Homologous involvement of striatum and prefrontal cortex in rodent and human water maze learning

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Abstract

The multiple memory systems hypothesis posits that dorsal striatum and hippocampus are central nodes in independent memory systems, supporting response-based and place-based learning, respectively. While our understanding of the function of hippocampus within this framework is relatively well established, the contribution of dorsal striatum is less clear. This in part, appears to be due to the heterogeneous nature of dorsal striatum, which receives extensive topographically organized projections from higher cortical areas. Here we quantified neural activity in the intact brain while mice and humans acquired analogous versions of the Morris water maze. We found that dorsomedial striatum and medial prefrontal cortex support the initial acquisition of what is typically considered a hippocampus-dependent spatial learning task. We suggest that the circuit involving dorsomedial striatum and medial prefrontal cortex identified here plays a more task-independent role in early learning than currently thought. Furthermore, our results demonstrate that dorsomedial and dorsolateral striatum serve fundamentally different roles during place learning. The remarkably high degree of anatomical overlap in brain function between mouse and human observed in our study emphasizes the extent of convergence achievable with a well matched multilevel approach.

Introduction

The multiple memory systems hypothesis posits that hippocampus and dorsal striatum are central nodes in independent memory systems, each supporting different aspects of learning and memory formation (1-4). In the context of spatial learning, the hippocampus supports place-based behavioral strategies relying on learning the general layout of the environment, whereas the dorsal striatum supports response-based behavioral strategies driven by task specific stimuli (5-12). Although the dorsal striatum is often referred to as a unitary structure within the multiple memory systems framework, there is considerable evidence in rodents and humans that it is composed of functional subdivisions that support different aspects of learning (13-20).

Here we conducted parallel experiments in mouse and human to test if dorsomedial and dorsolateral striatum make distinct contributions during early (initial acquisition) and late (overtraining) phases of place learning in the intact brain. Subjects performed the classic hippocampus-dependent hidden platform version of the Morris water maze which was matched between species with respect to behavioral processing demands (9). This widely used paradigm has served a critical role in the study of neurobiological aspects of learning and memory over the past 25 years (21, 22).

While it is well established that hippocampus supports the spatial processing demands of the water maze, much less is known about the precise contribution of dorsomedial and dorsolateral striatum during the early and late phases of learning in the hidden-platform version of the task. If dorsomedial striatum plays a general role in goal-directed learning, we predict that this region will support the non-spatial cognitive processing component of water maze learning important during initial task acquisition. We also targeted medial prefrontal cortex with the expectation that its contribution to water maze learning is concomitant with dorsomedial striatum, since together these structures form a corticostriatal network that has been implicated in goal-directed learning (16, 23). Although it remains controversial as to whether rodents possess prefrontal cortical regions similar to humans and other primates (24, 25), few studies have directly examined if corresponding behavioral processes in rodent and human are served by functionally homologous regions within prefrontal cortex (13). We also expected functional differences between dorsal striatum subdivisions since dorsolateral striatum plays an important role in habit learning, leading to the

prediction that it will support water maze performance once the task has been overtrained. In order to focus on non-spatial cognitive processes, we compared water maze learning to a free-swimming control condition known to also include a spatial processing component (26). To ensure that the comparison between place learning and free-swimming isolated these processes, we included hippocampus (CA1 in mouse, posterior hippocampus in human) as a control region where differences were not expected.

Complementary imaging techniques were used in each species to identify the involvement of target structures during learning. In mouse, expression of immediate early gene (IEG) *zif268* provided a molecular marker of learning-related neuronal activation. *zif268* plays a critical role in synaptic plasticity and the consolidation of long-term memories (27-29) and has been frequently used to visualize brain activation in rodents following behavioral training, including spatial learning tasks (30). In human, functional magnetic resonance imaging (fMRI) was used to measure the hemodynamic response to brain activation (31). Both techniques enable the quantification of distributed patterns of cortical and subcortical brain activity, while preserving the integrity of neural circuits and neuronal functioning.

Results

Behavioral learning profile in mouse

Two experimental groups of mice were trained on the hidden-platform version of the Morris water maze for 3 days (n = 7) and 30 days (n = 8). Behavioral performance after 3 days of training was characterized by a search pattern that was goal-directed but variable. In contrast, after 30 days of training the search pattern was highly focused on the hidden platform location (Fig. 1*A*). Both groups significantly decreased latency and search proximity over the course of training (3 day group: $F_{2, 12} \geq 4.06$, $P < 0.05$; 30 day group $F_{29, 203} \geq 33.8$, $P < 0.001$; Fig. 1*B*). A direct comparison between experimental groups indicates that the 30 day group performed significantly better than the 3 day group on the final training day ($T_{1, 13} \geq 7.7$, $P < 0.001$). Furthermore, performance of the 30 day group plateaued after 10-15 days (Fig. S1*AB*). These results confirm that the 3 day group and 30 day group represent early and late learning phases, respectively.

Dorsomedial striatum and medial prefrontal cortex are involved in early place learning in mouse

To identify *learning specific* changes in *zif268* expression experimental groups were compared to free-swimming control groups (3 day, n = 8; 30 day, n = 8), who explored the same environment except that the hidden platform and distal cues were not present. Experimental and free-swimming control groups were matched with respect to the overall amount of time spent swimming on each day. A non-swimming caged control group (n = 8) was also included to provide a baseline measure of *zif268* expression. In all brain regions experimental and control groups displayed significantly higher *zif268* expression compared to the caged control group (one-way between groups ANOVA; main effect of group: $F_{19, 124} = 69.5$, $P < 0.001$; post hoc tests: $P \leq 0.05$).

The strongest support for a phase-dependent contribution to place learning was observed in dorsomedial striatum. *zif268* expression decreased between early and late learning to a greater extent in the experimental groups than in the free-swimming control groups (condition x learning phase interaction: $F_{1, 27} = 5.3$, $P < 0.05$; Fig. 2*AB*), indicating a specific contribution to early place learning. Additionally, *zif268*

expression in the experimental groups was significantly higher than the free-swimming control groups in both learning phases (post hoc tests: $P \leq 0.05$), suggesting dorsomedial striatum remained involved in more general aspects of task performance. While dorsolateral striatum did not exhibit learning specific changes, *zif268* expression was significantly higher in the experimental groups during both phases of learning (main effect of condition: $F_{1, 27} = 36.0$, $P < 0.001$; Fig. 3*A*). Direct comparison between dorsomedial and dorsolateral striatum in the experimental groups confirmed that the pattern of learning related changes in *zif268* expression was different between dorsal striatum subdivisions (region x learning phase interaction: $F_{1, 26} = 53.5$, $P < 0.001$).

In medial prefrontal cortex *zif268* expression was significantly higher in the experimental groups compared to the free-swimming control groups (main effect of condition: $F_{1, 27} = 40.3$, $P < 0.001$), and decreased in both conditions from early to late learning (main effect of learning phase: $F_{1, 27} = 12.3$, $P < 0.01$; condition x learning phase interaction not significant: $F_{1, 27} = 2.5$, $P = 0.13$; Fig. 2*AC*). Although this pattern of results only indicates a general role in task performance, medial prefrontal cortex *zif268* expression was negatively correlated with search proximity during the early phase of learning in the experimental mice ($r = -0.84$, $P < 0.05$; Fig. S2). That is, higher levels of *zif268* corresponded to lower search proximity values (indicating better performance). Search proximity was not positively correlated with *zif268* late in learning ($r = 0.03$, $P = 0.94$). These findings suggest that in addition to dorsomedial striatum, involvement of the medial prefrontal cortex was also important during early place learning. In the CA1 region of hippocampus we did not find differences between experimental and free swimming control groups (main effect of condition: $F_{1, 18} = 0.3$, $P = 0.59$) or evidence of learning related changes (condition x learning phase interaction: $F_{1, 18} = 0.1$, $P = 0.74$; Fig. 3*B*) confirming that dorsomedial striatum together with medial prefrontal cortex subserve non-spatial cognitive aspects of place-based learning in the water maze.

Behavioral learning profile in human

Human subjects (n = 18) performed a virtual version of the Morris water maze task designed to closely match the processing demands of the rodent version in order to test if target brain areas in human display a similar phase-dependent function in place learning. Subjects learned the location of a hidden platform over the course of three training sessions that were 3-4 days apart. Task-related fMRI data were acquired in session 1 to capture early place learning and in session 3 to measure the late learning phase after subjects had been overtrained outside the scanner in session 2 (6 time series or 'runs' were acquired in each session). During ‘search’ trials subjects intercepted the hidden platform in order to learn its location, similar to the trials performed by the experimental mouse groups. The behavioral performance of subjects in session 1 was characterized by a search pattern similar to that observed during early learning in mouse, i.e., goal-directed but variable (Fig. 1*A*). Latency and search proximity decreased significantly over the course of the first scan session ($F_{5, 85} \geq 15.6$, $P < 0.001$; Fig. 1*B* and Fig. S1*CD*). Significant reductions were also found for both measures during the behavioral training session ($F_{5, 85} \geq 2.7$, $P < 0.05$; Fig. S1*CD*), suggesting that learning had not plateaued in the first scan session. The search pattern during the final scan session (session 3) was highly focused on the hidden platform location, again similar to that observed in mouse. Neither latency nor search proximity decreased further during this scan session ($F_{5, 85} \leq 0.92$, $P > 0.47$) indicating stable performance. A direct comparison between scan sessions revealed that performance was significantly better during the second scan session compared to the first ($F_{1, 17} \geq 35.5$, $P < 0.001$). These results confirm that the first and second scan sessions represent early and late learning phases, respectively.

Phase-dependent contribution to place learning in dorsomedial striatum and medial prefrontal cortex is similar in mouse and human

We first identified brain areas contributing to virtual Morris water maze performance by testing which areas responded more strongly to search trials than control trials. During control trials subjects freely explored the water maze environment in the absence of the hidden platform and distal cues. Task performance was associated with significant activations (statistical threshold: FWE corrected, $P < 0.05$) in bilateral dorsomedial striatum (caudate), bilateral dorsolateral striatum (putamen), and bilateral prefrontal cortex.

Based on prior knowledge of functional subdivisions within dorsomedial and dorsolateral striatum (32), these activations were subdivided into dorsal/ventral and anterior/posterior regions of interest. The resulting subdivisions were then subjected to further analysis to identify learning specific activations. The most striking finding in human was a phase-dependent contribution of the dorsal posterior subdivision of dorsomedial striatum to early learning, consistent with what was observed in mouse (Fig. 2*D*). Activation in this region decreased significantly from early to late learning on search trials, but not on control trials (trial type x learning phase interaction: $F_{1,\ 17} = 10.06$, $P < 0.01$; see Fig. S3*AB* for anterior and ventral subdivisions). In contrast to dorsomedial striatum, activation in dorsolateral striatum did not exhibit learning specific changes but remained significantly higher on search trials compared to control trials across both learning phases (trial type x learning phase interaction: $F_{1,\ 17} \leq 2.06$, $P \geq 0.17$; Fig. 3*C* and Fig. S2*CD*). Direct comparison between posterior dorsomedial and dorsolateral striatum on search trials confirmed that there was a difference in the evolution of learning related changes in activation between dorsal striatum subdivisions (region x learning phase interaction: $F_{1,\ 17} = 5.0$, $P < 0.05$).

Since the dorsal posterior subdivision of dorsomedial striatum was the only area that exhibited a clear pattern of learning specific activation, we tested if a functionally connected subregion of prefrontal cortex was activated in a similar manner. First we determined which voxels in prefrontal cortex were i) functionally connected to dorsomedial striatum (resting state fMRI data acquired at the start of session 1 served as an independent measurement) and ii) responded more strongly to search than control trials. Based on these criteria we identified a single region of interest in medial prefrontal cortex (40 voxels extending from pre-supplementary motor area into dorsal anterior cingulate cortex; Fig. 2*E*). In this region we found a significant decrease in activation on search trials, but not control trials (trial type x learning phase interaction: $F_{1,\ 17} = 14.7$, $P < 0.01$). This pattern of activation is indeed similar to that observed in the dorsal posterior subdivision of dorsomedial striatum and indicates that both areas support early place learning, a finding that is consistent across human and mouse experiments.

Finally, we tested if search trials were different to freely exploring the environment in the control condition with respect to hippocampus-dependent spatial processing demands. At the whole brain level we did not find any voxels in the hippocampus that responded more strongly to search trials than control trials (even at a more lenient statistical threshold of $P < 0.001$ uncorrected). In order to further increase our sensitivity to detect differences in activation we restricted our analysis to the posterior subdivision of hippocampus, the region most likely to be engaged in spatial processing tasks in human (5, 33). We defined hippocampal subdivisions by performing a cluster analysis on resting state data, a technique that parcellates a brain area based on its profile of spontaneous activity (34). Within the posterior hippocampus subdivision we did not observe a difference in activation between search and control trials (main effect of trial type: $F_{1,\ 17} = 2.28$, $P = 0.15$) or find any evidence of learning specific activity (trial type x learning phase interaction: $F_{1,\ 17} = 0.22$, $P = 0.64$; Fig. 3*D*).

Discussion

Here we have demonstrated that dorsomedial striatum and medial prefrontal cortex support the initial acquisition of what is typically considered a hippocampus-dependent spatial learning task. In the past, work on animal and human memory systems has mostly evolved in separate domains. However, it is now apparent that converging evidence from different model systems is required for a comprehensive understanding of learning and memory processes.

The multiple memory systems hypothesis has often treated the dorsal striatum as a unitary structure that is central to response-based learning (see, e.g., ref. 1). Contrary to this view, we found that dorsomedial but not dorsolateral striatum makes a critical contribution to the early phase of place learning (See SI Text: Additional Discussion). This result is consistent with previous reports of impaired expression of place learning following dorsomedial striatum lesions (18-20, 35-37). For example, when rats performed a water maze task designed to test for a preference between previously learned response-based and place-based strategies, dorsomedial striatum lesions resulted in a lower likelihood of choosing a place-based strategy (4, 19). Yin and Knowlton showed that dorsomedial and dorsolateral striatum make distinct contributions to plus maze performance, linking each subdivision to place-based and response-based strategies, respectively (20). Here we extend this line of evidence by demonstrating in both mouse and human that dorsomedial striatum contributes to place-based learning processes in the intact brain. This is important since most rodent studies investigating the multiple memory systems hypothesis have used maze tasks which require a choice between behavioral strategies when part of the brain is lesioned. While this approach is useful for identifying double dissociations, it is limited to the study of behavior that is produced by an impaired memory system. The IEG expression and fMRI activation we observed in dorsomedial striatum resulted from behavior produced by a fully intact memory system and provides a complementary form of evidence to the aforementioned lesion work. Furthermore, our data support Yin and Knowlton's recommendation that the multiple memory systems hypothesis should be revised to take into account functional subdivisions within the dorsal striatum (20).

It is worthwhile noting that dorsomedial striatum involvement during the early phase of place learning appears to contrast with the findings of other maze learning experiments in human (5, 6, 38, 39). Dorsomedial striatum activations have typically been associated with non-spatial behavioral strategies such as landmark based navigation (5), route following (6, 39) and habit learning (38). This is somewhat surprising given that these behaviors are now generally more associated with the dorsolateral striatum in rodents (17, 20). While the design of maze learning experiments in humans was often inspired by work in rodents, it remains a possibility that subtle task differences can lead to altered processing demands. Importantly, the experimental and control conditions in the present experiment were designed to be as similar as possible between species.

We also observed learning specific activation in medial prefrontal cortex, suggesting that together with dorsomedial striatum these areas form a cortico-subcortical loop that supports early place learning. What then, is the specific role of dorsomedial striatum and medial prefrontal cortex? Our experiments were designed to specifically target non-spatial processing during place learning in the water maze. A difference in IEG expression and fMRI activation between water maze learning and free-swimming control conditions in hippocampus would likely reflect a difference is spatial processing demands between conditions. However, differences were not observed in CA1 (mouse) and posterior hippocampus (human), indicating that the increased IEG expression and fMRI activation in dorsomedial striatum and prefrontal cortex during early learning is unlikely to be related to spatial processing demands. Others have shown that neurons in the dorsomedial striatum are most active during maze navigation at decision making locations, at reward

locations, and at the location of cues predicting reward delivery (40, 41). This is in contrast to hippocampal neurons that usually represent a single location in space. Thus, dorsomedial striatum appears to encode environmental information relevant to the successful outcome of a task that results in immediate or delayed reward. Interestingly, this suggests a more generalized role for dorsomedial striatum in navigation tasks, regardless of whether a spatial or non-spatial strategy is used. In human, evidence from instrumental conditioning tasks highlighted a similar role for the dorsomedial striatum in learning actions and their reward consequences (42, 43).

In addition to dorsomedial striatum we observed concomitant activity in medial prefrontal cortex during early place learning. Medial prefrontal cortex projects extensively to dorsomedial striatum and our data suggests these regions might interact to serve related processes during early place learning. In particular, imaging work in humans attempting to delineate the executive functions of medial prefrontal cortex has revealed its involvement in conflict monitoring, error detection, and processes driving reinforcement learning (44, 45). Brain activity reported in these studies was in close anatomical proximity to the location found in our place learning task. Recently, Alexander and Brown proposed a new model to reconcile previous theories: They posit that medial prefrontal cortex encodes action-outcome associations in relation to environmental information processed in a specific task context (46). This general function exhibits remarkable similarities to the properties identified for neurons in dorsomedial striatum during maze navigation described above. Most importantly, evidence suggests that medial prefrontal cortex activity reflects surprise resulting either from the occurrence of unexpected events or the non-occurrence of expected events (46). Even though experimental evidence for this theory has mainly been derived from classical reinforcement and decision making paradigms, the occurrence of these types of events is ubiquitous in many learning tasks including maze navigation. For example, consider a human or mouse approaching the hidden platform but suddenly realizing that it has been missed based on environmental information such as being too close to the pool wall. This would likely be an example of surprise resulting from not finding the platform (and the associated reward) at the expected location. Thus, our results in combination with recent findings give rise to the hypothesis that dorsomedial striatum in conjunction with medial prefrontal cortex process environmental information in order to detect deviations from an expected behavioral outcome. Consequently, the dorsomedial striatum – medial prefrontal cortex circuit identified here appears to support learning in many different task categories including reinforcement learning, motor skill acquisition and spatial learning.

In conclusion, we have demonstrated that dorsomedial striatum and medial prefrontal cortex play an important role during early place learning in a task typically thought to be hippocampus-dependent. Most strikingly, the pattern of activation observed in our target regions was remarkably similar between mouse and human, providing converging evidence from two model systems that are mostly studied independently. Our results provide further evidence that dorsomedial and dorsolateral striatum serve fundamentally different functions during place learning. Based on our findings and related work in humans and rodents using other learning tasks, we hypothesize that the identified dorsomedial striatum – medial prefrontal cortex circuit might play a much more task-independent role in early learning than currently thought.

Methods

Mouse experiment

Subjects

Eight week old female C57BL/6J mice (Centre D'Elevage Janvier) were group housed (5-7 mice per cage) in standard cages with wood-shaving bedding. Food and water were available *ad libitum* and mice were handled for 1 week (tail coloring) prior to the start of behavioral testing. The housing environment was temperature and humidity controlled with a 12 h light-dark cycle (lights on at 8 AM). Behavioral testing was performed during the light phase. All procedures were approved by the ethical research committee of KU Leuven in accordance with the Declaration of Helsinki.

Behavioral procedures

Mice were trained on the hidden platform version of the Morris water maze (see SI Text: Behavioural Procedures for details on apparatus; complete methods are included in SI Text.). Each trial began at one of four starting locations by placing the mouse at the edge of the pool facing toward the center. During trials the experimenter remained seated at a fixed location. When a trial was not completed in 2 min the mouse was guided to the platform and remained there for 15 s.

All mice arrived in the laboratory at the same time and were handled daily. From the start of the experiment all cages were transferred to the training room each day. Experimental mice were trained to find the hidden platform for 3 days (1 session of 4 trials per day) and 30 days (2 sessions of 4 trials per day for the first 25 days of training, then 1 session of 4 trials per day for the remaining 5 days; 5 consecutive training days were followed by 2 rest days). Trials in each session were separated by a 15 minute break, and when two sessions were performed on a single day they were separated by 2 hours. Free-swimming control mice (3 day and 30 day) explored the same environment except that the hidden platform and distal cues were removed. With distal cues present in the free-swimming condition goal-directed navigation and learning remains possible (albeit not learning of an escape platform location). Therefore the likelihood of achieving true free-swimming performance (i.e. not goal-directed) was optimised by the removal of distal cues. Non-swimming caged control mice did not receive any water maze training but were always transferred between housing and training rooms together with the other 4 groups during the 30 day testing period. All mice were 15 weeks old on the final day of training.

Behavior was recorded using Ethovision video tracking equipment and software (Noldus). Overall task performance was evaluated by calculating the time taken to find the hidden platform (latency). Spatial performance was evaluated by calculating the average distance between the mouse and the hidden platform (search proximity). A repeated measures one-way ANOVA was used to test for learning related changes in the experimental groups. The α-level was set to 0.05.

Quantitative in situ hybridization to determine zif268 expression

zif268 in situ hybridization was performed using previously established methods in our laboratory (47). Briefly, animals where sacrificed at the age of 15 weeks by cervical dislocation 45 minutes after the final training trial and brains were immediately frozen in 2-methylbutane (Merck) at a temperature of -40 °C. Coronal sections (25 µm) were cut on a cryostat (Microm HM 500 OM) and mounted onto 0.1% poly-L-lysine coated slides (Sigma-Aldrich). A series of brain sections covering the entire rostrocaudal extent of the striatum/anterior cingulate (medial prefrontal cortex) and hippocampus were collected (48) and kept at -30°C. Tissue was postfixed in 4% paraformaldehyde in 0.12M phosphoric acid in phosphate-buffered saline (PBS; 0.1 M, pH 7.4, 30 min, 4°C; 0.9% NaCl), dehydrated (50%, 70%, 98%, 100%, 5 min) and delipidated (100% chloroform, 10 min). The mouse specific synthetic *zif268* probe (NM_007913.5, sequence: 5'-

ccgttgctcagcagcatcatctcctccagyttrgggtagttgtcc-3') was end-labeled with 33P-dATP (NEN) using terminal deoxynucleotidyl transferase (Invitrogen). Unincorporated nucleotides were removed using mini Quick Spin columns (Roche Diagnostics). The radioactive labeled probe was mixed with a hybridization cocktail (50% formamide, 4x standard saline citrate, 1x Denhardt's solution, 10% dextran sulphate, 100 µg/ml Herring sperm DNA, 250 µg/ml tRNA, 60 mM dithiothreitol, 1% N-lauryl-sarcosine, 26 mM NaHPO4 pH 7.4) and applied to a series of dehydrated sections with overnight incubation at a temperature of 37 °C. The next day, the sections were rinsed in 1x standard saline citrate buffer at 42 °C, air-dried and apposed to an autoradiographic film (Kodak) together with a [14C] microscale (GE Healthcare). Films were developed 3 weeks later in Kodak D19 developing solution and fixed in Rapid fixer (Ilford Hypam).

Autoradiographic images were scanned (CanoScan LiDE 600F, Canon) and optical densities (mean gray value per pixel) were quantified with ImageJ (Image processing and analysis in Java, National Institutes of Health). Optical density was measured in three brain sections per mouse along the rostrocaudal axis for each target region. Striatum and medial prefrontal cortex slices were taken from +1.10 mm to +0.38 mm relative to bregma (Fig. S4*A*) and CA1 slices from -1.58mm to -2.54mm relative to bregma (48). Within striatum we targeted dorsolateral and superior dorsomedial subdivisions (Fig. S4*B*). The template of the striatal and medial prefrontal cortex compartments was drawn bilaterally over brain sections. Mean gray values were averaged across hemispheres and brain slices resulting in a single data point for each region per animal. A one-way between groups ANOVA was use to test differences in IEG expression between the caged control group and all experimental and control groups. To test for learning related changes in IEG expression mean gray values were entered into an ANOVA (2 conditions x 2 learning phases). For all analyses the α-level was set to 0.05 and Bonferroni correction applied to post-hoc tests. Statistical analyses were performed in Statistica 9 (StatSoft).

Corticosterone levels

Comparison of corticosterone levels ensured between group differences in IEG expression were not confounded by stress (see SI Text: Corticosterone levels). A one-way between groups ANOVA revealed that corticosterone levels did not differ significantly between groups ($F_{4,\ 26}$ = 1.462, P = 0.24; Fig. S5). Furthermore, previous work has demonstrated that *zif268* expression is generally not influenced by stress (49).

Human experiment

Subjects

Eighteen female subjects (aged 20-28, mean age 23.1) participated in the fMRI study. All were right-handed with no history of neurological disease. Prior to testing subjects were required to provide written informed consent to the procedures, which were approved by the Ethics Committee of the KU Leuven in accordance with the Declaration of Helsinki.

Task

A custom virtual environment analogous to the Morris water maze was constructed in Blender (www.blender.org) and rendered in MATLAB (2007b, The Mathworks) (see SI Text: Task for details on the virtual environment; complete methods are included in SI Text). Subjects viewed the room from a first-person perspective, and moved around by pressing buttons on an MRI compatible button box (Current Designs Inc.).

Trial procedures

Over the course of the experiment subjects performed 'search' and 'control' trials, which were designed to be compatible with our mouse water maze experiment. All trials began from one of four starting zones

(separated by 90°) located at the perimeter of the pool, with the exact position within a given starting zone varying by ±10° from trial to trial. Subjects always faced the center of the pool at the beginning of the trial.

The goal of search trials was to navigate to the hidden platform as quickly and directly as possible. When the goal location was successfully intercepted the walls of the room turned green for 1 second, after which the subject remained at the same location for a further 3 s. During this 4 s period forward movement and orienting were not possible. The maximum time limit for search trials was 45 s. If a trial reached the maximum time limit the walls of the room turned red for 1 s, after which the subject remained in their final unsuccessful location for a further 3 s (forward movement and orienting were again not possible during this 4 s period).

During control trials subjects moved freely within the pool. No distinguishing features were present on the walls, preventing any goal-directed navigation. Control trials were matched to the average duration of search trials (between 10 and 20 s) and finished in a similar manner, with the only difference being that the color of the walls always turned blue (which did not relate to feedback provided during other trials). See Fig. S6B for screenshots.

A third trial type, 'prediction' trials, required the subject to explicitly indicate where they thought the hidden platform was located via a button press. Analysis of prediction trials is not presented here since we focussed on the conditions closest to the mouse experiment.

Experimental protocol

Four testing sessions were completed, each on a separate day. The first session familiarized subjects with the experimental procedures and trial order prior to scanning. During this session a limited number of trials were performed in a different environment to that used in the main experiment.

One or two days later subjects returned for the first scan session. From this session onwards the environment and the location of the hidden platform was unchanged. Subjects performed 6 runs of trials, with each run lasting at least 8 min. See SI Text: Experimental protocol for the order of trial presentation.

A second identical scan session was performed 6-8 days after the first. Between scan sessions subjects performed a training session during which only behavioral data was acquired. The behavioral training session also consisted of 6 runs of trials each lasting 8 min.

Resting state protocol

In addition to acquiring task-related fMRI data, subjects were also scanned for 7 min in a resting state prior to the onset of task performance. Subjects were required to fixate on a white cross in the center of a black screen, and were instructed to relax and think of nothing in particular.

Behavioral analysis

The same behavioral measures as those previously described in mouse, i.e. latency and search proximity, were also used to quantify performance on the virtual water maze. To test for learning within each session we conducted a one-way repeated measures ANOVA (runs 1-6). Statistical analyses were performed in Statistica 9. The α-level was set to 0.05.

Statistical Analysis of FMRI Data

See SI Text: Image acquisition and Image preprocessing for scan parameters and preprocessing procedures. Search trials, control trials and rest following control trials were modeled for each subject as boxcar functions convolved with the canonical hemodynamic response function within a first-level general linear model. The time series in each voxel was high pass filtered at 1/160 Hz to remove low frequency drifts. The contrasts search>rest and control>rest were specified separately for each run.

Contrasts were entered into a second level random effects ANOVA model with the factors trial type (search>rest and control>rest) and run (runs 1-6 and 13-18). The model was estimated under the assumption of dependent measurements and unequal variances. The *t*-contrast identifying areas

responding more strongly to search than control trials was thresholded at $P < 0.05$, Family-Wise Error (FWE) corrected for multiple comparisons within the whole brain, and only included clusters above 30 voxels.

Further analyses focussed on the striatum, prefrontal cortex and hippocampus. Regions of interest (ROIs) were defined on the basis of a priori anatomical and functional criteria (see SI Text: ROI definition). For each of the ROIs created in striatum, medial prefrontal cortex and hippocampus, the marsbar toolbox (50) was used to extract the mean contrast value of all voxels, i.e. an estimate of the hemodynamic response to either search or control trials (compared to rest) in the area of interest. Unsmoothed images were used to avoid including signal from neighboring regions. To test for changes in activation over the course of learning contrast values were entered into an ANOVA (2 trial types x 2 learning phases). Statistical analyses were performed in Statistica 9. The α-level was set to 0.05. Post hoc tests were Bonferroni corrected.

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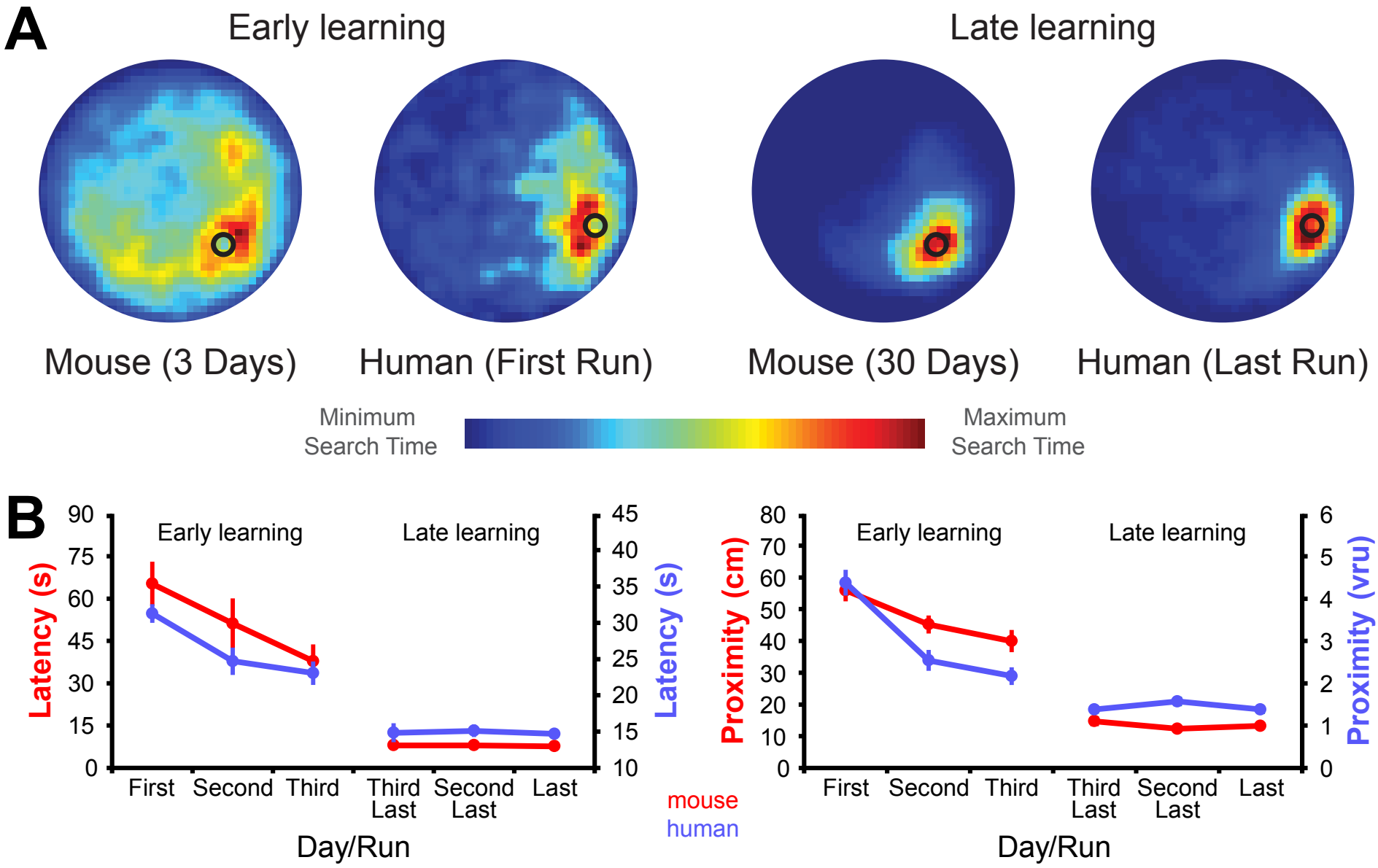
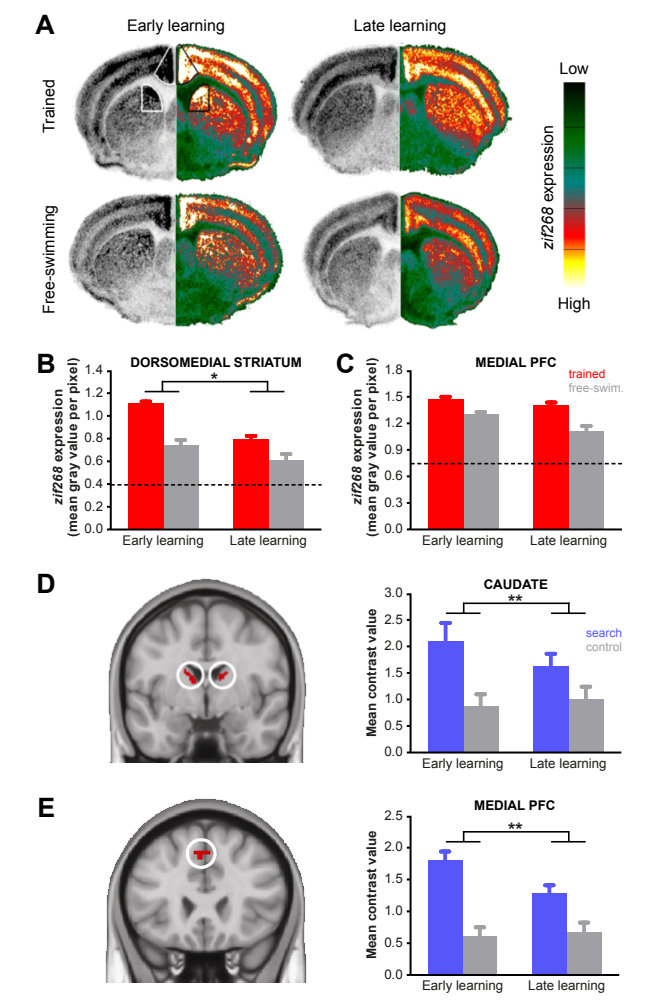
FIGURE LEGENDS

Fig. 1. Behavioral performance on mouse and human versions of the Morris water maze is closely matched. *A* Graphical representation of mouse and human search patterns during early and late learning (hidden platform displayed as back circle). *B* Reduction in latency and search proximity during initial acquisition of the water maze reflects an early learning phase in both species. Stable performance during overtraining is indicative of a late learning phase. Error bars in panel *B* represent SEM. See Fig. S1 for complete learning curves.

Fig. 2. Dorsomedial striatum and medial prefrontal cortex support early place learning in human and mouse. *A* Coronal sections from mouse displaying *zif268* expression during early and late learning in experimental and free swimming control groups. The left hemisphere shows the original autoradiogram in gray scale and is matched on the right by its pseudocolor counterpart. The color scale bar ranges from no signal (0, dark green) to maximum signal (255, white). Striatal and prefrontal subdivisions in mouse were based on known anatomical connectivity (Fig. S4*AB*). *B* A larger reduction in *zif268* expression between early and late learning was observed in the experimental groups than in the free swimming controls, suggesting a specific role for this region during early learning. *C zif268* expression in medial prefrontal cortex decreased from early to late in both experimental and free swimming control groups. *D* Posterior dorsomedial striatum in human (image displayed at MNI coordinate y = -3) responded more strongly to search trials than control trials ($P < 0.05$, FWE corrected). Subdivisions within human striatum were based on prior knowledge regarding functional differences (Fig. S4*CD*). *E* Medial prefrontal cortex in human (y = 24) responded more strongly to search trials than control trials ($P < 0.05$, FWE corrected) and was functionally connected to the dorsomedial striatum at rest ($P < 0.05$, FDR corrected). Mean contrast values were extracted from the activations shown in the left of *D* and *E* and are plotted to the right of each image. Error bars represent SEM. * indicates significant interaction $P < 0.05$. ** indicates significant interaction $P < 0.01$.

Fig. 3. Dorsolateral striatum (ventral anterior subdivision displayed in human; see Fig. S3*CD* for other subdivisions) and hippocampus did not show learning specific changes in mouse (*AB*) or human (*CD*). *AC* In dorsolateral striatum, activity during water maze learning and the free-swimming control condition was greater in comparison to baseline in both species (caged controls in mouse and rest in humans). Furthermore, a generalized increase in water maze learning compared to free-swimming was observed in both species across early and late phases of training. See SI Text: Additional Discussion. *BD* No differences were observed between experimental and free swimming control groups in CA1 (mouse) or posterior hippocampus (human). Error bars represent SEM.


A
Early learning
Late learning
Mouse (3 Days)
Human (First Run)
Mouse (30 Days)
Human (Last Run)
Minimum Search Time
Maximum Search Time
B
Early learning
Late learning
Latency (s)
Latency (s)
90
75
60
45
30
15
0
45
40
35
30
25
20
15
10
First
Second
Third
Third Last
Second Last
Last
Day/Run
mouse
human
Proximity (cm)
Proximity (vru)
80
70
60
50
40
30
20
10
0
6
5
4
3
2
1
0


A
Early learning
Late learning
Trained
Free-swimming
Low
zif268 expression
High
B
DORSOMEDIAL STRIATUM
zif268 expression
(mean gray value per pixel)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Early learning
Late learning
C
MEDIAL PFC
trained
free-swim.
zif268 expression
(mean gray value per pixel)
1.8
1.5
1.2
1.0
0.9
0.6
0.3
0.0
Early learning
Late learning
D
CAUDATE
Mean contrast value
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
search
control
Early learning
Late learning
E
MEDIAL PFC
Mean contrast value
2.5
2.0
1.5
1.0
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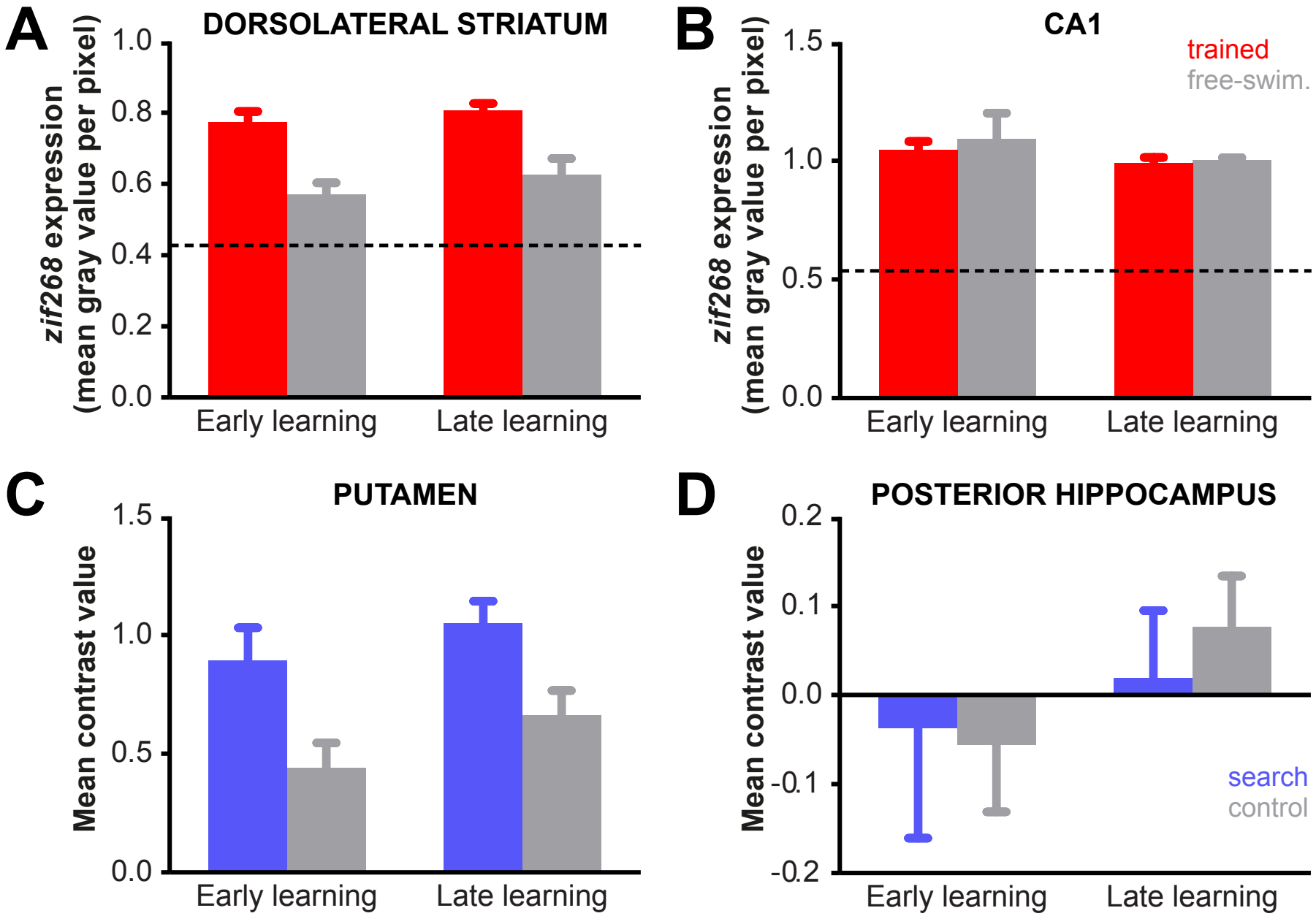
Early learning
Late learning


A
DORSOLATERAL STRIATUM
zif268 expression
(mean gray value per pixel)
1.0
0.8
0.6
0.4
0.2
0.0
Early learning
Late learning
B
CA1
trained
free-swim.
zif268 expression
(mean gray value per pixel)
1.5
1.0
0.5
0.0
Early learning
Late learning
C
PUTAMEN
Mean contrast value
1.5
1.0
0.5
0.0
Early learning
Late learning
D
POSTERIOR HIPPOCAMPUS
Mean contrast value
0.2
0.1
0.0
-0.1
-0.2
search
control
Early learning
Late learning

SUPPORTING INFORMATION

COMPLETE METHODS

Mouse experiment

Subjects

Eight week old female C57BL/6J mice (Centre D'Elevage Janvier) were group housed (5-7 mice per cage) in standard cages with wood-shaving bedding. Food and water were available ad libitum and mice were handled for 1 week (tail coloring) prior to the start of behavioral testing. The housing environment was temperature and humidity controlled with a 12 h light-dark cycle (lights on at 8 AM). Behavioral testing was performed during the light phase. All procedures were approved by the ethical research committee of KU Leuven in accordance with the Declaration of Helsinki.

Behavioral procedures

Mice were trained on the hidden platform version of the Morris water maze. The test apparatus consisted of a large circular pool (diameter 150 cm, height 33 cm) filled with water (25-26°C) to a depth of 16 cm. Water was made opaque with non-toxic white paint to prevent animals from seeing a transparent circular platform (diameter 15 cm, height 15 cm) submerged 1 cm beneath the surface. The platform was located at a fixed position 25 cm from the nearest pool wall. The pool was located in an elevated position in the center of a well-lit room with various distinct visual cues. Each trial began at one of four starting locations by placing the mouse at the edge of the pool facing toward the center. During trials the experimenter remained seated at a fixed location. When a trial was not completed in 2 min the mouse was guided to the platform and remained there for 15 s.

All mice arrived in the laboratory at the same time and were handled daily. From the start of the experiment all cages were transferred to the training room each day. Experimental mice were trained to find the hidden platform for 3 days (1 session of 4 trials per day) and 30 days (2 sessions of 4 trials per day for the first 25 days of training, then 1 session of 4 trials per day for the remaining 5 days; 5 consecutive training days were followed by 2 rest days). Trials in each session were separated by a 15 minute break, and when two sessions were performed on a single day they were separated by 2 hours. Free-swimming control mice (3 day and 30 day) explored the same environment except that the hidden platform and distal cues were removed. With distal cues present in the free-swimming condition goal-directed navigation and learning remains possible (albeit not learning of an escape platform location). Therefore the likelihood of achieving true free-swimming performance (i.e. not goal-directed) was optimised by the removal of distal cues. Non-swimming caged control mice did not receive any water maze training but were always transferred between housing and training rooms together with the other 4 groups during the 30 day testing period. All mice were 15 weeks old on the final day of training.

Behavior was recorded using Ethovision video tracking equipment and software (Noldus). Overall task performance was evaluated by calculating the time taken to find the hidden platform (latency). Spatial performance was evaluated by calculating the average distance between the mouse and the hidden platform (search proximity). A repeated measures one-way ANOVA was used to test for learning related changes in the experimental groups. The α-level was set to 0.05.

Quantitative in situ hybridization to determine zif268 expression

zif268 in situ hybridization was performed using previously established methods in our laboratory (1). Briefly, animals where sacrificed at the age of 15 weeks by cervical dislocation 45 minutes after the final training trial and brains were immediately frozen in 2-methylbutane (Merck) at a temperature of -40 °C. Coronal sections (25 µm) were cut on a cryostat (Microm HM 500 OM) and mounted onto 0.1% poly-L-lysine coated slides (Sigma-Aldrich). A series of brain sections covering the entire rostrocaudal extent of the striatum/anterior cingulate (medial prefrontal cortex) and hippocampus were collected (2) and kept at -30°C. Tissue was postfixed in 4% paraformaldehyde in 0.12M phosphoric acid in phosphate-buffered saline (PBS; 0.1 M, pH 7.4, 30 min, 4°C; 0.9% NaCl), dehydrated (50%, 70%, 98%, 100%, 5 min) and delipidated (100% chloroform, 10 min). The mouse specific synthetic *zif268* probe (NM_007913.5, sequence: 5'ccgttgctcagcatcatctcctccagyttrgggtagttgtcc3') was end-labeled with 33P-dATP (NEN) using terminal deoxynucleotidyl transferase (Invitrogen). Unincorporated nucleotides were removed using mini Quick Spin columns (Roche Diagnostics). The radioactive labeled probe was mixed with a hybridization cocktail (50% formamide, 4x standard saline citrate, 1x Denhardt's solution, 10% dextran sulphate, 100 µg/ml Herring sperm DNA, 250 µg/ml tRNA, 60 mM dithiothreitol, 1% N-lauryl-sarcosine, 26 mM NaHPO4 pH 7.4) and applied to a series of dehydrated sections with overnight incubation at a temperature of 37 °C. The next day, the sections were rinsed in 1x standard saline citrate buffer at 42 °C, air-dried and apposed to an autoradiographic film (Kodak) together with a [14C] microscale (GE Healthcare). Films were developed 3 weeks later in Kodak D19 developing solution and fixed in Rapid fixer (Ilford Hypam).

Autoradiographic images were scanned (CanoScan LiDE 600F, Canon) and optical densities (mean gray value per pixel) were quantified with ImageJ (Image processing and analysis in Java, National Institutes of Health). Optical density was measured in three brain sections per mouse along the rostrocaudal axis for each target region. Striatum and medial prefrontal cortex slices were taken from +1.10 mm to +0.38 mm relative to bregma (Fig. S4*A*) and CA1 slices from -1.58mm to -2.54mm relative to bregma (2). Within striatum we targeted dorsolateral and superior dorsomedial subdivisions (Fig. S4*B*). The template of the striatal and medial prefrontal cortex compartments was drawn bilaterally over brain sections. Mean gray values were averaged across hemispheres and brain slices resulting in a single data point for each region per animal. A one-way between groups ANOVA was use to test differences in IEG expression between the caged control group and all experimental and control groups. To test for learning related changes in IEG expression mean gray values were entered into an ANOVA (2 conditions x 2 learning phases). For all analyses the α-level was set to 0.05 and Bonferroni correction applied to post-hoc tests. Statistical analyses were performed in Statistica 9 (StatSoft).

Corticosterone levels

Comparison of corticosterone levels ensured between group differences in IEG expression were not confounded by stress. After decapitation, blood (0.3-0.5 mL) was collected in heparin coated eppendorf tubes (heparin lithium salt from porcine intestinal mucosa, Sigma-Aldrich; concentration coating: ~22 units per tube) and centrifuged (3000 rpm, 3°C, 15 min). Plasma was transferred to new tubes and stored at -20 °C. Plasma corticosterone levels were measured using a commercially available double antibody RIA (IDS Ltd.). The intra-assay coefficient for corticosterone was 3.9 %. A one-way between groups ANOVA revealed that corticosterone levels did not differ significantly between groups ($F_{4, 26} = 1.462$, $P = 0.24$; Fig. S5).

Human experiment

Subjects

Eighteen female subjects (aged 20-28, mean age 23.1) participated in the fMRI study. All were right-handed with no history of neurological disease. Prior to testing subjects were required to provide written informed consent to the procedures, which were approved by the Ethics Committee of the KU Leuven in accordance with the Declaration of Helsinki.

Task

A custom virtual environment analogous to the Morris water maze was constructed in Blender (www.blender.org) and rendered in MATLAB (2007b, The Mathworks). The environment consisted of a circular pool (diameter = 16 virtual reality units (vru), height = 0.5 vru) situated 0.5 vru above ground level in the center of a square room (length = 20 vru, height = 8 vru). Within the pool was a hidden platform 1.6 vru in diameter. There was only one distinguishing feature in the environment, a black cross located on a wall approximately half way between the floor and the ceiling in the opposite corner of the room to the quadrant in which the hidden platform was located. Subjects viewed the room from a first-person perspective, and moved around by pressing buttons on an MRI compatible button box (Current Designs Inc.). Movement was restricted to either forward displacement or orienting (i.e. rotating left and right in the same position). A single button press resulted in a forward movement of 0.1 vru or rotation of 1.5°. Data were recorded at 25 Hz (Fig. S6*A*).

Trial procedures

Over the course of the experiment subjects performed ‘search’ and ‘control’ trials, which were designed to be compatible with our mouse water maze experiment. All trials began from one of four starting zones (separated by 90°) located at the perimeter of the pool, with the exact position within a given starting zone varying by ±10° from trial to trial. Subjects always faced the center of the pool at the beginning of the trial.

The goal of search trials was to navigate to the hidden platform as quickly and directly as possible. When the goal location was successfully intercepted the walls of the room turned green for 1 second, after which the subject remained at the same location for a further 3 s. During this 4 s period forward movement and orienting were not possible. The maximum time limit for search trials was 45 s. If a trial reached the maximum time limit the walls of the room turned red for 1 s, after which the subject remained in their final unsuccessful location for a further 3 s (forward movement and orienting were again not possible during this 4 s period).

During control trials subjects moved freely within the pool. No distinguishing features were present on the walls, preventing any goal-directed navigation. Control trials were matched to the average duration of search trials (between 10 and 20 s) and finished in a similar manner, with the only difference being that the color of the walls always turned blue (which did not relate to feedback provided during other trials). See Figure S6B for screenshots.

A third trial type, 'prediction' trials, required the subject to explicitly indicate where they thought the hidden platform was located via a button press. Analysis of prediction trials is not presented here since we focussed on the conditions closest to the mouse experiment.

Experimental protocol

Four testing sessions were completed, each on a separate day. The first session familiarized subjects with the experimental procedures and trial order prior to scanning. During this session a limited number of trials were performed in a different environment to that used in the main experiment.

One or two days later subjects returned for the first scan session. From this session onwards the environment and the location of the hidden platform was unchanged. Subjects performed 6 runs of trials, with each run lasting at least 8 min. The order of presentation of search, prediction and control trials was determined as follows: Each sequence always started with a search trial. An unsuccessful search trial was repeated until the hidden target zone was successfully intercepted. Once a successful search trial was completed, a prediction and control trial were presented next. The order in which the prediction and control trials were presented was randomized. The sequence was then repeated. The current trial type was always displayed in small text at the top of the screen. Subjects rested for 5 to 10 s between trials and were required to fixate on a white cross in the center of a black screen.

A second identical scan session was performed 6-8 days after the first. Between scan sessions subjects performed a training session during which only behavioral data was acquired. The behavioral training session also consisted of 6 runs of trials each lasting 8 min.

Resting state protocol

In addition to acquiring task-related fMRI data, subjects were also scanned for 7 min in a resting state prior to the onset of task performance. Subjects were required to fixate on a white cross in the center of a black screen, and were instructed to relax and think of nothing in particular.

Behavioral analysis

The same behavioral measures as those previously described in mouse, i.e. latency and search proximity, were also used to quantify performance on the virtual water maze. To test for learning within each session we conducted a one-way repeated measures ANOVA (runs 1-6). Statistical analyses were performed in Statistica 9. The α-level was set to 0.05.

Image acquisition

A Siemens 3 T Magnetom Trio MRI scanner (Siemens) with 12 channel head coil was used for image acquisition. For all subjects, a high resolution T1-weighted structural image was acquired using a magnetization prepared rapid gradient echo sequence (MPRAGE; repetition time (TR) = 2300 ms, echo time (TE) = 2.98 ms, 1 x 1 x 1.1 mm voxels, field of view (FOV): 240 x 256, 160 sagittal slices). Functional data (fMRI) were acquired with a descending gradient echo planar imaging (EPI) pulse sequence for T2*-weighted images (TR = 3000 ms, TE = 30 ms, flip angle = 90°, 50 oblique axial slices each 2.8 mm thick, inter-slice gap 0.028 mm, in-plane resolution 2.5 x 2.5 mm, 80 x 80 matrix).

Image preprocessing

Image preprocessing was conducted using SPM8 (Wellcome Department of Imaging Neuroscience, University College London). Functional images were spatially realigned and unwarped, slice time corrected to the middle slice (reference slice = 25), normalized to the standard EPI template of the Montreal Neurological Institute (MNI), resampled into 2 mm isotropic voxels and spatially smoothed with an isotropic 8 mm full-width-at-half-maximum Gaussian kernel. Resting-state data were preprocessed in a similar manner, except functional images were not unwarped, resampled into 3 mm isotropic voxels and spatially smoothed with an isotropic 5 mm full-width-at-half-maximum Gaussian kernel.

Statistical Analysis of FMRI Data

Search trials, control trials and rest following control trials were modeled for each subject as boxcar functions convolved with the canonical hemodynamic response function within a first-level general linear model. The time series in each voxel was high pass filtered at 1/160 Hz to remove low frequency drifts. The contrasts search>rest and control>rest were specified separately for each run.

Contrasts were entered into a second level random effects ANOVA model with the factors trial type (search>rest and control>rest) and run (runs 1-6 and 13-18). The model was estimated under the assumption of dependent measurements and unequal variances. The *t*-contrast identifying areas

responding more strongly to search than control trials was thresholded at $P < 0.05$, Family-Wise Error (FWE) corrected for multiple comparisons within the whole brain, and only included clusters above 30 voxels.

Further analyses focussed on the striatum, prefrontal cortex and hippocampus. Regions of interest (ROIs) were defined on the basis of a priori anatomical and functional criteria (see SI Text: ROI definition). For each of the ROIs created in striatum, medial prefrontal cortex and hippocampus, the marsbar toolbox (3) was used to extract the mean contrast value of all voxels, i.e. an estimate of the hemodynamic response to either search or control trials (compared to rest) in the area of interest. Unsmoothed images were used to avoid including signal from neighboring regions. To test for changes in activation over the course of learning contrast values were entered into an ANOVA (2 trial types x 2 learning phases). Statistical analyses were performed in Statistica 9. The α-level was set to 0.05. Post hoc tests were Bonferroni corrected.

ROI definition

Caudate and putamen (defined by the Harvard-Oxford subcortical atlas (4)) activations were divided into dorsal/ventral and anterior/posterior subregions (Fig. S4*C*). Boarders were defined on the basis of prior knowledge regarding functional differences between subregions within the striatum (5). The dorsal/ventral division was defined as $z \geq 9$ (dorsal) and $z \leq 5$ (ventral) for the caudate and $z \geq 4$ (dorsal) and $z \leq 0$ (ventral) for the putamen. The anterior/posterior division was defined as $y \geq 2$ (anterior) and $y \leq -2$ (posterior) for both caudate and putamen. The gap between masks ensured that the signal from each voxel would only be included in one subdivision. According to these anatomical and functional criteria, the following bilateral striatal ROIs were created: dorsal posterior caudate, dorsal anterior caudate, ventral anterior caudate, dorsal posterior putamen, dorsal anterior putamen and ventral anterior Putamen (Fig. S4*D*).

The medial prefrontal cortex ROI in mouse was selected based on its connectivity to dorsomedial striatum. For the human data analysis we used resting state fMRI to identify voxels in prefrontal cortex functionally connected to dorsomedial striatum while at rest. Processing steps necessary to optimally prepare the data for functional connectivity analysis included band-pass filtering between 0.009 and 0.08 Hz, regression of global white matter and ventricle signals and their first derivatives, and regression of three-dimensional motion parameters and their first derivatives (6). Whole brain connectivity maps were created for all individual participants by calculating correlations between the average time course of voxels in dorsomedial striatum and all the time courses of the brain voxels (6). After applying Fisher's r-to-z transformation to each correlation map, a random effects analysis was performed in order to reveal a pattern of functional connectivity that was consistent across subjects (7). Statistical significance was assessed at the voxel level by means of one sample *t*-tests, with a statistical threshold of $P < 0.05$ corrected for multiple comparisons by False Discovery Rate (FDR) (8). An ROI was then created for those voxels in the prefrontal cortex with 1) significant functional connectivity with dorsal posterior caudate at rest and 2) a significantly higher response during search trials compared to control trials.

Although we did not find any voxels in hippocampus that responded more strongly to search trials than control trials in our whole brain analysis, we further tested for differences between trial types in this region using a more sensitive approach. We performed a hierarchical cluster analysis with temporal correlation as a similarity metric and the average linkage function to compartmentalize hippocampus into functional subdivisions characterized by distinct resting state activity (9, 10). This analysis revealed clusters in bilateral anterior, mid, and posterior hippocampus (Fig. S7). Subsequent analysis was only performed on the bilateral posterior hippocampus cluster, since this region is most likely to be engaged in spatial learning in human (11, 12).

ADDITIONAL DISCUSSION

Dorsolateral striatum:

In dorsolateral striatum we found increased activity in both species during early and late phases of place learning, suggesting a contribution to task performance that was not learning phase specific. The dorsolateral striatum is typically associated with habitual or automatized behavior (13-16). Yin and colleagues recently demonstrated with *in vivo* recordings that the dorsomedial and dorsolateral striatum were preferentially engaged during early and late learning phases of motor skill acquisition, respectively (17). On the basis of this evidence one would expect an increase in dorsolateral striatum activity specific to the late phase of water maze learning. However, they also found that lesions in dorsolateral striatum impaired both early and late phases of motor learning (17). Although the use of a motor task might have increased dependence on the dorsolateral striatum, simultaneous activity in dorsomedial and dorsolateral striatum was also recently observed during the early phase of a spatial learning task (18). Thus, our pattern of increased activation in both dorsomedial and dorsolateral striatum during early learning, followed by a decrease in dorsomedial striatum and no change in dorsolateral striatum in late learning is consistent with the interpretation that functionally distinct processes in these striatal subdivisions develop in parallel, and not serially (17).

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FIGURE LEGENDS

Fig. S1. Complete learning curves showing average performance across days for the 30 day group in mouse and runs for humans. Latency (*A*) and search proximity (*B*) are stable toward the end of training in the 30 day group. In human, latency (*C*) and search proximity (*D*) improve rapidly in scan session 1 (runs 1-6), show a small further improvement during overtraining outside the scanner (runs 7-12), and are stable in scan session 2 (runs 13-18). Error bars represent SEM.

Fig. S2. *zif268* expression in medial prefrontal cortex during early learning was positively correlated with search proximity.

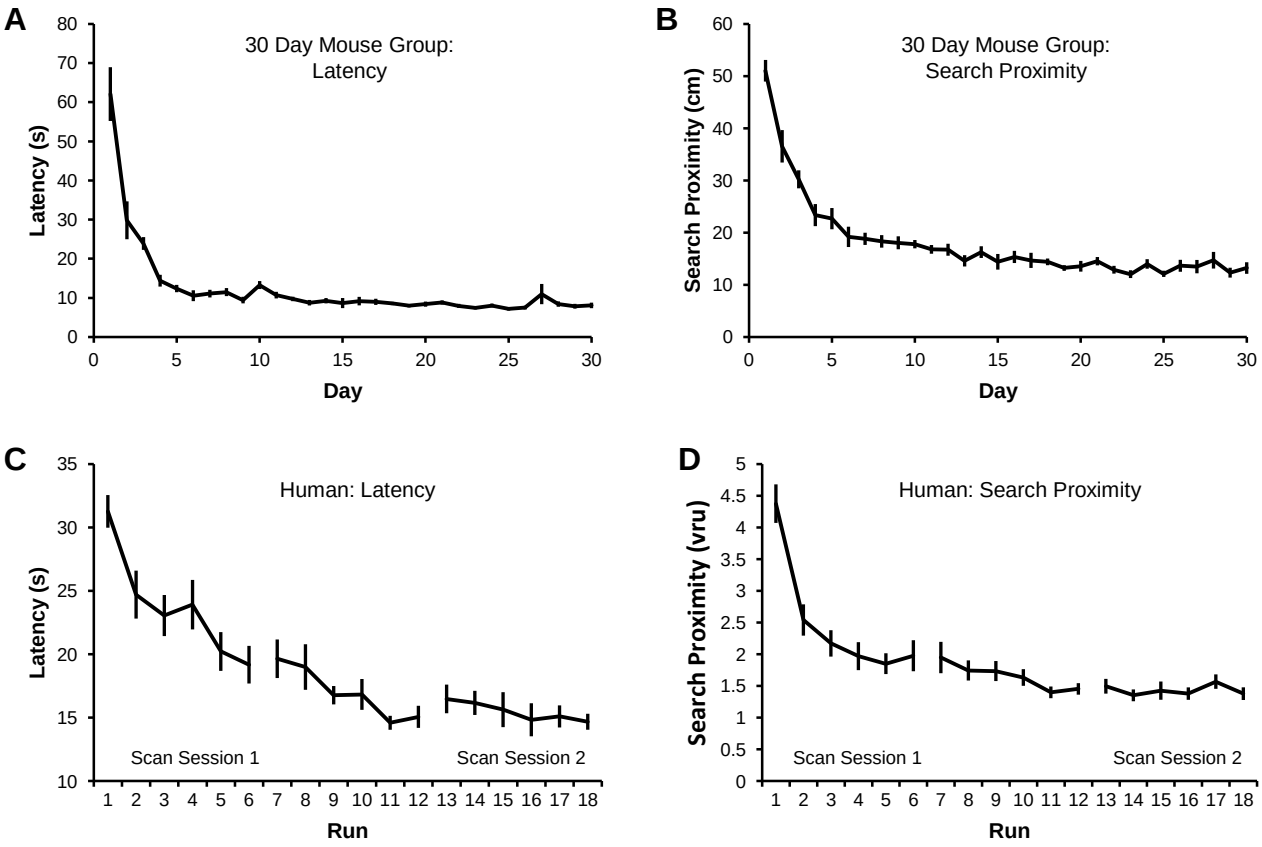
Fig. S3. Subdivisions in human striatum that did not show learning related changes in activity. Significant interactions were observed in dorsal anterior caudate ($F_{1, 17} = 7.52$, $P < 0.05$) (*A*) and ventral anterior caudate ($F_{1, 17} = 4.91$, $P < 0.05$) (*B*), however, these resulted from changes in activation on control trials and not search trials. Significant interactions were not observed in any of the dorsolateral subdivisions (*CD*). There was a significant main effect of trial type in all striatal subdivisions, which was expected since these ROIs were initially selected based on this contrast. * indicates significant interaction $P < 0.01$.

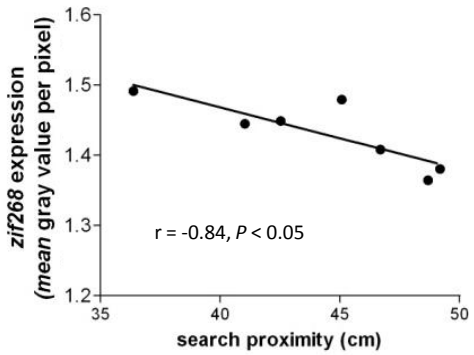
Fig. S4. Regions of interest. *A zif268* expression in the striatum and medial prefrontal cortex of mouse was measured in three brain sections along the rostrocaudal axis from +1.10 mm to +0.38 mm relative to Bregma. *B* Dorsomedial striatum is shaded in red, with diagonal red lines indicating the superior subdivision reported in the present study. Dorsolateral striatum is shaded in purple and the medial prefrontal cortex in yellow. *C* Caudate (x = 13; also referred to as dorsomedial striatum) and putamen (x = 24; also referred to as dorsolateral striatum) in human were each subdivided into three regions. The dorsal posterior region is shaded in red, the dorsal anterior region in blue, and the ventral anterior region in green. *D* Clusters of voxels in each anatomical subdivision that responded more strongly in search trials than control trials when compared to rest (FWE corrected, $P < 0.05$).

Fig. S5. Mouse corticosterone levels across all experimental and control groups were not significantly different. Solid black dots represent experimental animals (black dots), solid gray dots represent free swimming controls, and open gray dots represent caged controls.

Fig. S6. Virtual water maze environment *A* The virtual water maze viewed from an elevated position within the room showing the position of the hidden platform. Note that the platform was not visible at anytime during testing. *B* Order of events in search and control trials. Screenshots display a first person view of the environment seen by the subject during testing. Feedback during trials was provided by the walls of the room changing color.

Fig. S7. Three anatomical subdivisions were defined in hippocampus (x = -24) on the basis of a hierarchical cluster analysis. The posterior region of hippocampus is shaded in red, the mid region in blue, and the anterior region in green. Only the (bilateral) posterior subdivision was used for further analysis.


A
30 Day Mouse Group:
Latency
Latency (s)
Day
B
30 Day Mouse Group:
Search Proximity
Search Proximity (cm)
Day
C
Human: Latency
Latency (s)
Scan Session 1
Scan Session 2
Run
D
Human: Search Proximity
Search Proximity (vru)
Scan Session 1
Scan Session 2
Run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(mean gray value per pixel)
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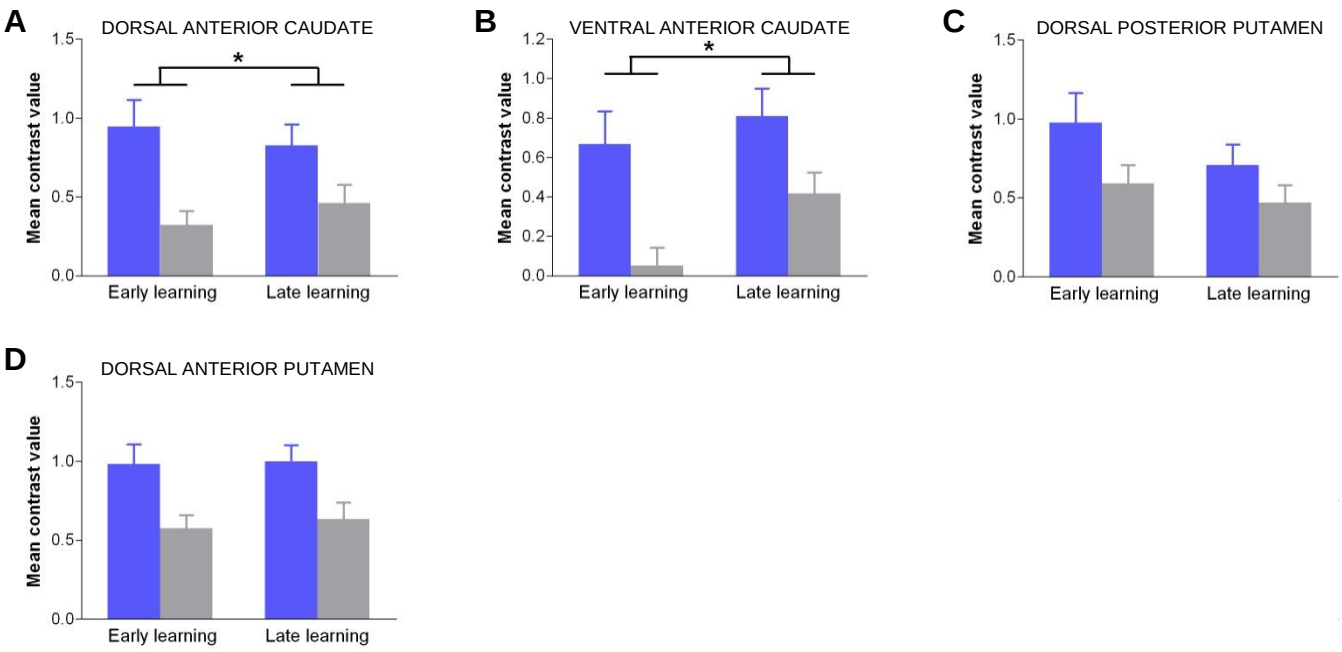
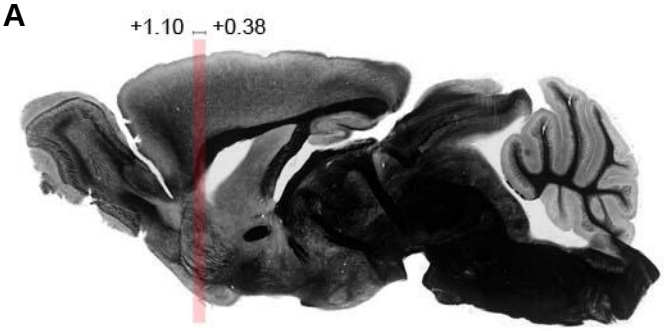
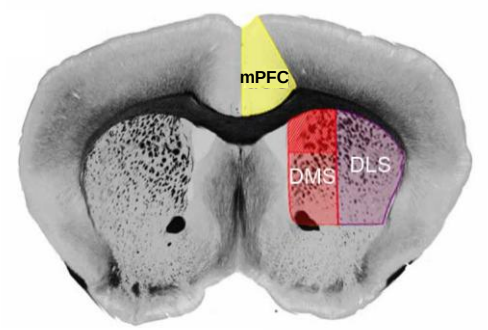
1.4
1.3
1.2
35
40
45
50
search proximity (cm)
r = -0.84, P < 0.05


A
DORSAL ANTERIOR CAUDATE
Mean contrast value
1.5
1.0
0.5
0.0
*
Early learning
Late learning
B
VENTRAL ANTERIOR CAUDATE
Mean contrast value
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Early learning
Late learning
C
DORSAL POSTERIOR PUTAMEN
Mean contrast value
1.5
1.0
0.5
0.0
Early learning
Late learning
D
DORSAL ANTERIOR PUTAMEN
Mean contrast value
1.5
1.0
0.5
0.0
Early learning
Late learning

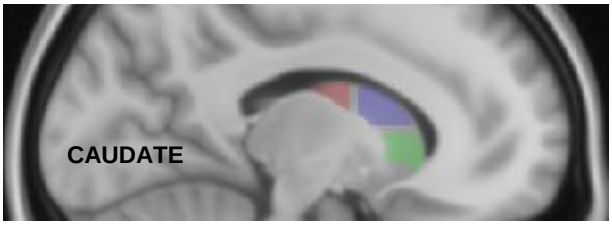
A

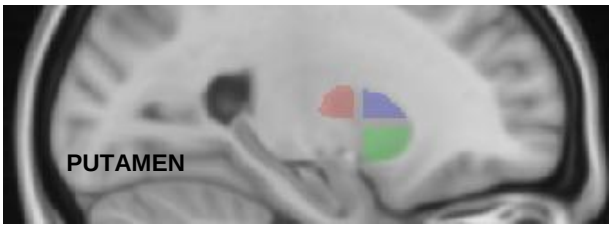


B ANATOMICAL SUBDIVISIONS IN MOUSE



C ANATOMICAL SUBDIVISIONS IN HUMAN STRIATUM





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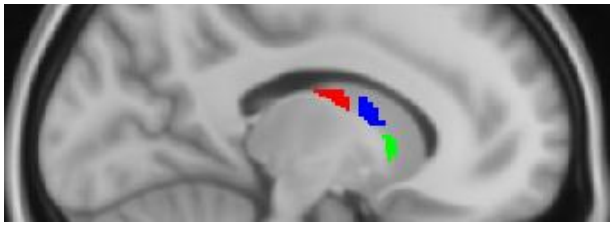

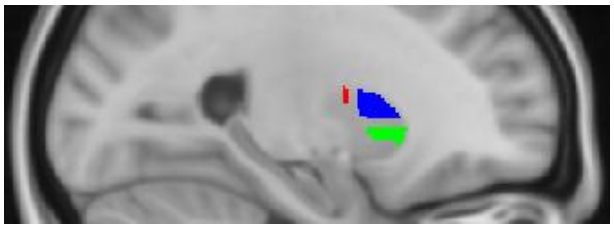

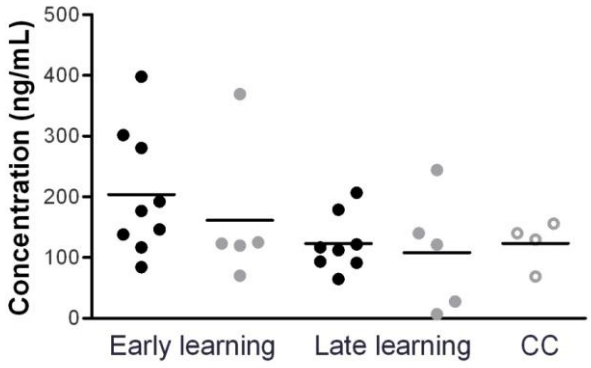
Concentration (ng/mL)
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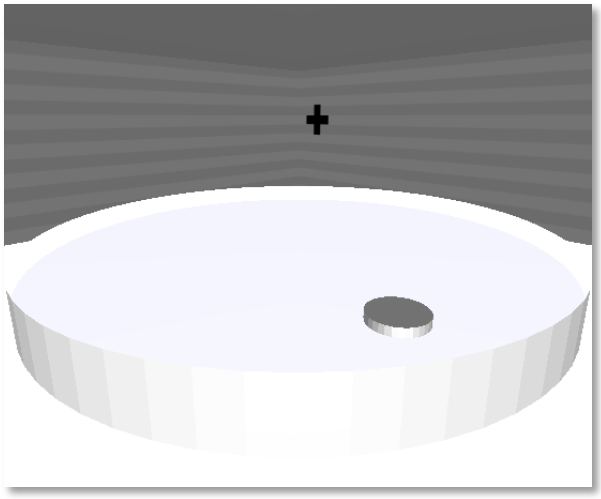
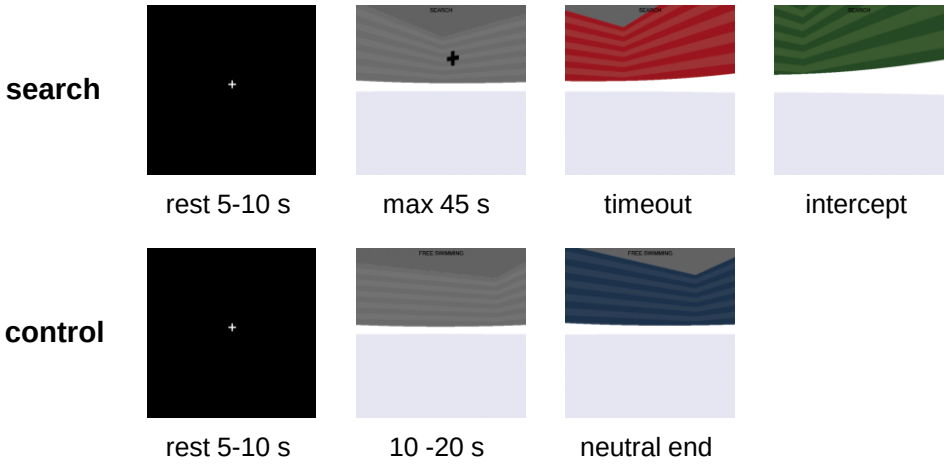
400
300
200
100
0
Early learning
Late learning
CC

A



B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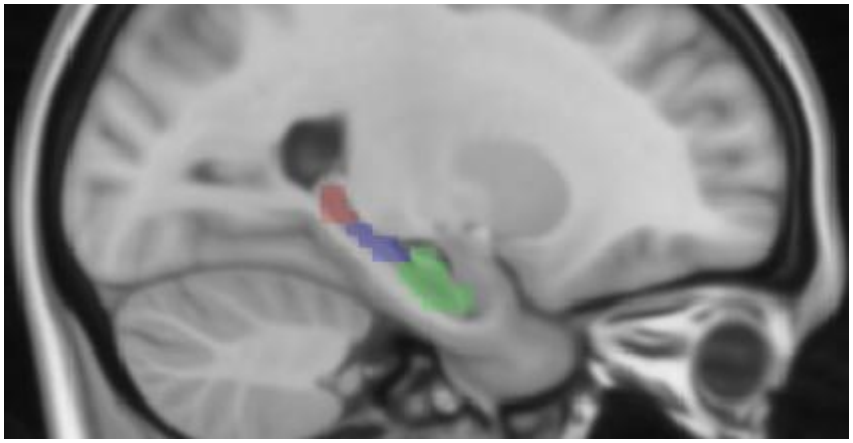
rest 5-10 s
max 45 s
timeout
intercept
control
rest 5-10 s
10 -20 s
neutral end



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