

Neonatal novelty exposure modulates hippocampal volumetric asymmetry in the rat

Timothy Verstynen,¹ Robert Tierney,¹ Tina Urbanski¹ and Akaysha Tang^{1,2,CA}

Departments of ¹Psychology and ²Neurosciences, University of New Mexico, Albuquerque, NM 87131, USA

CA,¹Corresponding Author and Address

Received 26 June 2001; accepted 24 July 2001

Early life environmental manipulations have been shown to affect hippocampal-dependent learning, hippocampal volume and cerebral lateralization. In this study, we investigated the effects of neonatal stimulation on hippocampal volumetric asymmetry. Long-Evans hooded rats were exposed to a novel non-home environment 3 min daily for the first 3 weeks of life. Histological measures of the left and right hippocampus were

made at 8 months of age. We found that neonatal novelty exposure resulted in a long-lasting change in hippocampal volumetric asymmetry. Specifically, this brief and transient early life stimulation increased the right hippocampal volumetric dominance at mid-adulthood. *NeuroReport* 12:3019–3022 © 2001 Lippincott Williams & Wilkins.

Key words: Asymmetry; Hippocampus; Neonatal novelty exposure; Rat

INTRODUCTION

The hippocampus is an important structure for spatial navigation [1–3]. In humans, it is well known that there is a functional dominance of the right hemisphere in spatial processing [4] and a parallel volumetric dominance of the right hippocampus [5–7]. Recently, a human structural MRI study revealed a positive correlation between the amount of time spent as a London taxi driver and the volume of the right posterior hippocampus [6]. This finding suggests that individual differences in the right hippocampal volumetric dominance may have functional consequences in spatial navigation. In rats, several lines of developmental studies suggest that the early life environment can influence hippocampal-dependent spatial learning [8–10], hippocampal volume [11] and hippocampal asymmetry in synaptic plasticity [12]. In this study, we examined the effect of early life stimulation on hippocampal volumetric asymmetry. Using a neonatal novelty exposure paradigm [9], we exposed neonatal rat pups to a novel environment for 3 min daily during the first 3 weeks of life and, histologically, measured their hippocampal volumes at adulthood. We found that neonatal novelty exposure led to a right-shift in this hippocampal asymmetry.

MATERIALS AND METHODS

Animals: Seven pregnant Long-Evans hooded dams arrived at our laboratory 7–11 days prior to giving birth (Harlan Sprague-Dawley Company, Indianapolis, IN). The litter size of each dam varied from 4 to 9 pups. A total of

31 male and four female pups born of these dams participated in this study. The dams and pups were housed in translucent plastic cages (51 × 25 × 22 cm) with a 12:12 h light:dark cycle (lights on 07.00 h) and food and water *ad lib*. Pups were housed with their natural mothers until weaning at postnatal day 21. After weaning, all animals were housed individually.

Novelty exposure: The details of this procedure are described elsewhere [9]. Briefly, the main experimental treatment was the exposure to a novel non-home cage. Using a split-litter design, we assigned approximately two halves of the pups within each litter to Novel and Home groups, with an approximately equal number of male and female pups in each of the groups. Each day, from post-natal day 1 to 21, the dam was first transferred to and remained in a separate housing cage that was placed next to the home-cage. The Novel pups were transferred to their individual new cages (29 × 19 × 12 cm) and remained there for 3 min while the Home pups stayed in the home cage. The new cages were lined with fresh sawdust (depth ~2 cm). To ensure that the only difference between the Novel and Home pups was the exposure to the new environment, each time a Novel pup was picked up by the experimenter, a Home pup was also picked up and replaced in its original location.

Histology: At ~8 months of age, all animals were euthanized with halothane and perfused with ~100 ml saline and a neutral 10% formalin/saline solution through the

ascending aorta. The brains were then removed from the skull and stored in the same formalin/saline solution for the first 24h, after which they were maintained on a 4% sucrose/formalin solution until slicing. On the day of slicing, the brains were frozen and cut coronally at 40 μ m. Every fifth slice was mounted onto a microscope slide (Fisher) and stained with cresyl violet (Aldrich). To prevent the effects of practice, the time of the day, and the delay between fixing and slicing from confounding the neonatal novelty effect, the order of all the above steps were counterbalanced between the Novel and Home rats.

Volumetric measures: The volumetric measurements of the hippocampus were made on all but one structure within the hippocampal formation [13]. We included subfields CA1–CA3, the dentate gyrus, and alveus of the hippocampus, the fornix, fimbria, and subicular complex. We excluded the entorhinal cortex (EC) from our analysis because the precise boundary between the EC and the rest of the cortex was not clearly defined with the specific stain used. Video images of individual tissue slices were captured using a Zeiss microscope with a $\times 4$ objective and Cohu high performance CCD camera. The relevant hippocampal areas were determined according to the stereotaxic atlas of the rat brain [14] and measured using a Scion Imaging program (version 1.59). The volume of each hippocampus was the sum of the product between the area on each slice and the inter-slice distance. In case a slice was missing or damaged, the area was estimated by taking the average of the areas from the slices immediately posterior and anterior to the missing slice (<3% of the slices required such estimation). All slice measurements were made blind with regard to their group affiliation.

Hippocampal asymmetry measures: Two asymmetry measures were computed: a directional lateralization score (L score) that measures both the direction and magnitude of hippocampal asymmetry and a non-directional L score that measures only the magnitude of asymmetry. The directional L score for an individual animal is defined as $(V_{\text{left}} - V_{\text{right}}) / (V_{\text{left}} + V_{\text{right}}) \times 100\%$ where $V = \text{volume}$ [15]. The volumetric difference ($V_{\text{left}} - V_{\text{right}}$) is first normalized by the total hippocampal volume of that individual ($V_{\text{left}} + V_{\text{right}}$) via division. This normalization removes the variance in the total hippocampal volume from the asymmetry measure. To express asymmetry in proportion (percentage) to the total hippocampal volume, the normalized asymmetry measure is multiplied by 100%. The resulting L score can be interpreted as hippocampal volumetric asymmetry expressed in percentage of the total hippocampal volume. For example, an L score of 1% means that the left hippocampus is 1% larger than the right. Defined as such, a positive value of L indicates a left dominance while a negative value a right dominance. If a manipulation produces a reduction in L, this reduction corresponds to a right-shift while an increase in L score indicates a left-shift.

The absolute lateralization score defined as $\text{abs}(L)$, measures the magnitude of asymmetry regardless of the direction of asymmetry. It is possible for two groups of animals to have the same average magnitude of asymmetry ($\text{abs}(L)$) but very different average directional asymmetry (L). For example, a group of left-dominant rats with L

values of 1, 1, 1 and 1 (%) would have an average L of 1 and average magnitude of L ($\text{abs}(L)$) of 1 (%), while a group of right-dominant rats with L values of -1, -1, -1 and -1 (%), would have an average L of -1 but the same average magnitude of 1.

RESULTS

The structures of the hippocampus were visible (Fig. 1) on an average of 29 ± 0.4 tissue slices. For the Novel rats, the measured volumes were $47.1 \pm 1.8 \text{ mm}^3$ and $47.1 \pm 1.7 \text{ mm}^3$ for the left and right hippocampi respectively. For the Home rats, the volumes were $48.0 \pm 1.2 \text{ mm}^3$ and $46.8 \pm 1.2 \text{ mm}^3$ for the left and right hippocampi respectively.

To test for a neonatal novelty effect on hippocampal volumetric asymmetry, average L scores were first computed for the novel and home pups from each litter. The difference score for each litter, $D_L = L_{\text{novel}} - L_{\text{home}}$, was computed as an index for the effect of novelty exposure. If neonatal novelty exposure does have an effect on hippocampal asymmetry, one would expect D_L to be significantly different from zero. A one-sample *t*-test performed on D_L revealed that D_L was significantly smaller than zero ($p < 0.005$), indicating that the neonatal novelty exposure led to a significant reduction in the directional L scores. This effect is very consistent across litters because, as shown in Fig. 2a, within every litter, the average L score of the home group was greater than that of the novel group. As a positive L score indicates a left dominance, this reduction in L score suggests a possible right-shift in hippocampal dominance.

A one-sample *t*-test performed on the L scores of the Home group alone revealed that the control rats had L scores significantly greater than zero (Fig. 2b, $p < 0.001$), indicating a baseline left-hippocampal volumetric dominance. The same test performed on the Novel group, however, yielded no significant difference from zero, consistent with a lack of asymmetry in the Novel pups. A two-sample *t*-test on the L scores pooled from all litters showed a significant reduction in the directional L scores ($p < 0.05$). Because this reduction in directional L scores could be due to a reduction in the magnitude of asymmetry or due to a right-shift in hippocampal asymmetry (see Fig. 2b), one must distinguish between the two possibilities.

To rule out the possibility of a reduction in the magnitude of asymmetry, a paired *t*-test was performed on the magnitude of directional L scores between the Novel and Home groups. We found no significant difference between

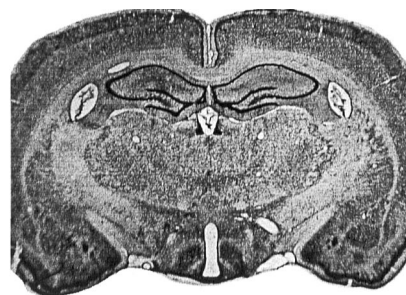


Fig. 1. An example of a coronal section through the rat hippocampus

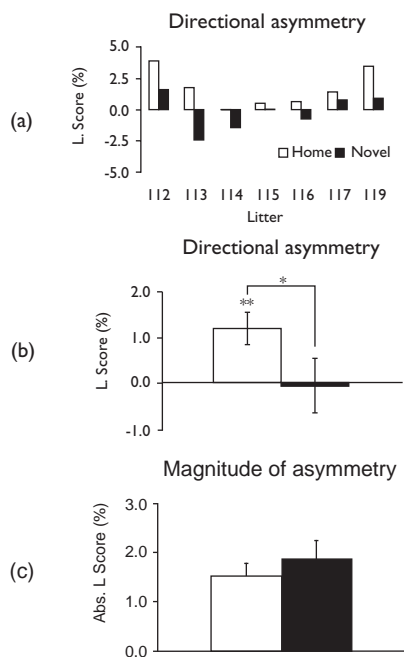


Fig. 2. Neonatal novelty exposure induced a right-shift in hippocampal volumetric asymmetry. (a) average directional L score of the Novel rats was significantly smaller than that of the Home rats for each litter. (b) The direction L score of the Home rats was significantly greater than zero (left dominance) and the directional L score of the Novel rats was not. (c) Magnitude of the directional L score (absolute value) of the Novel and Home rats did not differ significantly.

the Novel and Home groups in their magnitude of asymmetry ($p > 0.20$; Fig. 2c). Therefore, the reduction in the directional L scores is best explained by a right-shift in hippocampal volumetric asymmetry, rather than a reduction in the magnitude of such asymmetry.

To examine this right-shift in more detail, a Chi-square test was performed on the number of right-hippocampal dominant rats from the Novel and Home groups. By definition (see Materials and Methods), right-dominant rats were those whose L scores were smaller than zero. We found a significantly greater number of right hippocampal dominant animals in the Novel than in the Home group ($p < 0.05$), indicating that the novelty exposure increased the number of right-hippocampal dominant animals.

DISCUSSION

We found a significant difference in hippocampal volumetric asymmetry scores between the neonatal novelty exposed and the control rats. This finding indicates that hippocampal volumetric asymmetry is sensitive to a very brief early life environmental manipulation. Specifically, neonatal novelty exposure resulted in a right shift in hippocampal volumetric asymmetry (Fig. 2). This neonatal novelty effect is rather robust in that the same directional change (right shift) can be observed in every individual litter studied (Fig. 2a).

This increase in right-hippocampal volumetric dominance is consistent with a number of other findings from previous studies using the same neonatal novelty exposure. In a reaching task, neonatal novelty exposure resulted

in a left-shift in paw preference, suggesting an increase in the cerebral dominance that is contralateral to the left paw [16]. In studies of long-term potentiation (LTP), neonatal novelty exposure enhanced LTP selectively in the CA1 of the right hippocampus [12]. Finally, an earlier neonatal handling study also indicated a selective change in right-hemisphere function [17].

The mechanisms that mediate the observed changes in hippocampal volumetric asymmetry are not yet known. One possibility is a selective increase in the number of neurons in the right hippocampus. Neonatal handling and exposure to an enriched environment have both been shown to increase hippocampal neuron numbers [8,18]. However, the possible laterality effects of environmental manipulation on neuron numbers were not explored.

The neonatal novelty-induced change in asymmetry is $\sim 1.25\%$ relative to the total hippocampal volume (Fig. 2b), which is in the same order of magnitude as $\sim 2.5\%$ of right hippocampal dominance estimated from MRI studies of human hippocampal volume [5,6]. Using estimates of total hippocampal cell numbers in rats compiled from several studies [19–22] by O'Reilly and Rudy [23], our observed 1.25% shift can be translated into an increase of $\sim 20\,000$ cells in the rodent right hippocampus.

Modulation of cerebral asymmetry by neonatal stimulation has been found using the neonatal handling method and open field task [17]. Left and right complete neocortical lesions produced a differential effect on the open-field activity only in handled rats. Because such neocortical lesions were massive and could lead to reorganization of subcortical regions as well, the behavioral lateralization measure from the open field was both indirect and structurally non-specific. In contrast, our results provide direct measurements on the anatomy of the hippocampus.

Using a hippocampal thickness measure, a basal right hippocampal dominance has been found among rats of different ages except for the 400-day-old group [24]. In contrast, we found a left hippocampal dominance in the control rats. This discrepancy could be due to a variability in the level of stimulation experienced, as differential stimulation during infancy has been shown to affect cerebral asymmetry [12,16,17]. This difference could also be due to differences in the measurement used (volume *vs* thickness).

Finally, although we did not find any sex differences in the above reported data, our data should not be considered as evidence for a lack of sex difference in hippocampal asymmetry or in early environmental effects on hippocampal asymmetry. In this study, we included only a few females for the purpose of maintaining sufficiently large litter sizes. Therefore, we did not have the statistical power to address important issues concerning sex differences [25,26].

CONCLUSION

The effects of neonatal novelty exposure on the volumetric asymmetry of the adult hippocampus were investigated. We found that neonatal novelty exposure led to a right-shift in this hippocampal asymmetry. This modulation was seen both at the group level and across all litters studied. This finding suggests that early life stimulation can influence the development of hippocampal asymmetry, which

may have functional consequences for spatial navigation and other hippocampal dependent functions.

REFERENCES

1. O'Keefe J and Nadel L. *The Hippocampus as a Cognitive Map*. Oxford: Oxford University Press; 1978.
2. Morris R, Garrud GMP, Rawlings J and O'Keefe J. *Nature* **297**, 681–683 (1982).
3. Sutherland RJ, Kolb B and Whishaw IQ. *Neurosci Lett* **31**, 271–276 (1982).
4. Maguire EA, Burgess N, Donnett JG *et al.* *Science* **280**, 921–924 (1998).
5. Pruessner JC, Collins DL, Pruessner M and Evans AC. *J Neurosci* **21**, 194–200 (2001).
6. Maguire EA, Gadian DG, Johnsrude IS *et al.* *Proc Natl Acad Sci USA* **97**, 4398–4403 (2000).
7. Bilir E, Craven W, Hugg J *et al.* *Neuroradiology* **40**, 138–144 (1998).
8. Meaney M, Aiken D, Bhatnager S *et al.* *Science* **239**, 766–769 (1988).
9. Tang AC. *Learn Mem*, in press (2001).
10. Caplan M, Verstynen T and Tang AC. *J Cogn Neurosci*, abs., in press (2001).
11. Cramer CP. *Dev Brain Res* **42**, 151–155 (1988).
12. Zou B and Tang AC. *J Cogn Neurosci*, abs., in press (2001).
13. Amaral DG and Witter MP. *Neuroscience* **31**, 571–591 (1989).
14. Paxinos G and Watson C. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press; 1982.
15. Rosen GD, Sherman GF and Galaburda AM. *Prog Neurobiol* **39**, 507–515 (1992).
16. Tang AC and Verstynen T. *Behav Brain Res*, in press (2001).
17. Denenberg VH, Garbanati J, Sherman G *et al.* *Science* **201**, 1150–1152 (1978).
18. Kempermann G, Kuhn HG and Gage FH. *Nature* **386**, 493–495 (1997).
19. Squire LR, Haist E and Shimamura AP. *J Neurosci* **9**, 828–839 (1989).
20. Boss BD, Turlejski K, Stanfield BB and Cowan WM. *Brain Res* **406**, 280–287 (1987).
21. Boss BD, Peterson GM and Cowan WM. *Brain Res* **338**, 144–150 (1985).
22. Barnes CA, Menaughton BL, Mizumori SJY *et al.* *Prog Brain Res* **83**, 281–300 (1990).
23. O'Reilly RC and Rudy JW. *Psychol Rev*, in press (2001).
24. Diamond MC, Murphy GM, Akiyama K and Johnson RE. *Exp Neurol* **76**, 553–565 (1982).
25. Juraska JM. *Psychoneuroendocrinology* **16**, 105–119 (1991).
26. Lewis D and Diamond M. The influence of gonadal steroids on the asymmetry of the cerebral cortex. In: Davidson R and Hugdahl K (eds). *Brain Asymmetry*. Cambridge, MA: The MIT Press; 1995, pp. 31–50.

Acknowledgements: We would like to thank Roxanne Jensen, Delaine King, Linda Alvarado, Leona Zamora, Jennifer Jones for assistance and Dr. Robert Sutherland and Janice Hoelsing for technical advice. This project was funded in part by a NIMH-COR fellowship to T. Verstynen.