurane, injected with 100,000 units of penicillin intramuscularly and either 5 mg/kg Rimadyl or 2.5 mg/kg Flunixin subcutaneously, and placed in a stereotaxic frame while receiving continuous isoflurane though a nose-piece. The skull was exposed through a small incision in the scalp and cleared of blood and fascia. Small burr holes were made in the skull overlying the hippocampus or striatum for guide cannulae (9 mm long, 350 µm diameter, BASi, West Lafayette, IN) and more laterally for hardware to attach the headcap. To permit the later insertion of a glucose biosensors near the time of training, the guide cannulae were placed just dorsal to the hippocampus (AP: -3.8 mm from bregma, ML: + or - 2.5 mm, 0.9 mm ventral to dura) or striatum (AP: 0.2 mm from bregma, ML: + or - 2.0 mm, 1.5 mm ventral to dura, angled 15 degrees away from midline) (Paxinos and Watson, 2008). The angled approach for the striatum cannulae was needed to accommodate the head stage and later insertion of glucose biosensors (see Newman et al., 2017). Four skull screws were inserted as anchors for dental acrylic cement used to affix the cannula to the skull and to mount the bottom of the biosensor head stage, which contained a potentiostat, circuitry, and battery used to measure glucose levels with wireless telemetry. Several injections of 0.9% sterile saline (10 ml total, i.p.) were given to the rats post-surgery to provide hydration during recovery. In addition, ibuprofen (Children's Motrin, 50 mg in 250 ml water bottle) was provided for 24 hrs in the rats'

drinking water for analgesia support after surgery. Rats were allowed to recover for seven days after surgery with free access to food and water and with daily monitoring of health status.

Place and Response Learning

Rats were either food- or water-restricted depending on the reward used during training; water reward was included here to avoid possible confounds of food restriction and reward on brain glucose concentrations. Food-restricted animals received a daily aliquot of rat chow for one week to reduce their body weights to 85% of baseline. During food restriction, rats received several reward pellets (~1/3 Frosted Cheerio®) that would be used during training. Water-restricted rats were given water once a day for one week in the same cup that would be used later in the learning tasks. The water restriction schedule reduced the rats' body weights to 85% of baseline at the end of one week. All rats were handled for 3 min on each of the five days prior to training.

One day prior to testing, rats were briefly anesthetized with 2-4% isoflurane while a glucose biosensor probe (Pinnacle Technology, Inc., Lawrence, KS) was inserted into the guide cannula. The probe was then connected to a wireless potentiostat and the top of the biosensor head stage was attached to enclose the probe system. The rat was returned to its cage overnight to allow the readings of glucose levels to stabilize. The biosensor data were recorded using Pinnacle Technology Laboratory v. 1.6.7 software.

The following day, the rat was placed in the testing room and allowed to acclimate to the room for approximately one hour. A four-arm radial maze was used with one arm blocked, forming a T-shaped maze as used and detailed in past papers (e.g.: Gold, 2016; Newman et al., 2017; Pisani et al., 2012; Pych et al., 2006). The arms

of the maze were 45 cm long x 14 cm wide x 7.5 cm high and extended from a 14 cm^2 center area. For food-rewarded training, each arm had a food cup with perforations at the bottom, permitting placement of small Frosted Cheerio® pieces below the cup to mitigate the use of odor cues to find the correct arm; the reward pellet in the goal arm sat above the perforated bottom of the cup. For water-rewarded training, the same plastic cups in which the rats had been given water during water restriction were used in the maze. Rats were allowed to drink from the cup during reinforcement for 2-3 seconds. In both tasks, training continued until rats made 9/10 correct turns or until they reached a maximum of 100 trials. Untrained control rats were fed the food reward every 1.5 minutes or had access to water for 52.5 minutes while in their home cages.

Biosensor measurements of extracellular glucose levels

The biosensors extended 3 mm past the end of the guide cannula with the distal 1 mm of the probe coated with glucose oxidase. The current generated by glucose reaction with the enzyme was monitored in 1-sec bins. The responses of extracellular glucose reported here were normalized within rat to the baseline extracellular glucose measures.

After training, the biosensor probes were removed and calibrated to ensure functional integrity. The probes were placed in 8 ml of 0.1 M phosphate-buffered saline until a stable baseline was reached. Small amounts of glucose (range: 0.5 - 7 mM) were then added incrementally every 1.5 min to create a linear standard curve. The line generated from the standards was used to translate the change recorded during testing from current (amps) to molar concentration of the substrate of glucose. The biosensors were also coated with ascorbate oxidase to prevent ascorbate interference with the

glucose probe; if the coating is compromised, readings will reflect both glucose and ascorbic acid. Therefore, after glucose calibration, ascorbic acid (range: 200-400 μM) was added to the solution. Results from probes that malfunctioned during *in vivo* measurements, that did not have linear responses to glucose, or that responded to ascorbic acid (N = 9) were excluded from analysis.

Histology

Upon completion of testing, the rats were anesthetized with 2-4% gas isoflurane followed by an overdose of pentobarbital (75 mg/kg) (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI). The brain was removed and the hemisphere containing the cannula was immersed in 10% formalin for at least 2 days, followed by 20% glycerol for 2 days at 4°C. Brains were sectioned in a cryostat and stained with cresyl violet for histological assessments of cannula placements.

Statistical Analyses

Because several rats had trials to criterion scores at the maximum 100 trials allowed, the data for trials to criterion were truncated. Therefore, medians and interquartile ranges were used to display central tendencies for trials to criterion and differences on this learning score were evaluated with non-parametric Mann-Whitney U-tests. For neurochemical analyses, samples were collected from rats every second before, during, and after training and collapsed into 10-second bins. Using these bins, changes in extracellular glucose from baseline were charted across time to portray the full dynamic of glucose response to training. To capture training-induced depletion and elevation of glucose, the averaged values during two epochs are reported: Minutes 1-3 after training, when brain glucose was near its maximum decrease from baseline, and

minutes 15-25 during training, when glucose was near its maximum rise during training. Training- and food/water intake-related changes in mean extracellular glucose levels were evaluated within groups with paired sample t-tests comparing baseline values just before training with values at 1-3 min and at 15-25 min during training, with alpha = 0.05. The changes in glucose levels at each epoch after training were also evaluated across groups with two-way ANOVAs to identify main effects of and interactions between brain area and task within each reward condition and additionally by planned paired contrasts. One outlier in the food-untrained group min 15-25 was removed from data analyses ($Grubb_4$ = 1.47, p=0.05 critical value). A two-way ANOVA also tested the effects of reward condition (food, water) by brain area (hippocampus, striatum) in rewarded-untrained rats.

Results

Learning scores depicted as trials to criterion are shown in Figure 1 for rats in the four training-reward conditions: place trained-food, place trained-water, response trained-food, or response trained-water. When trained for either food or water, the number of trials to reach criterion did not differ significantly across place vs. response training (Food, $U_{12,12}$ = 54.5, p>0.2; Water, $U_{10,9}$ = 50.5, p>0.2).

INSERT FIGURE 1 ABOUT HERE

The neurochemical findings revealed two main characteristics of the changes in extracellular glucose levels during training. During the first several minutes of training, extracellular glucose levels decreased from baselines in trained rats. After that time, the levels increased substantially throughout the rest of training. To show these different portions of the results graphically, Figures 2-5 focus on the first 5 min of

training and Figures 6-9 show the results across the first 40 min of training. Across training conditions,there was variability in the amount of time required to learn; however all rats required at least 40 min to reach criterion.

Initial glucose responses to training

The left panels of Figures 2-5 show the responses in 10-sec bins during that time. The right panels show the mean responses during minutes 1-3 of training, i.e. at the time when the responses are near their asymptotic decreases.

Food reward.

When rats were trained with food reward, there was no interaction between brain area (hippocampus, striatum) and task (place, response) for the early glucose decline (Figures 2 and 3; $F_{1,17}$=0.13, p>0.7). In addition, the early dips in extracellular glucose for food-rewarded rats during the initial phase of place and response training were not different either in the hippocampus (Figure 2; t_7=0.07, p>0.9) or striatum (Figure 3; t_7=0.47, p>0.6) samples. Given the similar results across place and response training, the values were combined into 'trained' groups within the food reward condition for comparison with the values obtained in untrained controls.

INSERT FIGURES 2 and 3 ABOUT HERE

Although the glucose responses did not vary significantly by task, food-rewarded rats exhibited hippocampal glucose values that decreased significantly below baseline during minutes 1-3 of training (t_8=3.57, p<0.01) (Figure 2). In contrast, food-rewarded, untrained rats had hippocampal glucose values that did not significantly change from baseline (t_3=0.253, p>0.8). The decreases in extracellular glucose levels in food-

rewarded trained rats approached significance when compared to those of food-rewarded untrained rats (t_{11}=2.16, p<0.06).

For striatum, food-rewarded trained rats had glucose values during minutes 1-3 (Figure 3) that were again significantly below baseline (t_8=2.54, p<0.05), while untrained food-rewarded rats had striatal values that did not differ significantly from baseline (t_3=0.11, p>0.9). There was a trend for greater decreases in striatal glucose levels in food-rewarded trained rats compared to those of food-rewarded untrained rats that was not statistically significant (t_{11}=1.46, p>0.1).

Water reward.

Like those trained with food rewards, rats rewarded with water showed no interaction between brain area and task in extracellular glucose levels during early training (Figures 4 and 5; $F_{1,15}$=0.03, p>0.8). The decreases in glucose levels between water-rewarded rats receiving place and response training were not significantly different in either the hippocampus (Figure 4; t_6=0.18, p>0.9) or striatum (Figure 5; t_6=0.31, p>0.7). As with food training above, given the similar results across place and response training when rewarded with water, those values were combined into 'trained' groups within the water-reward condition.

As shown in Figure 4, water-trained rats had hippocampal glucose values from minutes 1-3 that dropped below baseline but these did not reach statistical significance (t_7=2.13, p<0.08). However, these values were significantly different from rats that were untrained but received a water reward (t_{12}=2.76, p<0.02). Glucose in the hippocampus samples of water-rewarded untrained rats was slightly higher, not lower, than baseline; however, this increase was not statistically significant (t_5=1.77, p>0.1).

For striatal samples (Figure 5), glucose levels in the water-rewarded trained rats from minutes 1-3 were significantly below baseline values (t_7=4.27, $p<0.005$) and glucose levels in water-rewarded untrained rats were similar to baseline levels (t_5=0.61, $p>0.5$). The changes in striatal glucose in water-rewarded trained rats were not significantly different than those of water-rewarded untrained rats (t_{12}=1.47, $p>0.1$).

INSERT FIGURES 4-5 ABOUT HERE

Late glucose responses to training

Figures 6-9 show the changes in extracellular glucose levels in hippocampus and striatum across 40 minutes of training. The left panels show the responses in 10-sec bins during that time. The right panels show the mean responses during minutes 15-25 of training, i.e. a time when the responses are near their asymptotic maxima.

Food reward.

When rats were trained for food reward, the interaction between brain area (hippocampus, striatum) and task (place, response) was not statistically significant for the minute 15-25 measurements ($F_{1,18}$=1.10, $p>0.3$), although mean glucose values were higher in the hippocampus during place than during response training and values in the striatum were higher during response than during place training. In addition, the results from rats receiving place and response training for food rewards were not different for either hippocampus (t_8=0.66, $p>0.5$) or striatum (t_8=0.89, $p>0.4$). The results of place- and response-trained rats were therefore pooled within the food reward condition to generate “trained” hippocampus and striatum groups.

For hippocampus samples (Figure 6), the food-rewarded trained rats had glucose values from minutes 15-25 that were significantly higher than baseline (t_8=6.40,

$p<0.0002$). The food-rewarded untrained rats also had hippocampal values that significantly increased from baseline ($t_2=6.83$, $p<0.05$). The increases in glucose levels in food-rewarded trained rats were greater than those of food-rewarded untrained rats ($t_{11}=2.36$, $p<0.05$), showing an effect of training on glucose levels beyond that of food intake *per se*.

For striatum samples (Figure 7), the food-rewarded trained and untrained rats had glucose values from minutes 15-25 that were significantly above baseline (trained: $t_8=6.40$, $p<0.0002$; untrained: $t_3=5.27$, $p<0.02$). The increases in striatal glucose levels in food-rewarded trained rats did not differ significantly from those of food-rewarded untrained rats ($t_{11}=0.66$, $p>0.5$), showing no effect of training on glucose levels beyond food ingestion. Thus, training with food reward resulted in significant increases beyond those seen in food-untrained rats in the hippocampus but not striatum.

INSERT FIGURES 6 and7 ABOUT HERE

Water reward.

When rats were trained for water reward, the interaction between brain area (hippocampus, striatum) and task (place, response) was not statistically significant for the minute 15-25 measurements ($F_{1,16}=0.92$, $p>0.3$). Therefore, the glucose results of place- and response-trained rats were pooled as above, generating “trained” hippocampus and striatum groups.

Hippocampal glucose levels with water reward rose significantly above baseline in both the trained and untrained conditions (trained: $t_8=5.27$, $p<0.001$; untrained: $t_4=2.56$, $p<0.01$; Figure 8). Of note, trained rats had increases in hippocampal glucose levels that were significantly greater than those of untrained rats ($t_{12}=2.57$, $p<0.05$).

For the striatum, glucose levels increased significantly above baseline in water-rewarded trained rats (t_7=7.78, p<0.0001) but not in untrained rats (t_5=1.03, p>0.3; Figure 9). The trained rats had glucose levels higher than those seen in untrained rats (t_{12}=3.22, p<0.01). Thus, with water reward, training resulted in increases in both hippocampal and striatal glucose levels significantly above those seen in untrained water-rewarded controls.

INSERT FIGURES 8 and 9 ABOUT HERE

Glucose responses to rewards in untrained rats.

Untrained rats were given continuous food or water rewards to match the pace of consumption in maze-trained rats. In measurements early in reward provision, there were no significant changes in extracellular glucose levels in either the hippocampus or striatum. These early times likely preceded increases in blood glucose levels after food (or water) ingestion during initial consumption and therefore were not evident in brain measures (Newman et al., 2017). However, at minutes 15-25 after the start of reward consumption in untrained rats, hippocampal and striatal glucose responses to the rewards differed by food vs. water and by brain area. For clarity to compare untrained groups across brain region and reward condition, the changes in glucose levels 15-25 min after the start of reward presentation to untrained groups are replotted from earlier figures and shown in Figure 10. In untrained-rewarded rats, there was a significant interaction of reward by brain area ($F_{1,17}$=11.22, p<0.005), driven mainly by the large differences in the striatal glucose response to food and water. The food reward increased hippocampal and striatal glucose, as did water reward in the hippocampus (p's<0.05); however, there was no change in striatum extracellular glucose in water-

rewarded rats ($p>0.2$). Of note, the increase in striatal glucose levels after water reward was significantly lower than that of each of the other three groups ($p's<0.02$), whereas the magnitude of changes in glucose levels in the other groups did not differ significantly from each other ($p's>0.1$).

INSERT FIGURE 10 ABOUT HERE

In all rats, accurate placements into hippocampus and striatum were histologically confirmed under light microscopic evaluation; see Figure 11 for examples of hippocampus and striatal placements.

INSERT FIGURE 11 ABOUT HERE

Discussion

Extracellular glucose levels in the hippocampus and striatum exhibited biphasic responses to training on both the place and response versions of the 4-arm radial maze under both food and water training conditions. Glucose levels decreased below baseline at the start of training and then increased well above baseline beginning at ~10 min into training.

The early decreases seen in all trained groups are consistent with previous results obtained with both microdialysis and biosensor measurements of brain glucose levels when rats are tested for spatial working memory on a non-rewarded spontaneous alternation task (McNay et al., 2000; Newman et al., 2011). The findings that initial decreases in brain extracellular glucose values are evident in both food- and water-rewarded training conditions suggests that the declines were not limited to a condition of reduced glucose availability. In that case, the decrease in glucose levels might be expected to be greater in rats under food restriction than in those under water

restriction. Rather, the decreases likely reflect increased brain utilization of glucose in response to the demands of information processing, i.e. uptake of extracellular glucose into neurons and astrocytes (Beland-Millar and Messier, 2018; Dienel and Cruz, 2016; Fox and Raichle, 1986; Li and Freeman, 2015; Magistretti et al., 1999). Consistent with this view, simultaneous recordings of activated multiunit activity and biosensor measures of glucose concentrations in neocortex reveal an immediate decrease in extracellular glucose concentrations during neural activation (Li and Freeman, 2015).

The initial decline in extracellular glucose levels during training was followed by an increase in glucose levels that reached a maximum rise at ~15-25 min after the start of training. The rise suggests that brain activation recruits additional glucose into extracellular space when needed to support neural activity important for processing learning and memory. The late increase in hippocampal and striatal glucose levels during training above what results from reward alone was clearly evident in water-rewarded rats and for hippocampus with food reward but did not reach significance in food-rewarded trained rats. We have previously reported in untrained rats that food, but not water, intake increases blood glucose (Newman et al., 2017), suggesting that food rewards may have masked training-related increases in glucose during training. However, the use of water reward makes it clear that glucose levels in both the hippocampus and striatum increase as a consequence of place or response training, rather than increasing as a function of food or water consumption*, per se*. The increase in extracellular glucose levels is consistent with evidence that stimulation of brain areas results in increased blood flow, glucose uptake into neurons and astrocytes, and glycolysis (Bak and Walls, 2018; Díaz-García et al., 2017; Díaz-García and Yellen,

2019; Hung et al., 2011) and that protein and mRNA levels of cerebral glucose transporter 1 (GLUT1) increase immediately after operant training (Choeiri et al., 2005). GLUT1 expression is tightly correlated with local cerebral glucose utilization (Duelli and Kuschinsky, 2001; Vannucci et al., 1998; Zeller et al., 1997), possibly by regulating brain glucose uptake. The plasticity of GLUT1 may contribute to the increase in glucose levels in extracellular fluid samples seen here in the hippocampus and striatum during place and response training.

Because the present experiment mirrored the design of our previous study (Newman et al. 2017) of changes in extracellular hippocampal and striatal lactate levels during place and response training, it is possible to compare the time-dependent responses of the two energy substrates. Like glucose in the present report, the earlier experiment showed that lactate levels increased in both the hippocampus and striatum during both place and response training. However, lactate levels exhibited task-related differences in the magnitude of increases in lactate levels in the hippocampus and striatum that depended on the type of reward, i.e. food vs. water. In contrast, in the present experiment neither the decreases in glucose early in training nor the increases in glucose later in training were related to task-dependent changes across brain areas. The absence of dissociations of task by brain area responses of glucose diverge from dissociations evident when measuring acetylcholine (Pych et al., 2005), cFos, or cJun (Teather et al., 2005) in place and response tasks such as those used here. The findings are also different that those seen in dual-solution tasks in which the strategy selection can be either place or response based. With these training procedures, acetylcholine release (Chang et al., 2003a; McIntyre et al., 2003), pCREB and c-Fos

expression (Colombo et al., 2003), Arc expression (Gardner et al., 2016), and histone acetylation (Dagnas and Mons, 2013) each reveal differences by strategy in concert with the hippocampus and striatum measures. Thus, the data in the present report suggest that changes in glucose levels are less discriminate than are lactate and other neurochemical measures in terms of marking the neural system predominantly engaged by different training procedures and effective learning strategies. Interestingly, in untrained rats, the striatal glucose response was substantially larger to food versus water, suggesting that striatum may contribute to detecting or distinguishing reward consequences. The findings also indicate that the relationship between glucose and lactate is complex, varying by task, reward, and brain area, with relative changes in the substrates diverging across conditions.

The different responses of glucose, reported here, and lactate (Newman et al., 2017) do not readily fit a model directly linking the two energy substrates. That changes in glucose and lactate levels support learning and memory processing is consistent with previous findings showing that direct infusions of either glucose (Canal et al., 2005; Morris and Gold, 2013; Pych et al., 2006; Ragozzino et al., 1996, 1998; Stefani and Gold, 2001) or lactate (Newman et al., 2011; Suzuki et al., 2011; Hamling et al., 2016; Wang and Korol, 2017) into the brain enhance learning and memory in young and aged rats. Reinforcing the view that these substrates are used to support energy metabolism needed during cognitive processing is evidence that direct infusions of other substrates, e.g. pyruvate or the ketone body, β-hydroxybutyrate (B3HB), at the time of inhibitory avoidance training or spontaneous alternation testing also enhance memory functions (Descalzi et al., 2019; Ragozzino et al., 1995).

An alternative view to one in which glucose is the primary substrate for neuronal energy provision is that, upon stimulation of a brain area, glucose preferentially enters astrocytes (Chuquet et al., 2010), where it can be stored as glycogen. According to the astrocyte-neuron lactate shuttle hypothesis, glycogen can then be broken down to lactate, which is delivered to neurons for energy needed to support neural plasticity, learning, and memory (Gold, 2014; Gold and Korol, 2014; Magistretti et al., 1999; Pellerin and Magistretti, 2012; Steinman et al., 2016). However, reports vary regarding whether neural activation leads to preferentially increased uptake of glucose into astrocytes (Chuquet et al., 2010; Jakoby et al., 2014; Loaiza et al., 2003; Magistretti and Allaman, 2018; Pancani et al., 2011) or into neurons (DiNuzzo et al., 2010; Ivanov et al., 2014; Lundgaard et al., 2015; Patel et al., 2014). An important caveat in interpreting the dynamic extracellular responses of glucose as described in the present report is that the levels evident at any time during behavioral testing reflect momentary equilibria of glucose delivery and its consumption. The methods used here do not distinguish the movement of glucose into or out of different cell types or movement into brain from blood.

Of particular interest, a consistent finding across measurements of lactate in (Newman et al., 2017) and glucose is that extracellular levels of both glucose and lactate increase after the first few minutes of training and do so robustly. Using zero-net-flux microdialysis measurements of extracellular glucose, resting glucose levels are ~1 mM in the hippocampus and ~0.7 mM in the striatum of male rats (McNay et al., 2001). Baseline lactate levels in male rats are estimated to be ~0.8 mM in hippocampus (Samala et al., 2011) and ~1 mM in striatum (Kuhr and Korf, 1988;

Kiewert et al., 2010). Across the present and our prior experiments (Newman et al., 2017), training for water reward without a confound of the effects of food ingestion resulted in approximate 50-300% increases in glucose and lactate values during maze training; of note, glucose and lactate rise in concert during both place and response training. The lactate response has been described as reflecting 'energy on demand', i.e. provision of an energy substrate to neurons to supplement glucose availability (Gao et al., 2016; Gibbs et al., 2008; Magistretti, 2006; Magistretti et al., 1999; Moraga-Amaro et al., 2014; Steinman et al., 2016). Therefore, the findings of large glucose and lactate increases during training, together with the ability of the energy substrates to enhance learning and memory, support the idea that neurons require additional energy substrates to engage in optimal processing of learning and memory information. During early training, the net demand for and use of glucose is evident as an initial decrease in extracellular levels. A later increase in extracellular glucose during training supports the neuronal energy needs of brain areas engaged in learning and memory to promote mechanisms of neural plasticity. The decrease in available glucose may trigger an increase in extracellular lactate levels from astrocytic glycogenolysis, with lactate then serving as a supplemental fuel for neurons, with the simultaneous increase in both substrates together fueling neuronal activity. Alternatively, if glucose and not lactate is a preferred energy substrate in neurons, the increase in lactate levels may serve its many potential roles beyond supporting the metabolic needs of neurons (Chih et al., 2001; Dienel, 2012, 2019; Dienel and McKenna, 2014; Fryer and Brown, 2015; Mangia et al., 2009; Mergenthaler et al., 2013; Sonnewald, 2014). For example, lactate actions include support for the energetic needs of astrocytes, perhaps thereby sparing

glucose consumption by astrocytes in support of glucose use by neurons (Dienel et al., 2018; Dienel and McKenna, 2014; DiNuzzo et al., 2010, 2012; Sonnewald, 2014). Lactate also activates GPR81 (or HCAR1) receptors (cf.: Dienel, 2017; DiNuzzo, 2016), which can regulate neural excitability (de Castro Abrantes et al., 2019; Herrera-López and Galván, 2018) and possibly modulate neural plasticity. GPR81 receptors are also found on epithelial cells where lactate can serve as a vascular signal (Barros, 2013; Bergersen and Gjedde, 2012; Bozzo et al., 2013; Morland et al., 2015). In this way, lactate might coordinate region-specific regulation of neurovascular coupling to increase glucose availability in a brain area by task manner. As such, lactate would serve as a mediator of a feed-forward process to promote increases in glucose availability upon demand (Gordon et al., 2008; Lauritzen et al., 2014). From this perspective, the findings open the possibility that initial use of glucose, shown here as decreased extracellular levels early in training, leads to increased extracellular lactate levels. Lactate, in turn, may act on GPR81 receptors to signal the vasculature to increase brain glucose uptake, shown here as increased glucose levels late in training. Interacting in this manner, the increases in both glucose and lactate could support increased neuronal energy capacity needed for cognitive processing.

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Figures

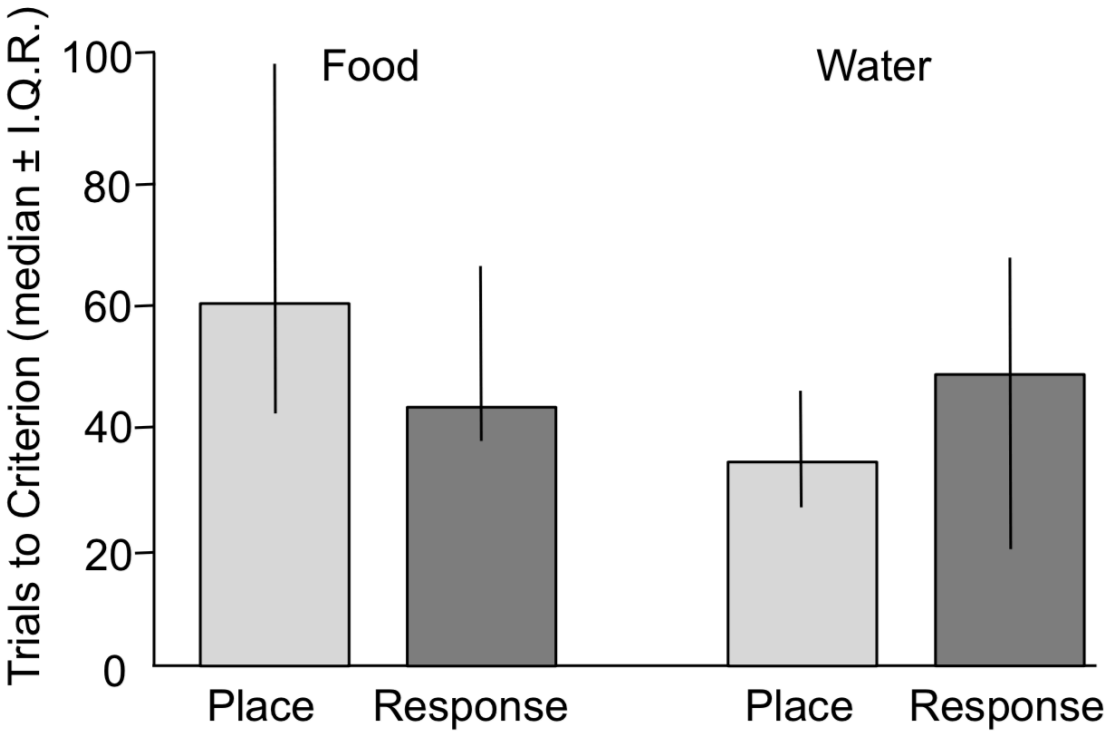

Figure 1. Trials to reach 9/10 correct choices under the four training conditions. Note that rats solved the place and response versions of the maze in similar numbers of trials when trained to find either food or water.

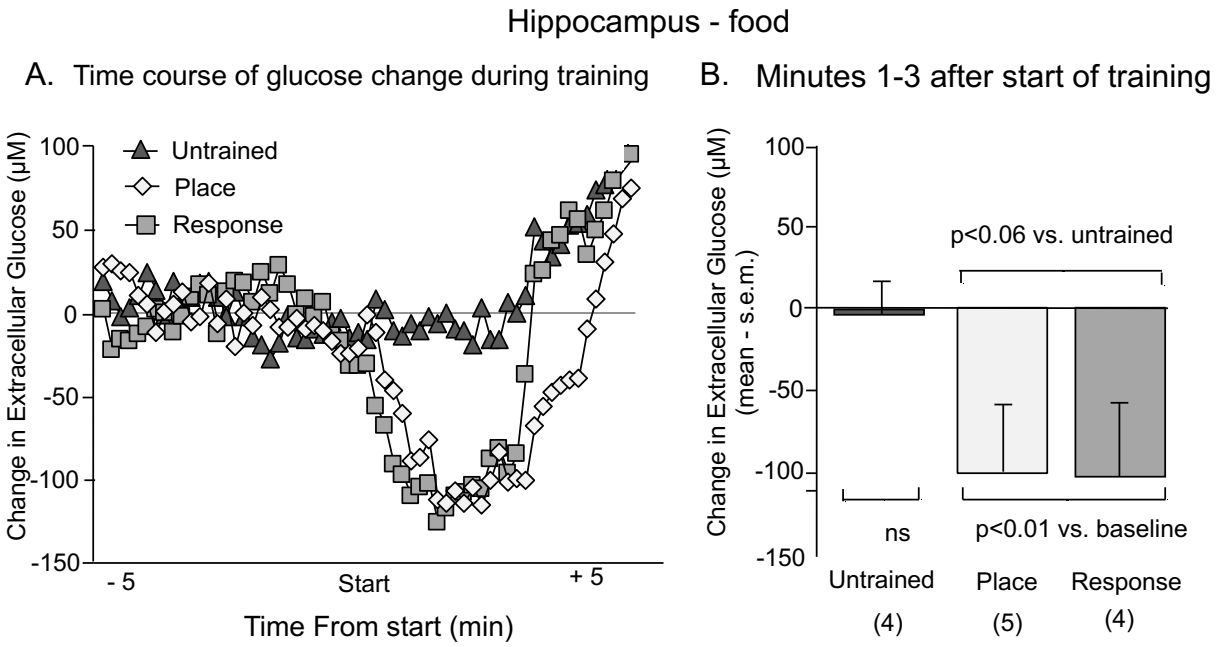

Figure 2. A. Change in extracellular glucose in the hippocampus from 5 min before to 5 min after the start of training for food reward. B. Mean changes in glucose levels during minutes 1-3 after the start of training. Note that the trained groups exhibited significant decreases in glucose levels at this time.

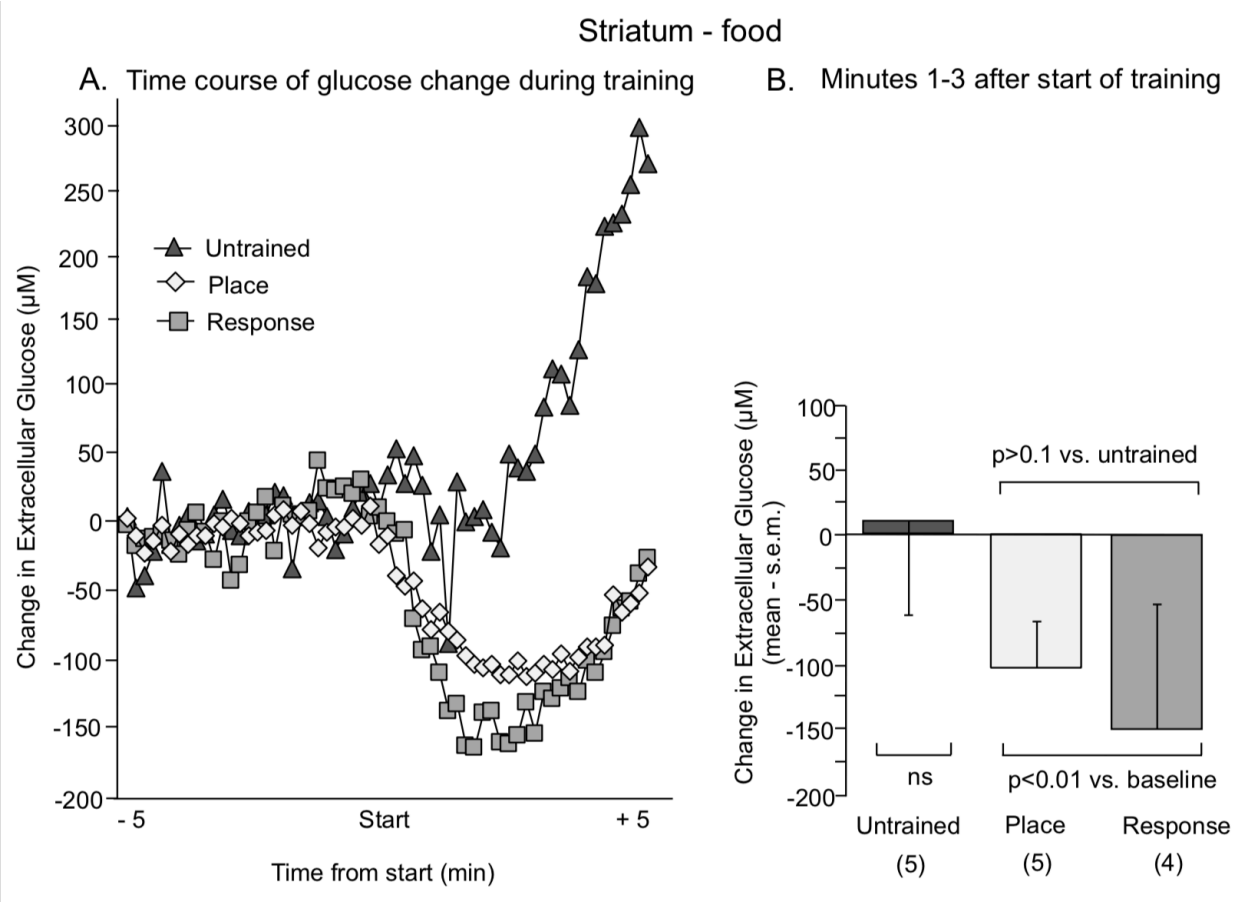

Figure 3. A. Change in extracellular glucose in the striatum from 5 min before to 5 min after the start of training for food reward. B. Mean changes in glucose levels during minutes 1-3 after the start of training. Note that the trained groups exhibited significant decreases in glucose levels at this time.

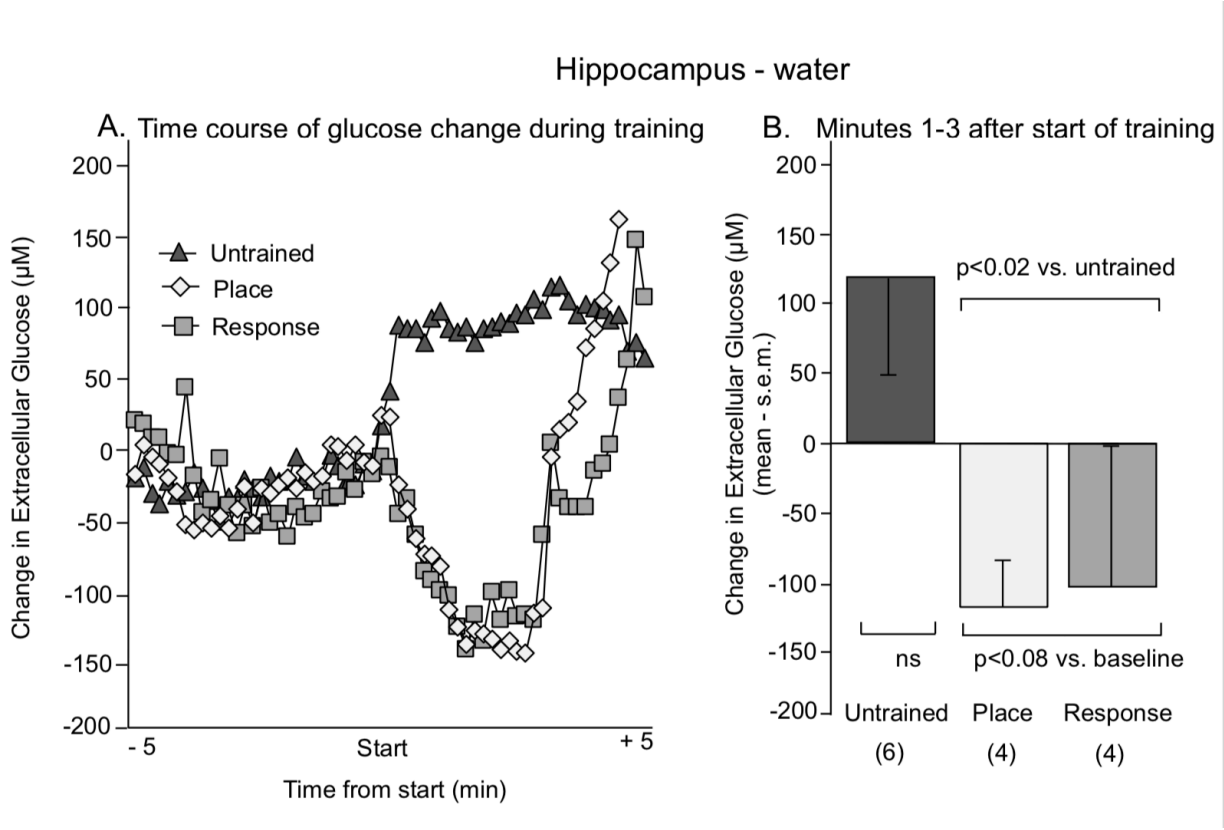

Figure 4. A. Change in extracellular glucose in the hippocampus from 5 min before to 5 min after the start of training for water reward. B. Mean changes in glucose levels during minutes 1-3 after the start of training. Note that the trained groups exhibited decreases in glucose levels at this time. However, this difference was not statistically significant vs. either baseline or vs. untrained groups.

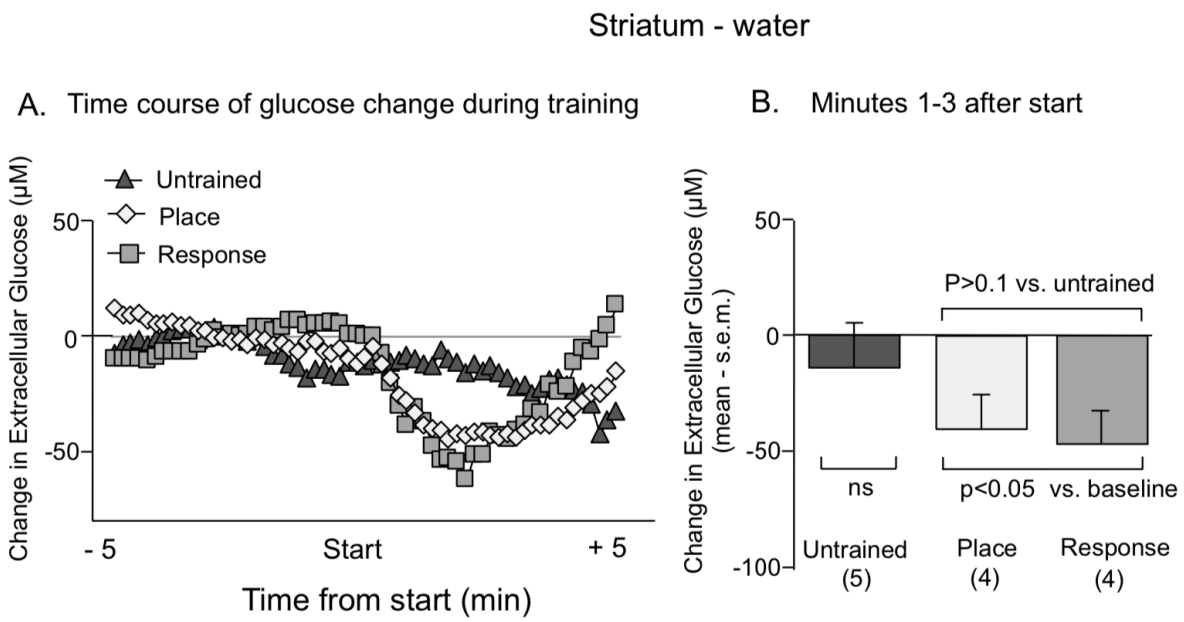

Figure 5. A. Change in extracellular glucose in the striatum from 5 min before to 5 min after the start of training for water reward. B. Mean changes in glucose levels during minutes 1-3 after the start of training. Note that both trained groups but not the untrained group exhibited decreases in glucose levels at this time.

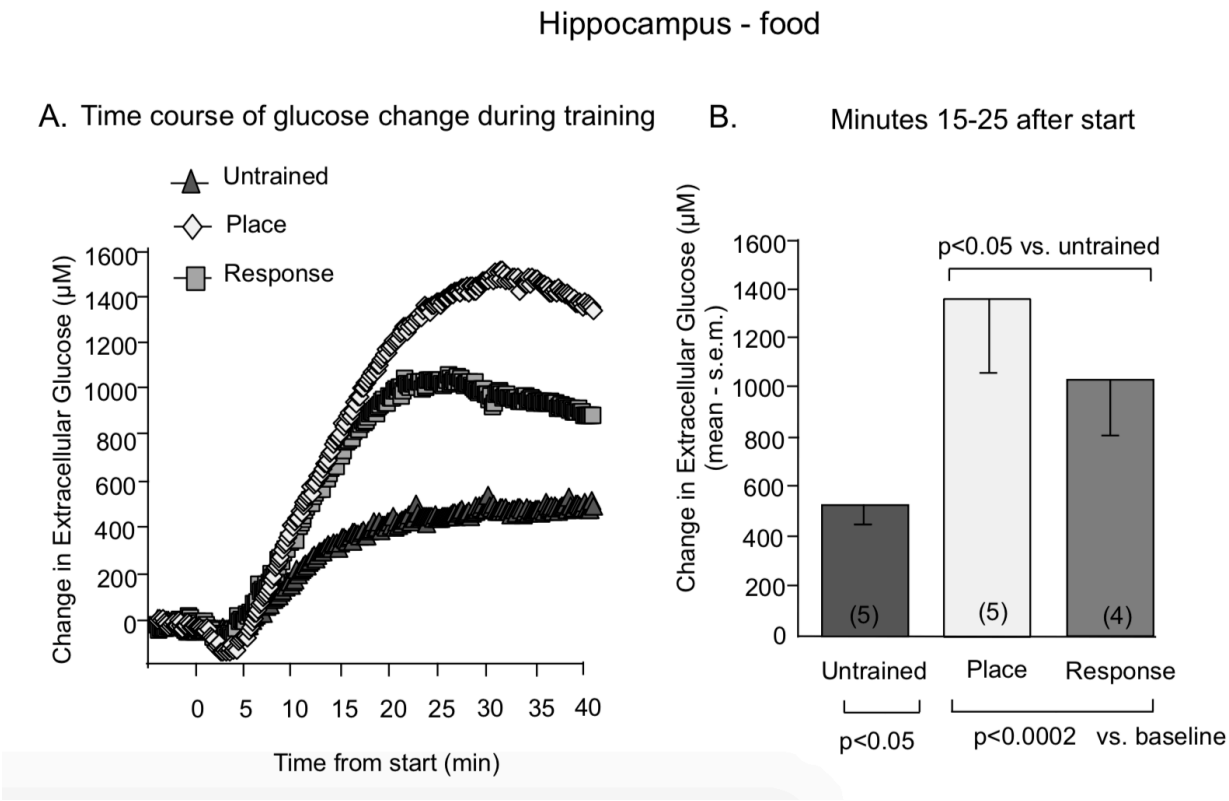

Figure 6. A. Change in extracellular glucose in the hippocampus from 5 min before to 40 min after the start of training for food reward. B. Mean changes in glucose levels during minutes 15-25 after the start of training. All groups, including the untrained group, exhibited significant increases in hippocampal glucose levels above baseline Note that glucose levels in trained rats were significantly higher than those of untrained rats.

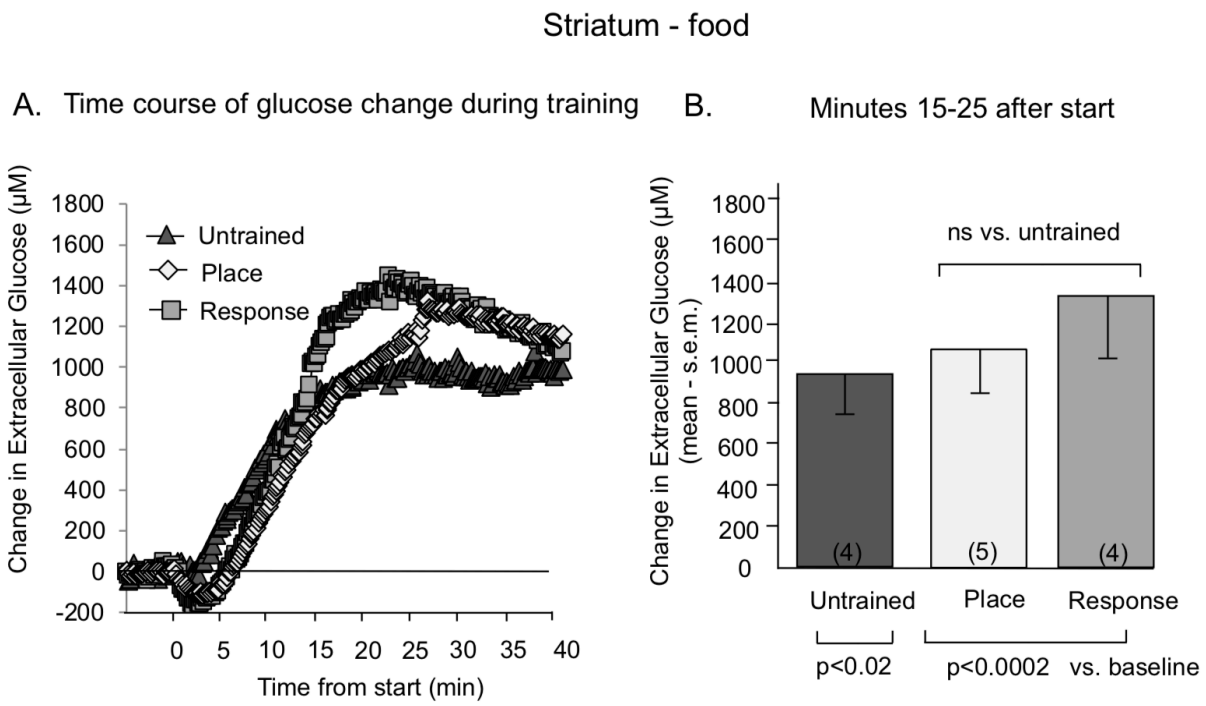

Figure 7. A. Change in extracellular glucose in the striatum from 5 min before to 40 min after the start of training for food reward. B. Mean changes in glucose levels during minutes 15-25 after the start of training. All groups, including the untrained group, exhibited significant ncreases in striatal glucose levels above baseline. Note that glucose levels in trained rats were only slightly higher than those of untrained rats; the difference were not statistically significant.

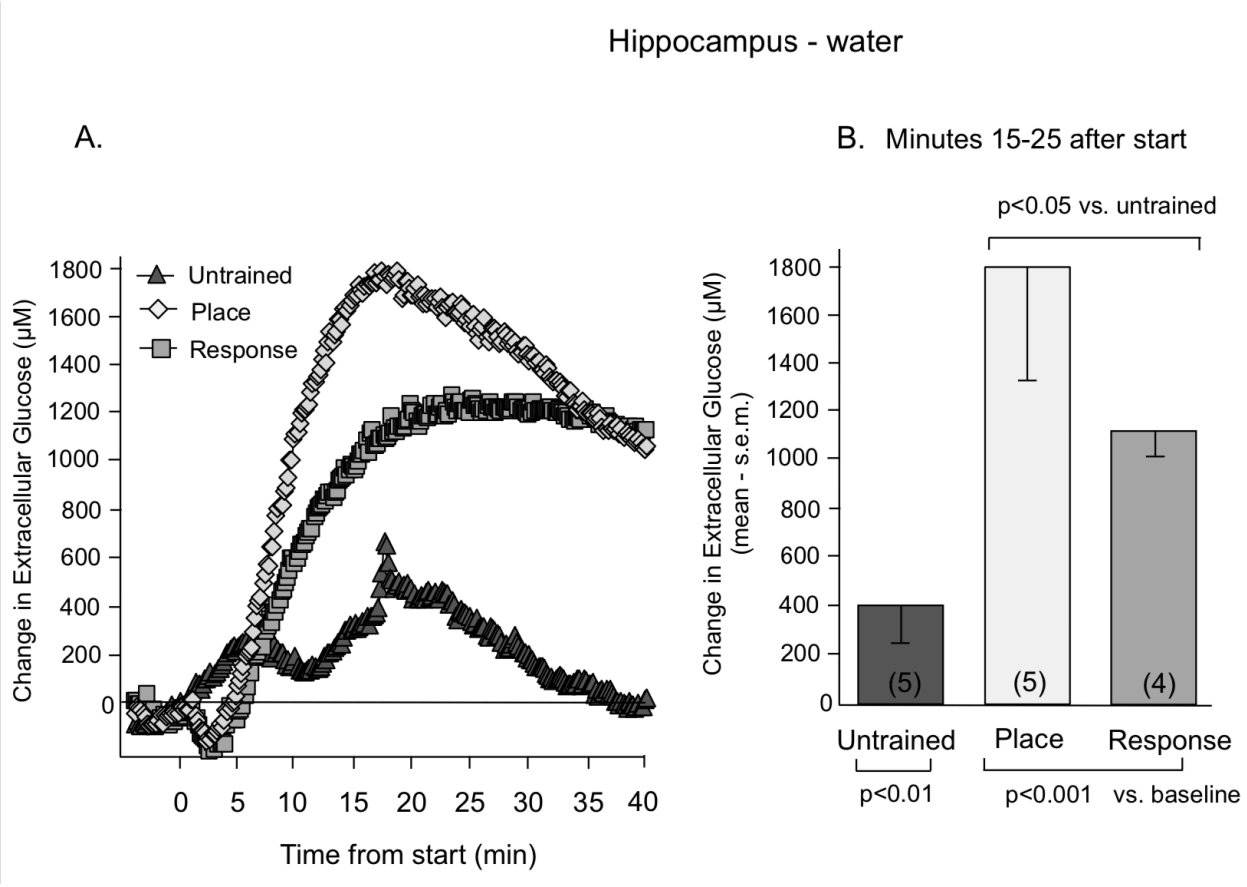

Figure 8. A. Change in extracellular glucose in the hippocampus from 5 min before to 40 min after the start of training for water reward. B. Mean changes in glucose levels during minutes 15-25 after the start of training. All groups, including the untrained group, exhibited significant increases in hippocampal glucose levels above baseline. Note that glucose levels in trained rats were significantly higher than those of untrained rats.

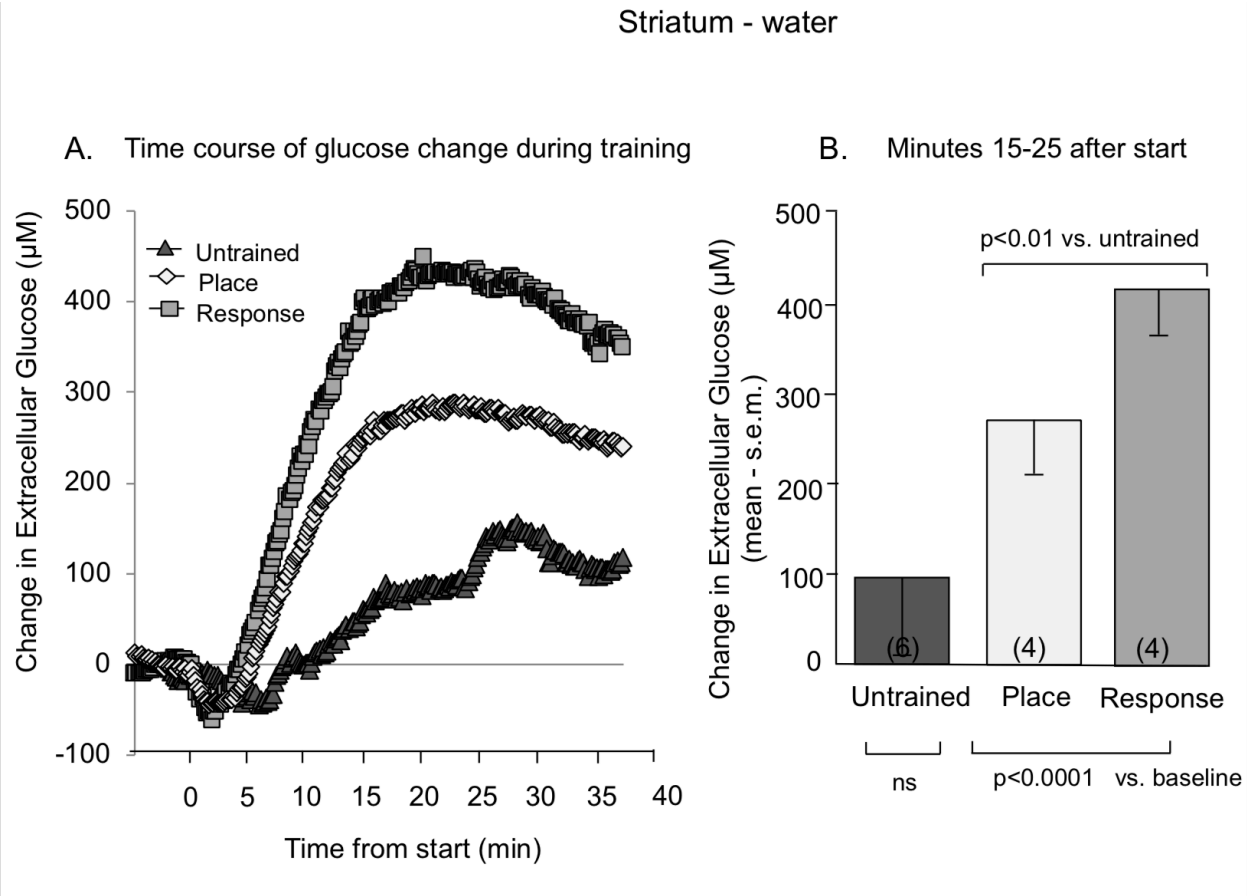

Figure 9. A. Change in extracellular glucose levels in the striatum from 5 min before to 40 min after the start of training for water reward. B. Mean changes in glucose levels during minutes 15-25 after the start of training. The trained groups but not the untrained group had a significant increase in striatal glucose levels above baseline. Note that glucose levels in trained rats were significantly higher than those of untrained rats.

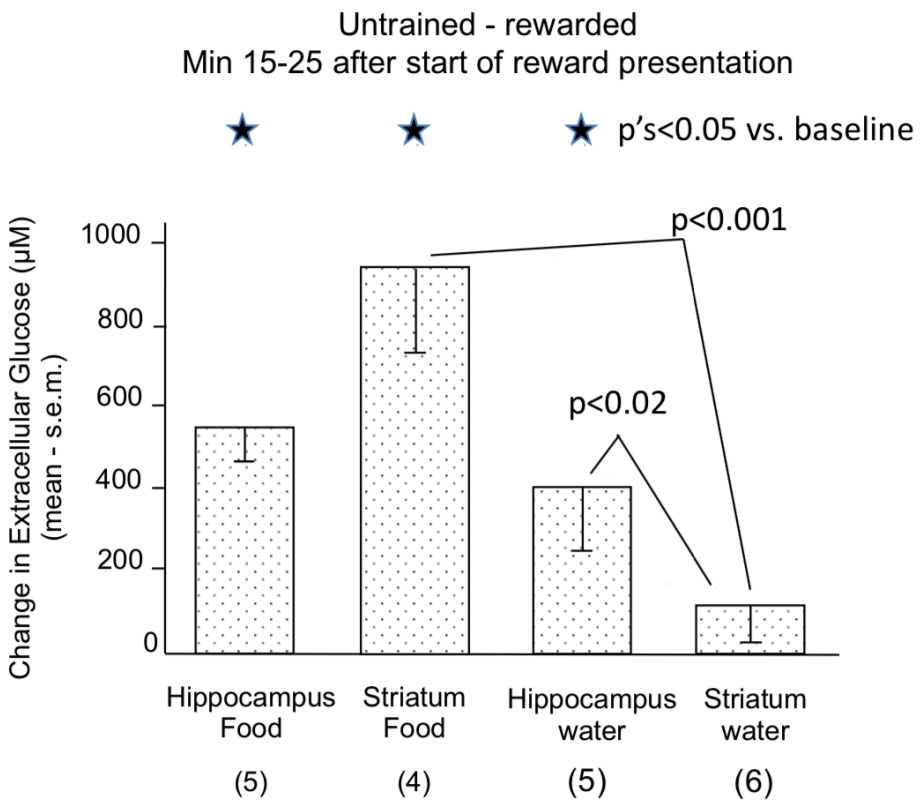

Figure 10. Increases in extracellular glucose levels in the hippocampus and striatum 15-25 min after the start of reward presentation. Food rewards significantly increased extracellular glucose levels in both brain areas. Water rewards significantly increased extracellular glucose levels in the hippocampus but not striatum. In the hippocampus, the increases after food or water did not differ significantly. However, in the striatum, the increase to food was significantly above that to water.

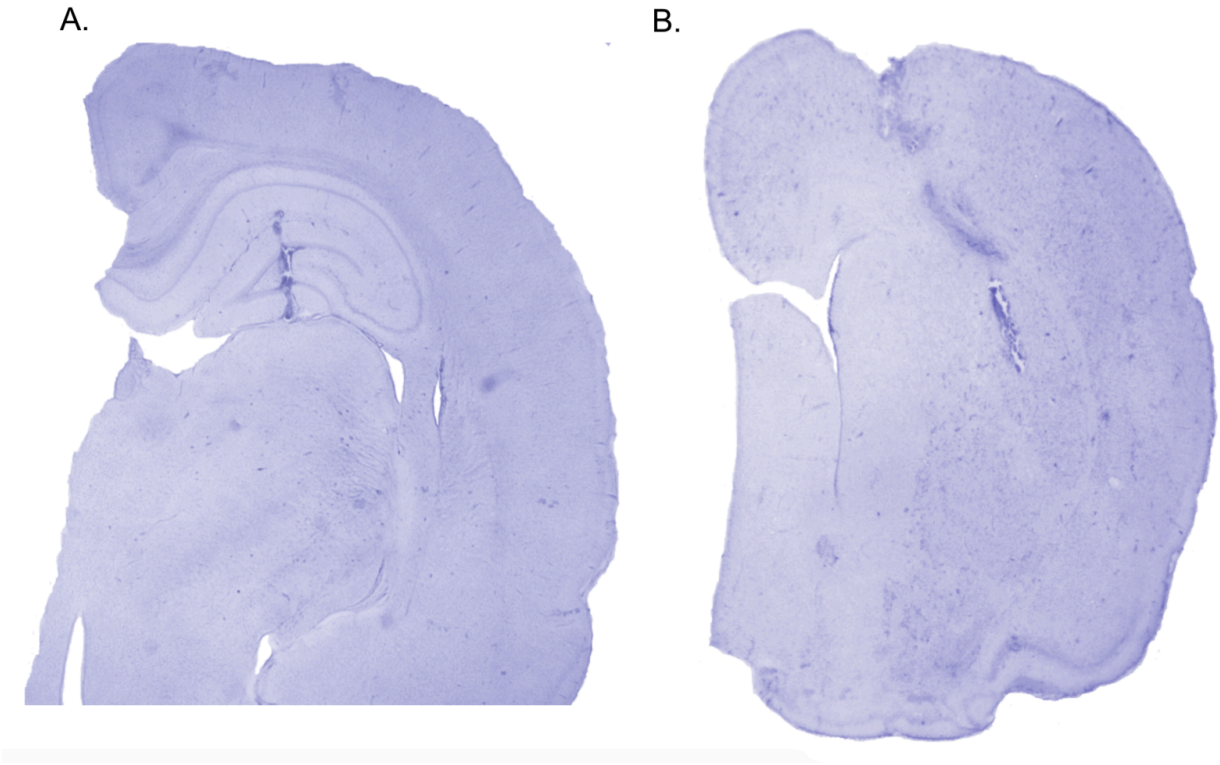

Figure 11. Histological examples of biosensor probe placements in the hippocampus (A) and striatum (B). Probes were located accurately in all rats in this study.