Available online at www.sciencedirect.com
ScienceDirect
Behavioural Brain Research 177 (2007) 358–363
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Short communication

Inhibition of lesion-induced neurogenesis impaired behavioral recovery in adult ring doves

Gang Chen, Mei-Fang Cheng *

Department of Psychology, Rutgers University, Newark, NJ 07102, United States

Received 15 August 2006; received in revised form 20 November 2006; accepted 21 November 2006
Available online 27 December 2006

Abstract

We have previously shown that electrolytic lesion-induced neurogenesis in the ventromedial nucleus of the hypothalamus (VMN) is significantly correlated with the recovery of courtship behavior in adult male ring doves. Here we revealed that reduction of lesion-induced neurogenesis in VMN impaired the behavioral recovery suggesting therefore a causal link between neurogenesis and behavioral recovery. Our results lend support that adult neurogenesis is latent in repair of injured brain.

Keywords: Adult neurogenesis; Ara-C; Courtship behavior; Recovery; DiI; Lesion

In adult vertebrates, including birds and mammals, neurogenesis occurs only in restricted brain regions. Neurogenesis, however, was induced in the brain regions that sustained injury, the neostriatum of adult rats after stroke [1], the mouse neocortex with photolytic lesion [2], the rat hippocampal CA1 region with ischemia injury [3]. A portion of these lesion-induced neurons were found to survive at the lesion site and eventually integrated into the damaged brain circuitry [2,3], suggesting that they may play a role in brain repair. However, compelling evidence linking new neurons to recovery of behavior is still lacking. We have used the ventromedial nucleus (VMN) of the hypothalamus that mediates the nest coo behavior of adult ring doves in search for more direct evidence. We have shown incorporation of new neurons into the hypothalamus after electrolytic lesion in the VMN, a brain region that is not normally neurogenic in the adult ring dove [4]. The VMN contains coo-sensitive neurons [5] and projection neurons that mediate nest coo performance [6], we reason therefore that destruction of these cells are responsible for the deficit of male nest coo seen after bilateral VMN lesions [7]. Indeed, we found recovery of behavior (the nest coo) followed resurfacing of coo-responsive units [5] and projection neurons [8], some of which were born after lesion. We have further shown that increased level of newly generated neurons, facilitated by cohabitation with a receptive female, resulted in a greater recovery in male ring dove's courtship behavior, specifically the nest coo [8]. These correlational data support the hypothesis that lesion-induced VMN new neurons are involved in behavioral recovery. Since recovery of behavior is a collective effect of various factors on the damaged brain, the specific contribution of lesion-induced neurogenesis to behavioral recovery remains to be elucidated. For this reason, and our desire to test the hypothesis of causal relationship between adult neurogenesis and behavioral recovery, in this study, we evaluate the effect of inhibition of neurogenesis on the behavioral recovery.

Adult ring doves (*Streptopelia risoria*), male, 1–3 years old, 150–180 g, were used as subjects. All subjects received surgical procedures in the following sequence in one setting: (1) bilateral electrolytic or sham lesion in VMN; (2) implantation of an osmotic minipump for infusion of an anti-mitotic agent or vehicle onto the surface of the brain; (3) infusion of DiI into the lateral ventricle. Briefly, each subject was anesthetized with Chloropent (2.5 ml/kg) and the head of the bird was fixed on a stereotaxic instrument at an angle of 45 °C. An insulated 0.3 mm o.d. tungsten wire electrode with only the tip conductive was lowered to the VMN area on one hemisphere and then the other (AP = −2.6, $L = \pm 0.5$, $D = -8.5$). Constant positive current with 1 mA was then applied for 30 s. The same procedure was carried out in the sham birds with the exception that no current

* Corresponding author at: Department of Psychology, Rutgers University, 101 Warren Street, Newark, NJ 07102, United States.
Tel.: +1 503 220 8262x54145; fax: +1 503 220 3411.
E-mail address: chenga@ohsu.edu (G. Chen).

0166-4328/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2006.11.041

was applied. After lesion, an osmotic minipump (Alzet 2004, flow rate 0.25 μl/h, 4 weeks) was implanted subcutaneously to infuse Cytosine-Darabinofuranoside (Ara-C, 4%, Sigma) in 0.9% saline or saline alone on the surface of the left brain via a cannula. The position of cannula was AP = 0 relative to the intraaural line and $L = -1.0$ left to the midline. Ara-C solution was infused onto the brain surface to avoid damaging the ventricle cavity or the subventricular zone. This method of Ara-C infusion has been shown to successfully deplete the germinal cells in the subventricular zone [9] or new neurons in the substantia nigra [10] in adult rodents. Immediately after implantation and while the subjects were still anesthetized, the birds received a single injection of 2.5 μl of 0.2% (w/v) 1,1′-dioctadecyl-6,6′-di-(4-sulfophenyl)-3,3,3′,3′ tetramethylindo-carbocyanine (DiI, Molecular Probes) in dimethyl sulfoxide into the right lateral ventricle (AP = −0.8, $L = 0.3$, DV = −4.0) with a 5 μl Hamilton syringe. DiI was used to stain the adult neural stem cells lining the ventricles [9–11].

There were four groups: lesion + Ara-C ($n = 5$), lesion + saline ($n = 4$), sham + Ara-C ($n = 5$), and sham + saline ($n = 4$). The male courtship behavior was tested with a receptive female before surgery (pre-test), 1 week after surgery (post-test) and 6 weeks after surgery (final test). Each male was housed with a female throughout the experiment. One day before the behavioral test, an opaque divider was inserted vertically in the middle of a cage. The test with a stimulus female took 20-min beginning after the divider was pulled out. When encountering a stimulus female, the male bird displayed a number of bouts of courtship behavior consisting of bow coo (BC), nest coo (NC), wing-flip (WF), preen (PR) and cackle (CA) [7]. The frequencies of bow coo, nest coo (which occurred with wing-flip) were tallied while wing-flip (which occurred with or without nest coo) was recorded in duration. If the female was not generally receptive to the male or even fought with the male, a new female was used later on.

Upon the completion of final behavior test at 6 weeks post-lesion, each subject was sacrificed with an overdose of Chloropent. The brain was perfused, fixed, sectioned with a cryostat, and the sections were mounted. The sections were processed with immunofluoresent staining and the newborn neurons were counted. Briefly, sections were incubated in blocking solution with 5% normal donkey serum and 0.5% Triton X-100 in phosphate-buffered saline (PBS) for 1 h at room temperature. Sections were subsequently incubated in primary antibodies: mouse anti-NeuN (Chemicon, 1:200) or rabbit anti-glia fibrillary acidic protein (GFAP, Dako, 1:1000) overnight at 4 °C. The secondary antibodies (1:200, all from Molecular Probes) were then applied to the sections: Alexa 488-conjugated donkey anti-mouse IgG or Alexa 488-conjugated donkey anti-rabbit IgG for 2 h. Sections were counterstained with DAPI. A selection of slides were subjected to simultaneous staining of antibodies NeuN and GFAP. The sections were incubated with pooled solution of primary antibodies including mouse anti-NeuN (1:100) and rabbit anti-GFAP (1:500) overnight at 4 °C, then followed by a pooled solution of secondary antibodies, including Alexa 350-conjugated donkey anti-mouse IgG (1:200) and Alexa 488-conjugated donkey anti-rabbit IgG (1:200) for 2 h.

We used the optical disector technique to conduct unbiased counting of the labeled cells. All the counting of fluorescent labeling was done on a computer monitor using Image-Pro 4.0 software. The images were captured under a Nikon ECLIPSE E800M microscope attached to a Sony DKC-5000 digital photo camera. The DiI+ cells count was limited to the VMN on the hemisphere contralateral to the DiI injection. This precaution was taken to avoid counting cells that were labeled by potential "leak" of DiI along the track of the electrode on the ipsilateral lesion site. Using "counting brick" of 86 μm × 66 μm × 30 μm at 100× objective, we sampled 32 "counting bricks" on the left side of VMN for each subject. For each subject, the number of the DiI+ labeled and DiI+/NeuN+ double labeled cells in the area of VMN was determined, on every 8th immunofluorescent-stained, 30-μm-thick coronal sections taken 240 μm apart. Both DiI+ cells and DiI+/NeuN+ cells were counted. The average density of double labeling was derived by sum of all counts of DiI+/NeuN+ double labeling divided by sum of sampled volume. The percent of new neurons within the newborn cells was calculated by sum of all counts of double labeling divided by sum of all counts of DiI+ cells. Statistical values were expressed as the mean ± S.E.M. Two-way ANOVA and ANOVA with repeated measures were used to analyze behavioral and cytological data. When appropriate, post hoc comparisons between groups were applied using Bonferroni method. p-Values <0.05 were considered statistically significant.

One week post-surgery, there was a significant main effect of lesion on all three major displays of courtship behavior ($F(1, 14) = 15.09$ BC, 5.08 NC, 30.36 WF, $p < 0.05$), whereas the infusion of Ara-C did not show a significant main effect on the three major displays ($F = 0.38$ BC, 0.39 NC, 2.94 WF, $p > 0.10$). No significant interaction was found between lesion and Ara-C on all three displays. In other words, the disruption of courtship behavior 1 week after surgery resulted entirely from VMN lesion; Ara-C had no direct effect on the behavior of the sham or lesion birds (Fig. 1). However, 6 weeks post-lesion, the level of courtship behavior showed distinct group differences depending on the type of display (Fig. 2). There was no significant difference in BC across four groups: the disruption of BC seen at 1 week had disappeared. The treatment of Ara-C did not affect BC in either sham birds or lesioned birds. In contrast, there was a main effect of lesion ($F(1, 14) = 18.78$, $p < 0.001$) and Ara-C treatment ($F = 5.53$, $p < 0.05$) on the NC display. Lesion birds receiving Ara-C treatment continued to show the deficit in NC, compared to lesion birds receiving vehicle ($t = 3.05$, $p < 0.05$) or sham birds receiving Ara-C ($t = 4.52$, $p < 0.01$), whereas Ara-C alone did not show any effect on NC in sham birds ($t = 0.28$, $p > 0.10$). A similar result was found in WF as well: Ara-C treated lesion birds were significantly different from the birds received lesion without Ara-C treatment ($t = 4.204$, $p < 0.01$) or birds received Ara-C but no lesion ($t = 5.835$, $p < 0.01$). In addition, there was a significant interaction of lesion and Ara-C treatment on WF ($F = 4.62$, $p < 0.05$). When compared to birds' own presurgery courtship, only the lesion birds receiving Ara-C treatment displayed significantly different levels of NC (repeated measures, $F(3, 14) = 4.15$, $t = 3.477$, $p < 0.05$) and WF ($F = 5.79$, $t = 4.423$, $p < 0.01$).