Title: Behavioral state-dependent lateralization of dorsal dentate gyrus c-Fos expression in mice

Authors: Jake T. Jordan[1,2,*], Carolyn L. Pytte[1-4]

1 – Biology PhD Program, The Graduate Center, CUNY, New York, NY 11016
2 – CUNY Neuroscience Collaborative, The Graduate Center, CUNY, New York, NY 11016
3 – Psychology PhD Program, The Graduate Center, CUNY, New York, NY 11016
4 – Psychology Department, Queens College, CUNY, Flushing, NY 11367

*Correspondence should be addressed to:
Jake Jordan
65-30 Kissena Blvd, Psychology Department, Queens College, Flushing, NY 11367
jake.jordan@qc.cuny.edu

Abstract

Hemispheric lateralization is a fundamental organizing principle of nervous systems across taxonomic groups with bilateral symmetry. The mammalian hippocampus is lateralized anatomically, physiologically, and chemically; however, functional asymmetries are not yet well understood. Imaging studies in humans have implicated the left and right hippocampus in specialized processing. However, it is not clear if lateralized activity occurs in the rodent hippocampus. c-Fos imaging in animals provides a measure of neuronal activity with a resolution at the level of single cells. The aim of this study was to determine whether lateralized activity-dependent c-Fos expression occurs in the rodent hippocampus. To understand functional lateralization of hippocampal processing, we compared interhemispheric expression of c-Fos in the dentate gyrus (DG), a structure involved in encoding new experiences, in mice that ran on a wheel, encoded a novel object, or remained in home cages. We found that wheel running induced the greatest amount of DG c-Fos expression in both hemispheres, with no difference between hemispheres. Object exploration resulted in left-lateralized DG c-Fos expression, whereas control mice were not lateralized. We then sought to determine whether differential consideration of hemispheres might influence the conclusions of a study by simulating common cell quantification methods. We found that these different approaches led to different conclusions. These data demonstrate lateralization of neuronal activity in the mouse DG corresponding to the experience of the animal and show that differentially considering hemisphere leads to alternative conclusions.

Abbreviations: dentate gyrus (DG), immediate early gene (IEG)

Introduction

Lateralization in the brain refers to interhemispheric asymmetry in the presence or dominance of a neural substrate [1]. Many forms of anatomical and physiological lateralization in the hippocampus have been well described; however, understanding functional hippocampal lateralization has been more elusive. In humans, both hippocampal hemispheres are recruited during spatial navigation, although activity in only the right hippocampus corresponds to navigation accuracy [2]. Conversely, the left entorhinal cortex appears to be specialized for object encoding [3] and the left hippocampus is specialized for object-cued spatial memory [4]. Spatial navigation involves actual or imagined self-motion through a real or virtual environment, whereas object memory consists of the processing of externally-originating sensory stimuli; invoking a long-held dichotomy in the characteristics of information integrated by the hippocampus described in the cognitive map theory [5].

Although direct functional comparisons cannot be made across paradigms, it seems that rodents likewise use both hemispheres for spatial memory yet also show hemispheric specializations in particular tasks. Spatial memory as tested on the Barnes Maze has been reported to be right-lateralized [6], while long-term spatial memory storage on the Y-Maze has been suggested to be left-lateralized [7, 8]. Interhemispheric asymmetries have also been reported in hippocampal physiology in mice [7, 9], suggesting lateralization in *how* information is processed, in addition to lateralization in which types of information are processed. Despite these data, one of the most crippling obstacles to translational hippocampal research is the widely held view that the rodent hippocampus does not exhibit the same inter-hemispheric differences that are seen in humans. Thus, a large majority of rodent hippocampal studies do not consider hemisphere.

Imaging immediate early gene expression (IEG) in animals provides several advantages over human imaging, such as single-cell resolution and the ability to examine activity during freely moving behaviors, no study has compared IEG expression in the left and right dorsal hippocampus (see [10], for ventral hippocampal imaging). Further, no study has examined lateralization in the dentate gyrus (DG), a structure important for the encoding of new hippocampus-dependent memories [11]. We examined how the behaviors of wheel running and exploration of a novel object are associated with IEG expression in the left and right dorsal DG. Interestingly, two studies that examined DG IEG expression but that differed in respect to treatment of hemispheres during cell quantification arrived at conflicting conclusions [12, 13]. Therefore, we simulated these and other common cell quantification approaches that are indifferent to hemisphere to determine whether such approaches may arrive at different conclusions.

Methods and Experimental Design

This work was approved by the Queens College Institutional Animal Care and Use Committee (Protocol #177).

Animals and Housing. Adult male (n=20) and female (n=15) C57Bl6 mice (Jackson Labs) were group housed (3-4) in standard shoebox plastic caging with bedding on a 12 hr light/dark cycle with free access to food and water.

Behavior. Group-housed littermates were distributed roughly evenly across three treatment groups: 1) wheel running (WR, n= 4 males, 3 females), 2) exploration of a novel object in the home cage (OB, n= 4 males, 3 females), and 3) control home cage (CON, n= 9 males, 6 females). We sought to emphasize the relative salience of internally-generated motor

information versus externally-originating environmental cues by training and testing the WR group in the dark to reduce visual input. In contrast, the OB mice were exposed to novel objects in the light. CON animals were housed in the dark for 24 hrs prior to sacrifice. In a follow-up experiment to test the effect of the difference in lighting between WR and OB groups, we determined c-Fos expression in a group that similarly experienced a novel object, but did so in the dark (OB-D, 3 males, 3 females).

Behavioral training and testing was conducted in a small, dedicated behavior room. WR mice were trained to run on a wheel 24 hours prior to testing. For training, WR mice were individually brought to the behavior room in their home cage and within a few minutes placed in a cage identical to that of their home cage except containing a wheel. They were permitted 2 hours of housing in the wheel-cage, during which time they all ran to varying extents. They were then transferred to their home cage and dark housed in the behavior room overnight. The next day, WR mice were transferred from their home cage to the wheel-running cage, allowed 30 minutes of running, then transferred back to their home cage in the behavior room. Sixty-five minutes later, they were brought to a perfusion room and sacrificed. The entire procedure prior to transportation to the perfusion room (25 hr 35 min) was conducted in the dark.

OB mice were transported individually in their home cage to the behavior room and housed in the dark for 24 hours. The following day, the room light was turned on and a novel object (small PVC pipe) was placed in their home cage. After 30 minutes of exposure, the object was removed and the room light turned off. They remained in the dark for 65 minutes and then were transported to a perfusion room and sacrificed.

CON mice were transported individually in their home cages to the behavior room and housed undisturbed for 25 hr 35 min, time-matched to the behavioral experiments of the WR and OB groups. They were then transported to a perfusion room and sacrificed. Individual times spent wheel running and exploring the object were not quantified.

To determine whether the behavior room light contributed to differences in c-Fos expression between WR and OB groups, we repeated the object exploration treatment in an OB-D group in which the behavior room was dark throughout the experiment. Immunohistochemistry and c-Fos quantification of DG c-Fos expression for the OB-D mice was conducted separately from the first three groups and therefore was not included in the analysis of DG c-Fos+ cell densities across the other behavioral conditions.

Immunohistochemistry. Seventy-five minutes following the end of behavior, mice were deeply anesthetized with 0.2 mL of Euthasol (Virbac) and perfused intracardially with 0.1 M phosphate buffered saline (PBS) and 4% paraformaldehyde in PBS. The brain was bisected and the dorsal hippocampus was coronally sectioned at 18 µm (two CON brains and one OB brain were cut at 30 µm) one hemisphere at a time. Cutting order of hemisphere was pseudorandomized. The brains of mice that explored an object in the dark were not bisected. Instead, one hemisphere was marked by removing a segment of ventral neocortex. Marked hemispheres were pseudorandomized. For all brains, every third section was mounted directly onto positively charged microscope slides with a random orientation in both axes such that hemisphere could not be determined by the section orientation. Slides were stored at -20°C until processed.

Tissue from WR, OB, and CON was processed together and distributed equally across immunohistochemistry batches. The OB-D group was processed separately. Frozen sections were rinsed once in 0.1 M PBS for 10 minutes. A block containing 10% donkey serum and 3% Triton-X in phosphate buffer (PB) was applied for 30 minutes, followed by a 48-hour incubation in anti-c-Fos made in rabbit (Millipore, ABE457, 1:1000) in block. Sections were then rinsed in

0.1 M PBS three times for 10 min each and then incubated with Cy3-conjugated donkey anti-rabbit for 2 hours (Millipore, AP182C, 1:200) in block or PB/S). Sections were rinsed in 0.1 M PBS three times for 10 min each before a serial dehydration in ethanols followed by immersion in xylene and cover slipping with Krystalon (Harleco).

Cell Quantification. Microscopy was conducted blind to hemisphere for all mice. Microscopy was conducted blind to treatment for groups WR, OB, CON, but aware of treatment for OB-D as this group was processed independently after the others. The DG was traced under 4x magnification using Neurolucida software (Microbrightfield) coupled to an Olympus BX51 microscope. Cy3-labeled cells within the DG were counted under 60x magnification using a rhodamine filter. For brains sectioned at 18 µm, 3-5 sections (54-90 µm) were quantified per hemisphere per mouse; for brains sectioned at 30 µm, 2-4 sections (60-120 µm) were quantified per hemisphere per mouse. Amount of tissue sampled did not correlate with c-fos+ cell counts. Densities of c-Fos expression within hemispheres were calculated by determining the number of c-Fos+ cells within the DG boundary divided by the volume sampled.

To simulate commonly used cell quantification methods that ignore possible hemispheric asymmetries, we conducted three additional computations. First, to simulate experiments that look at expression unilaterally we quantified cells using primarily one hemisphere and occasionally the other, simulating occasions of tissue loss. Second, we simulated experiments that look at expression unilaterally but that disregard which hemisphere is quantified by randomly selecting a hemisphere for each animal by coin toss, differing across animals, and analyzing expression using only that hemisphere. Finally, we pooled hemispheres to determine bilateral DG c-Fos expression: (left c-Fos + right c-Fos) / (left volume + right volume).

Because the amount of c-Fos expression may vary more widely among individuals than between hemispheres, we also calculated a lateralization index (LI), which shows hemispheric asymmetry normalized by the total number of c-Fos cells in each animal (as in Tsoi et al., 2014), and compared LI values across groups (Figure 1B).

$$\text{LI} = \frac{\text{Left Hemisphere c-Fos+ cells/mm}^3 - \text{Right Hemisphere c-Fos+ cells/mm}^3}{\text{Left + Right c-Fos+ cells/mm}^3}$$

Positive values indicate more c-Fos+ cells in the left hemisphere relative to the right and negative values indicate more cFos+ cells in the right hemisphere.

Statistics

WR, OB, and CON groups: A two-way mixed-model ANOVA was used to compare c-Fos in the right and left hemisphere (repeated measure) between treatments. One-way ANOVAs were used to compare treatment effects within either the left or right hemisphere, total c-Fos expression in pooled hemispheres, and treatment effects for randomly drawn unilateral DG c-Fos expression.

WR, OB, CON, and OB-D groups: A one-way ANOVA was used to determine if lateralization indices differed across treatments. A one sample t-test was used to determine if c-Fos lateralization indices differed from zero (non-lateralized expression) for each group. Paired t-tests were used to compare numbers of c-Fos+ cells between left and right hippocampi within groups WR, OB, CON and OB-D. For all tests, differences were considered significant at P values less than 0.05. P values reported for t-tests are two-tailed.

Results

Object exploration, but not wheel running, lateralized DG c-Fos expression to the left hemisphere.

To determine whether wheel running and object exploration resulted in hemispheric differences in DG c-Fos expression across hemispheres, we compared the density of c-Fos expressing cells in these treatment groups and standard-housed controls. We found a main effect of behavioral condition ($F(2,24)=43.96$; $P<0.0001$) but not of hemisphere ($F(2,24)=0.02$; $P=0.888$). There was a trend toward an interaction between these two factors ($F(2,24)=3.05$; $P=0.065$, two-way mixed ANOVA, Figure 1A).

We were interested in potential hemisphere-specific effects of behavioral conditions. Therefore, we compared densities of c-Fos expression across behavioral groups in the left hemisphere and right hemisphere separately. As expected, across left hemispheres, we found an effect of treatment ($F(2,26)=37.569$, $P<0.0001$, one-way ANOVA, Figure 1A). More interestingly, post-hoc tests revealed that both OB and WR mice differed from CON mice ($Q=4.45$, $P=0.011$; $Q=12.24$, $P=0.001$, Tukey's HSD) and that OB and WR mice differed from each other ($Q=6.67$, $p=0.001$, Figure 1A, Tukey's HSD). We then compared DG c-Fos expression in the right hemisphere across behavioral groups. Again, we found a significant treatment effect ($F(2,26)= 40.92$, $P<0.0001$, one-way ANOVA). In addition, WR mice had greater expression than either CON or OB mice ($Q=12.59$, $P=0.001$; $Q=9.21$, $p=0.001$, Tukey's HSD). However, unlike in the left hemisphere, right hemisphere expression of c-Fos did not differ between OB and CON mice ($Q=1.83$; $P=0.414$, Tukey's HSD Figure 1A).

To understand how behavioral state was reflected in relative DG activity across hemispheres, we compared left and right c-fos expression within treatment groups. Within CON or WR mice, numbers of c-Fos expressing cells did not differ between hemisphere (df 14, $t=-1.426$, $P=0.176$; df 6, $t=0.020$, $P=0.985$, paired t-test). However, there were significantly more c-Fos expressing cells in the left than in the right hemisphere in the OB group (df 6, $t=3.223$, $P=0.018$, paired t-test, Figure 1A).

To determine whether room lighting contributed to the lateralization of DG c-Fos expression in OB mice, we examined DG c-Fos expression in OB-D mice, which underwent the same behavioral treatment as OB mice, but in the dark (as were CON and WR mice). Like the OB group, OB-D mice had significantly higher DG c-Fos expression in the left as compared to the right hemisphere (df 5, $t=6.401$, $P=0.001$, paired t-test, Figure 1B). Overall levels were lower in OB-D than in the other groups; however, we cannot determine whether this is due to the treatment or differences in tissue processing.

Finally, we used a lateralization index to compare relative hemispheric c-Fos expression normalized to individual expression levels. There was no difference in the lateralization index between OB and OB-D (df=11, $t=1.792$, $P=0.101$, t-test, Figure 1C). There was also no difference in the lateralization index across all groups (CON, WR, OB, OB-D, df=3, $F=1.349$, $P=0.277$, Figure 1C). The lack of difference in LI was expected due to the high variability in the control group. Therefore, we also tested whether the degree of lateralization within each group differed from zero. A lateralization index of zero indicates bilaterally equal c-Fos expression. We found that the degree of lateralization in the CON and WR groups did not differ from zero (one sample t-test, $t=0.761$, $P=0.460$; $t=0.086$, $p=0.934$) whereas the lateralization index in the OB and OB-D groups were significantly left dominant (one sample t-test, $t=-4.452$, $P=0.004$; $t=-5.013$, $P=0.004$, Figure 1C).

Widely-used cell quantification methods produced different results.

A majority of hippocampal cell quantification studies do not account for potential hemispheric asymmetries and either pool hemispheres, reporting total cells across hemispheres, or only use one hemisphere. In the latter case, the hemisphere used may be consistent (i.e. the left or the right hemisphere is quantified for each animal) or it may not be identified (cell counts may include the left hemisphere of some subjects and the right hemisphere from others, or cell counts may include mixed hemispheres from some or all subjects). As we found above, analysis of just the left or just the right hemisphere can lead to different conclusions regarding the effects of certain experiences on DG c-Fos expression. To further assess whether differing approaches with respect to hemisphere can lead to different conclusions, we simulated methods in which DG c-Fos+ cells were 1) quantified unilaterally, using primarily one hemisphere and occasionally the other; 2) quantified unilaterally, randomly choosing which hemisphere was used, and 3) quantified bilaterally.

First, we reanalyzed the data from our initial experiment using the right hemisphere from 80% of mice and the left hemisphere from the remaining 20% of mice (6 animals total, 2 from each group; Figure 2A). We found a main effect of behavioral condition ($F(2,26)=43.19$, $P<0.0001$; one-way ANOVA; Figure 2A). Post-hoc analysis revealed that WR mice had higher DG c-Fos expression then both CON ($Q=9.30$, $P=0.001$; Tukey's HSD; Figure 2A) and OB mice ($Q=12.97$, $P=0.001$; Tukey's HSD; Figure 2A). However, this analysis revealed no difference between OB and CON mice ($Q=2.11$, $P=0.312$; Tukey's HSD; Figure 2B).

We then repeated this analysis using the left hemisphere from 80% of mice and the right hemisphere from the remaining 20% of mice. We found a main effect of behavioral condition ($F(2,26)=53.54$, $P<0.0001$, one-way ANOVA, Figure 2B). Unlike in the previous analysis, post-hoc analysis revealed significant differences between all three behavioral groups (WR vs. CON: $Q=14.62$, $P=0.001$; WR vs. OB: $Q=9.00$, $P=0.001$; OB vs. CON: $Q=4.11$, $P=0.020$; Tukey's HSD; Figure 2B).

To simulate studies in which cell counts were made unilaterally while ignoring hemisphere, we randomly drew a hemisphere for each animal and then compared c-Fos densities among treatment groups. We found a significant effect of behavioral condition ($F(2,26)=30.15$, $P<0.0001$; one-way ANOVA; Figure 2C) with WR mice having significantly greater levels of expression than either CON ($Q=10.98$, $P=0.001$; Tukey's HSD; Figure 2C) or OB mice ($Q=6.12$, $P=0.001$; Tukey's HSD; Figure 2C). We also found significantly more expression in OB than in CON mice ($Q=3.83$, $P=0.030$; Tukey's HSD; Figure 2C).

Finally, we analyzed DG c-Fos expression bilaterally. For each mouse, we summed cell counts across hemispheres and divided by the summed volume sampled in both hemispheres, producing a measure of bilateral c-Fos density. This analysis yielded an effect of behavioral condition on bilateral DG c-Fos expression ($F(2,26)=46.38$, $P<0.0001$; one-way ANOVA; Figure 2D). Post-hoc analysis revealed significantly greater expression in WR mice compared to CON ($Q=13.59$; $P<0.0001$; Tukey's HSD; Figure 2D) and OB mice ($Q=8.63$, $P<0.0001$; Tukey's HSD; Figure 2D). There was a trend towards greater expression in OB mice compared to CON ($Q=3.51$, $P=0.0502$; Tukey's HSD; Figure 2D).

Discussion

We quantified neuronal activity-related c-Fos expression in the left and right DG following wheel running, object exploration, and home cage housing. Wheel running in the dark upregulated c-Fos expression bilaterally and showed no hemispheric asymmetry. Interestingly,

exploration of a novel object with the room lights on upregulated c-Fos expression in the left, but not the right hemisphere compared with controls housed in the dark. With the room lights off, mice similarly exposed to an object showed lower DG c-Fos expression than the controls, however, the tissue for the OB-D mice was processed and expression quantified separately (and using a primary antibody from a different lot), and thus, we cannot directly compare expression levels of this group across treatments. More importantly for our objectives, object exploration in either the light or dark resulted in left-dominant DG c-Fos expression compared to controls.

Although the rodent hippocampus has generally been considered to be functionally symmetric, accumulating evidence suggests that there are indeed rodent hippocampal functional asymmetries (see [14] for review). The assumed lack of hemispheric specialization in rodents has in part been based on substantial interhemispheric connectivity between the two hippocampi as subfields send both ipsi- and contralateral projections. In contrast, it is widely believed that humans have only ipsilateral projections from CA3 to CA1 [15]. Although the presence of bilateral projections in humans has been argued for [16, 17, 18], this position has not been widely adopted [19]. Rather, it has been suggested that in humans and other primates, hemispheric isolation due to the absence of interhippocampal pathways has resulted in a higher degree of hemispheric processing specialization than in other mammals [20]. However, the anatomy of inter-hemispheric projections need not require or predict functional asymmetry.

A recent study showed that sharp-wave ripples in the rat hippocampus, thought to function in memory consolidation, have coordinated activity within either, but not across, hemispheres [21]. This suggests that even though information may be shared across hemispheres in rodents, cell assemblies corresponding to a particular experience may be intrahemispheric and their information consolidated in isolation to each other. This is consistent with other work indicating that spatial memory may be lateralized to one hemisphere or the other ([22], rats; [6, 7. 8], mice). Further, neurophysiological studies in mice have identified lateralization in some of the key neural substrates, such as LTP and NMDA receptor subunits, thought to underlie hippocampal function [7, 9, 23, 24, 25]. Taken together, this suggests that despite potential differences in direct interhemispheric communications between humans and rodents, hippocampal hemispheric specialization may be a shared property.

It is not yet clear whether rodent and human hippocampal lateralization show the same patterns of functional division of labor. In humans, a role of the right hippocampus in allocentric spatial navigation has been widely documented [2, 26, 27, 28, 29] whereas a clear understanding of left hippocampus processing remains more elusive. The role of the left side has been suggested to relate to the transfer of recently formed memories to long term storage (in rats, [22]) or function in autobiographical memory formation (in humans, [30]). More recently, the left hippocampal formation in humans has been shown to be preferentially active during an object recognition task [3, 4] whereas the right hippocampus showed greater response in a place task [4]. It is intriguing that we found similar left side preference for object processing in mice, suggesting perhaps greater functional parallels between humans and rodents than has been recognized.

In addition, we found that the use of common cell quantification methods resulted in different conclusions regarding the effects of experience on neuronal activity in the rodent hippocampus. In this context, it is intriguing that two similar studies examining IEG expression in the rodent DG arrived at contradictory conclusions. Deng et al. [12] found that re-exposure to a fear-conditioned context did not reactivate DG neurons that were active during the conditioning trial. However, Denny et al., [13] found that re-exposure to a fear-conditioned

context in a similar task paradigm did in fact reactive the same population of neurons that were active during initial encoding. The two studies used different genetic mouse models, which may have contributed to their differing results. However, it is intriguing that the two reports also differed in their approaches to cell quantification with respect to hemisphere. Deng et al. [12] quantified unilaterally, primarily using the right hemisphere for all mice, but on occasion using the left hemisphere if the right was damaged (personal communication). When primarily using the right hemisphere, our data set led to the finding that DG c-Fos expression was not different between OB and CON mice, while primarily using the left hemisphere lead to the finding that DG c-Fos expression was indeed significantly different between the same groups. Denny et al. [13] pooled hemispheres and quantified bilaterally (personal communication). This approach in our study led to a trend (P=0.05) when comparing OB and CON DG c-Fos expression. Thus, considering hemispheric differences in rodent models may potentially resolve discrepancies in reported findings. Moreover, considering hemisphere may in addition provide critical insight into the mechanisms of hippocampal function.

Acknowledgements
We would like to thank M. Regis Shanley for help with a pilot study. We thank Jeff Beeler and Daniel McCloskey for input on experimental design.

Conflicts of Interest
We declare no conflicts of interest (financial or otherwise).

Funding
This work was supported by the Alfred P Sloan Foundation (76332-28) and PSC-CUNY TRADA-43-687 to CLP and CUNY Doctoral Student Research Grant and Mina Rees Dissertation Fellowship to JTJ.

Author Contributions
JJ designed the study. JJ performed the experiments. JJ and CP analyzed the data and wrote the manuscript.

Data Accessibility
All data for this manuscript will be made available upon email request.

References

1. Concha ML, Bianco IH, Wilson SW. 2012. Encoding asymmetry within neural circuits. *Nat Rev Neurosci*, 13: 832-843. https://doi.org/10.1038/nrn3371

2. Maguire EA, Burgess N, Donnett JG, Frackowiack RSJ, Frith CD, O'Keefe J. 1998. Knowing where and getting there: A human navigational framework. *Science*, 280: 921 – 924. https://doi.org/10.1126/science.280.5365.921
3. Bellgowan PSF, Buffalo EA, Bodurka J, Martin A. 2009. Lateralized spatial and object memory encoding in entorhinal and perirhinal cortices. *Learn Mem*, 16(7): 433-438. https://doi.org/10.1101/lm.1357309
4. Lee CH, Ryu J, Lee SH, Kim H, Lee I. 2016. Functional cross-hemispheric shift between object-place paired associate memory and spatial memory in the human hippocampus. *Hippocampus*, 26(8): 1061-1077. https://doi.org/10.1002/hipo.22587.

5. O'Keefe J & Nadel L (1978). *The Hippocampus as a Cognitive Map*. Oxford University Press, Oxford.

6. Shinohara Y, Hosoya A, Yamasaki N, Ahmed H, Hattori S, Eguchi M, Yamaguchi S, Miyakawa T, Hirase H, Shigemoto R, 2012. Right hemispheric dominance of spatial memory in split-brain mice. *Hippocampus*, 22, 117–121. https://doi.org/10.1002/hipo.20886.
7. Shipton OA, El-Gaby M, Apergis-Schoute J, Deisseroth K, Bannerman DM, Paulsen O, Kohl MM. 2014. Left-right dissociation of hippocampal memory processes in mice. *Proc Natl Acad Sci USA*, 111(42), 15238-15243. https://doi.org/10.1073/pnas.1405648111.
8. El-Gaby M, Zhang Y, Wolf K, Schwiening CJ, Paulsen O, Shipton OA, 2016. Archaerhodopsin selectively and selectively and reversibly silences synaptic transmission through altered pH. *Cell Reports*, 16: 2259-2268. https://doi.org/10.1016/j.celrep.2016.07.057
9. Kohl M, Shipton OA, Deacon RM, Rawlins JNP, Deisseroth K, Paulsen O. 2011. Hemisphere-specific optogenetic stimulation reveals left-right asymmetry of hippocampal plasticity. *Nat Neurosci*, 14: 1413-1415. https://doi.org/10.1038/nn.2915.

10. Sakaguchi Y, Sakurai Y. 2017. Left-right functional asymmetry of ventral hippocampus depends on aversiveness of situations. *Behav Brain Res*, 325 (Pt A), 25-33. https://doi.org/10.1016/j.bbr.2017.02.028
11. Kheirbek, M.A., Drew, L.J., Burghardt, N.S., Costantini, D.O, Tanneholz, L., Ahmari, S.E., Zeng, H., Fenton, A.A., Hen, R. (2013). Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron*, 77, 955-968. https://doi.org/10.1016/j.neuron.2012.12.038.
12. Deng W, Mayford M, Gage FH. 2013. Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. *eLife*, 2:e00312. https://doi.org/10.7554/eLife.00312.
13. Denny CA, Kheirbek MA, Alba EL, Tanaka KF, Brachman RA, Laughman KB, Tomm NK, Turi GF, Losonczy A, Hen R. 2014. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron*, 83(1): 189-201. https://doi.org/10.1016/j.neuron.2014.05.018.
14. El-Gaby M, Shipton OA, Paulsen O, 2015. Synaptic plasticity and memory: New insights from hippocampal left–right asymmetries. *Neuroscientist*, 21(5): 490-502. https://doi.org/10.1177/1073858414550658.
15. Wilson CL, Isokawa-Akesson M, Babb TL, et al: A comparative view of local and interhemispheric limbic pathways in humans: an evoked potential analysis, in Fundamental Mechanisms of Human Brain Function: Opportunities for Direct Investigation in Association With the Surgical Treatment of Epilepsy. Edited by Engel JJ, Ojemann GA, Lüders HO, et al. Stuttgart, Germany, London, UK, Raven, 1987, pp 27–38.
16. Phelps EA, Hirst W, Gazzaniga MS. 1991. Deficits in recall following partial and complete commissurotomy. *Cerebral Cortex*, 1, 492-498. https://doi.org/10.1093/cercor/1.6.492
17. Gloor P, Salanova V, Olivier A, Quesney LF. The human dorsal hippocampal commissure: An anatomically identifiable and functional pathway. *Brain*, 116, 1249-1273. https://doi.org/10.1093/brain/116.5.1249

18. Rosenzweig I, Beniczky S, Brunnhuber F, Alarcon G, Valentin A. 2011. The dorsal hippocampal commissure: when functionality matters. *J Neuropsychiatry Clin Neurosci*, 23(3), E45-48. https://doi.org/10.1176/appi.neuropsych.23.3.E45
19. Amaral D, Lavenex P. 2007. Hippocampal Neuroanatomy. In: Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J, eds. The Hippocampus Book. Oxford University Press, Oxford
20. Zaidel, DW. 1995. The case for a relationship between human memory, hippocampus and corpus callosum. *Biol Res*, 28(1): 51-57.
21. Villalobos C, Maldonado PE, Valdés JL. 2017. Asynchronous ripple oscillations between left and right hippocampus during slow-wave sleep. *PLoS One*, 12(2): e0171304. https://doi.org/10.1371/journal.pone.0171304
22. Klur S, Muller C, Pereira de Vasconcelos A, Ballard T, Lopez J, Galani R, Certa U, Cassel JC. 2009. Hippocampal-dependent spatial memory functions might be lateralized in rats: An approach combining gene expression profiling and reversible inactivation. *Hippocampus*, 19, 800–816. https://doi.org/10.1002/hipo.20562.
23. Kawakami R, Shinohara Y, Kato Y, Sugiyama H, Shigemoto R, Ito I, 2003. Asymmetric allocation of NMDA receptor ε2 subunits in hippocampal circuitry. *Science*, 300: 990-994. https://doi.org/10.1126/science.1082609
24. ShinoharaY, Hosoya A, Hirase H. 2013. Experience enhances gamma oscillations and interhemispheric asymmetry in the hippocampus. *Nat Comm*, 4, 1652. https://doi.org/10.1038/ncomms2658
25. Benito N, Martín-Vázquez G, Makarova J, Makarov VA, Herreras O. 2016. The right hippocampus leads the bilateral integration of gamma-parsed lateralized information. *eLife*, 5. pii: e16658. https://doi.org/10.7554/eLife.16658
26. Spiers HJ, Burgess N, Maguire EA, Baxendale SA, Hartley T, Thompson PJ, O'Keefe J. 2001. Unilateral temporal lobectomy patients show lateralized topographical and episodic memory deficits in a virtual town. *Brain*, 124: 2476 – 2489
27. Burgess N, Maguire EA, O'Keefe J. 2002. The human hippocampus and spatial and episodic memory. *Neuron*, 35: 625-641. https://doi.org/10.1016/S0896-6273(02)00830-9
28. Iaria G, Petrides M, Dagher A, Pike B, Bohbot VD. 2003. Cognitive strategies dependent on the hippocampus and caudate nucleus in human navigation: variability and change

with practice. *J Neurosci*, 23:5945–5952 https://doi.org/10.1523/JNEUROSCI.23-13-05945.2003

29. Igloi K, Doeller CF, Berthoz A, Rondi-Reig L, Burgess N. 2010. Lateralized human hippocampal activity predicts navigation based on sequence or place memory. *Proc Natl Acad Sci USA*, 107:14466– 14471. https://doi.org/10.1073/pnas.1004243107
30. Maguire EA, Frith CD. 2003. Lateral asymmetry in the hippocampal response to the remoteness of autobiographical memories. *J Neurosci*, 23(12): 5302 – 5307. https://doi.org/10.1523/JNEUROSCI.23-12-05302.2003

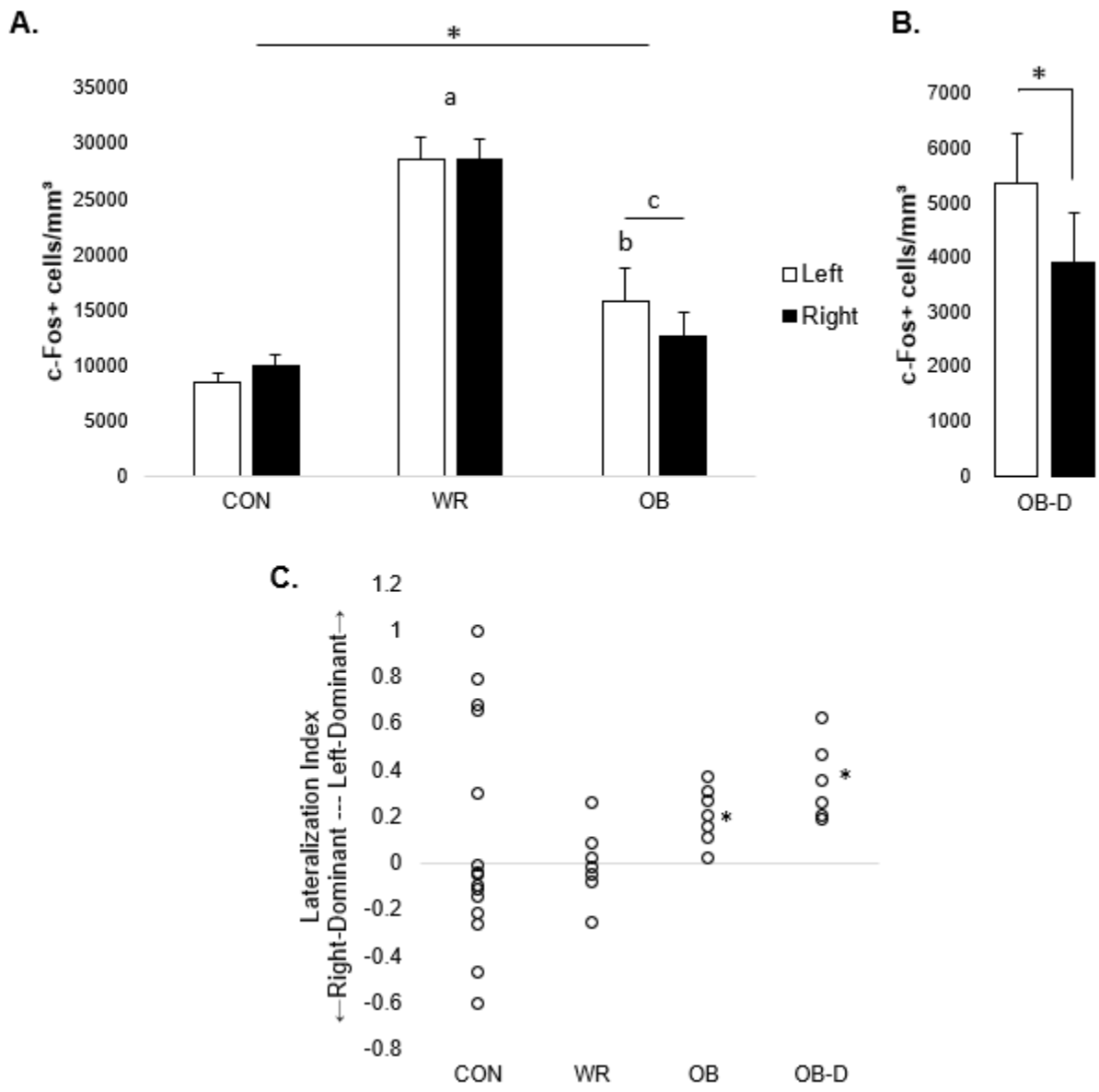

Figure 1. Exposure to a novel object preferentially recruits the left DG, while wheel running equally recruits both DG. (A) Dorsal DG c-Fos expression across hemispheres and behavioral groups shows an overall effect of wheel running on left- and right-hemisphere c-Fos expression. *a* Post hoc tests show that cell counts in the WR group is greater than those in either the CON or OB groups; *b* indicates an effect of object exploration on left-hemisphere c-Fos expression compared to controls; *c* indicates a difference between the left and right DG c-Fos expression within the object exploration group. * indicates main effect, P<0.05; All letters indicate P<0.05. (B) Left and right densities of DG c-Fos expression in OB-D mice. * indicates a difference between hemispheres, P<0.05. (C) Degree of hemispheric lateralization for individuals in each behavioral group. * indicates a group whose mean lateralization index differs from zero, P<0.05.

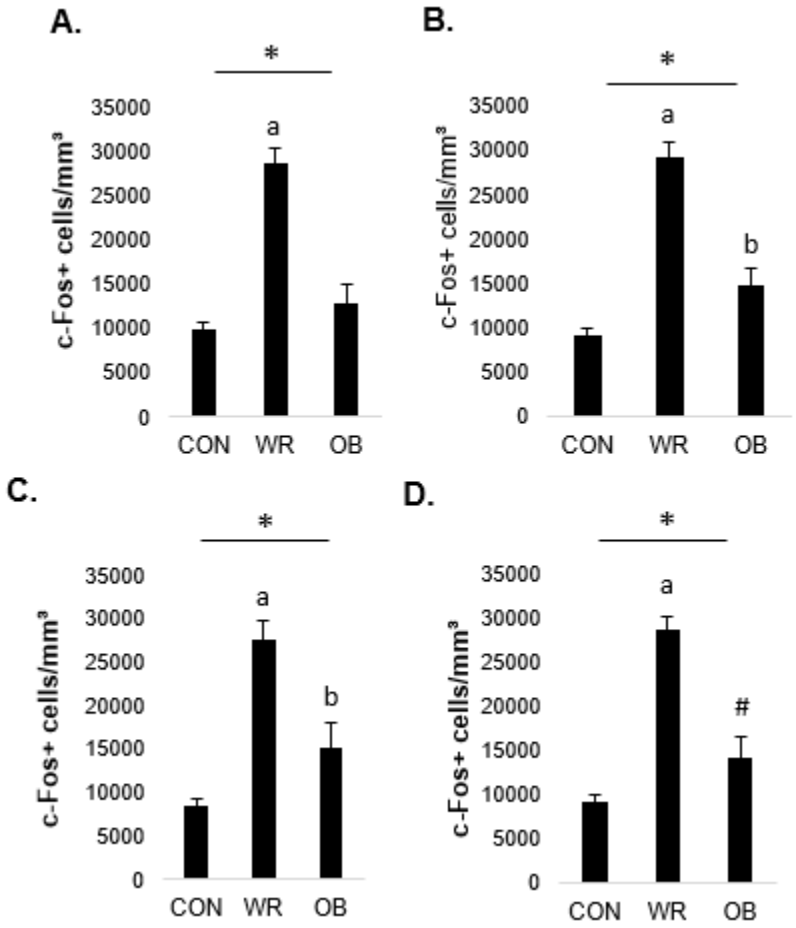

Figure 2. Ignoring hemispheres during cell quantification can lead to incomplete conclusions. (A) DG c-Fos expression when the right hemisphere was used for quantification 80% of the time. (B) DG c-Fos expression when the left hemisphere was used for quantification 80% of the time. (C) DG c-Fos expression when hemisphere was randomly chosen for quantification. (D) DG c-Fos expression when hemispheres were pooled bilaterally. * indicates a main effect of behavioral group, $P<0.05$; [a] indicates a significant difference between WR vs. OB and WR vs. CON, $P<0.05$; [b] indicates a significant difference between OB vs. CON, $P<0.05$; [#] indicates a trend towards a significant difference between OB vs CON, $P=0.05$.