Interplay of Maternal Care and Genetic Influences in Programming Adult Hippocampal Neurogenesis

Muriel Koehl, Rixt van der Veen, Delphine Gonzales, Pier Vincenzo Piazza, and Djoher Nora Abrous

Background: Adult hippocampal neurogenesis, which is involved in the physiopathology of hippocampal functions, is genetically determined and influenced by early life events. However, studies on the interaction of these determining forces are lacking. This prompted us to investigate whether adult hippocampal neurogenesis can be modulated by maternal care and whether this influence depends upon the genetic background of the individual.

Methods: We used a model of fostering that allows singling out the influence of the genetic make-up of the pups on the outcome of maternal behavior. Mice from two different inbred strains (C57BL/6J and DBA/2J) known to differ in their baseline neurogenesis as well as in their sensitivity to the influence of environmental experiences were raised by nonrelated mothers from the AKR/Ola (AKR) and C3H/He (C3H) strains exhibiting low- and high-pup-oriented behavior, respectively. Neurogenesis was then assessed in the dentate gyrus of the adult adopted C57BL/6J and DBA/2J mice.

Results: We show that both the number and the morphological features of newborn granule cells in the dentate gyrus are determined by the maternal environment to which mice were exposed as pups and that this sensitivity to maternal environment is observed only in genetically vulnerable subjects.

Conclusions: Altogether, our data indicate interplay between early environment and the genetic envelop of an individual in determining adult hippocampal neurogenesis. Our experimental approach could thus contribute to the identification of factors determining the neurogenic potential of the adult hippocampus.

Key Words: Dendrite, hippocampus, maternal behavior, mice strain, neurogenesis, resiliency

The development of neural systems of the brain and subsequent behavior depends on continuous interaction of genes with environmental factors, among which the ontogenetic niche provided by the mother is a determinative force. Thus clinical and epidemiological studies point to an important role of early life experiences in determining vulnerability to psychiatric disorders in adulthood (1). During the early postnatal period, several brain structures involved in cognitive and emotional processing—such as the dentate gyrus (DG) of the hippocampus—still develop (2). It is thus very likely that interferences with this neuronal development might lead to long-lasting structural and functional consequences and increase the risk of developing psychopathology.

In particular, prenatal stress and maternal deprivation studies have highlighted the importance of mother–pup interactions in regulating neural systems subserving cognitive functions, including adult hippocampal neurogenesis. Indeed both manipulations were consistently found to decrease cell proliferation in the DG of the offspring, resulting in a decreased level of adult neurogenesis (3–6). Because these manipulations involve to a certain degree a disruption of mother–pups interactions, and because pups are not weaned and depend on the presence of their mother for survival, the previous dataset suggested that level of maternal care is instrumental in determining the setpoint for adult neurogenesis.

From Institut National de la Santé et de la Recherche Médicale (MK, DG, PVP, DNA); University of Bordeaux (MK, DG, PVP, DNA), Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale, Bordeaux, France; and the Leiden University Medical Pharmacology Department (RvdV), Leiden, The Netherlands.

Address correspondence to Muriel Koehl, Ph.D., Neurogenesis and Physiopathology Laboratory, INSERM U862, 146 rue Leo Saignat, 33077 Bordeaux Cedex, France; E-mail: muriel.koehl@inserm.fr.

Received Nov 18, 2011; revised Feb 16, 2012; accepted Mar 1, 2012.

However, the maternal mediation of this determinism has not been directly tested, due to lack of an appropriate model that would distinguish parental effects that are nongenomic in nature from those associated with genetic transmission. Indeed, despite the common assumption in both psychology and biology that parental behavior exerts a pervasive influence on phenotype, it rarely determines per se the nature and outcome of psychopathology, because the genetic makeup of an individual can highly modulate the capacity of an environmental risk factor to give rise to mental illness (7).

The aim of this study was thus to establish a model allowing singling out the influence of the genetic make-up of the pups on the outcome of maternal behavior to determine the influence of maternal care on adult neurogenesis. To this end, we selected two widely used inbred strains of mice (C57BL/6J and DBA/2J) known to differ in their baseline neurogenesis (8–11) as well as in their sensitivity to the influence of environmental experiences (12,13). These mice were raised by nonrelated mothers from the AKR/Ola (AKR) and C3H/He (C3H) strains exhibiting low and high levels of maternal care, respectively (14). Neurogenesis was then assessed in the DG of the adult adopted C57BL/6J and DBA/2J mice.

We hypothesized, on the basis of their reported differential sensitivity to environmental factors (12,13), that neurogenesis in DBA mice would be dependent on the level of maternal care received during development, whereas that of C57 would be unaffected or at least less affected by the rearing environment. Such a result would: 1) demonstrate unequivocally the involvement of maternal care in the developmental control of adult neurogenesis; and 2) constitute the first step in the isolation of the biological factors, both genetic and environmental, that control individual differences in adult neurogenesis.

Methods and Materials

Subjects

All mice used in the experiments were bred in our animal facilities. Three of the original strains (C57BL/6JOlaHsd, AKR/OlaHsd,

0006-3223/$36.00
doi:10.1016/j.biopsych.2012.03.001

C3H/HeNHsd) were obtained from Harlan Laboratories (Gannat, France), whereas the DBA/2J@Ico mice were obtained from Charles River Laboratories (Arbresle, France). All experiments were carried out in accordance with the European Community council directive 2010/63 and institutional regulations.

Breeding and Cross-Fostering

Cross-fostering was conducted between 4 and 8 hours after both biological and adoptive dams had given birth (Supplement 1). Four experimental groups/pup strain were constituted—pups of the C57 and DBA strains raised by: 1) their biological mother, 2) a mother of the same strain (a control of the cross-fostering procedure), 3) a mother of the AKR strain, or 4) a mother of the C3H strain (Figure 1A). For each experimental group, a maximum of two pups/foster mother was used (Table 1) to sample an adequate number of litters and thus prevent any uncontrolled mother effect.

Maternal Behavior

Maternal behavior was continuously recorded on postnatal days (PDs) 2, 4, 6, and 9. Three pup-oriented (pup licking, nursing posture, and nest scattering) and three non-pup-oriented (self-grooming on the nest, passive nest presence, and out of nest) behaviors were scored (Supplement 1).

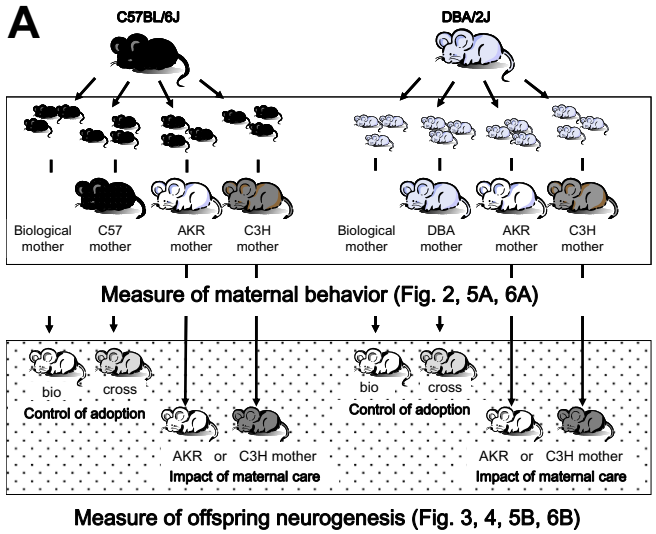

Figure 1. Experimental design of the study. **(A)** Experimental setup: manipulation of maternal care levels was achieved by fostering pups of the C57 and DBA strains with mothers of their own strains (biological mothers [bio], or fostered [cross] mothers from the same strain) or mothers from the AKR or C3H strain. **(B)** Time course of the study: all fostering procedures took place on postnatal day (PD) 0. Maternal behavior was recorded from PD 2 to PD 9. Fostered mice were left undisturbed until 11 months of age when they were injected with bromodeoxyuridine (BrdU) (1 daily injection of 50 μg/g for 12 days) and sacrificed 24 hours after the last injection to measure their baseline neurogenesis levels.

Table 1. Experimental Design: Number of Animals Studied and Number of Litters Sampled/Experimental Group

	Number of Pups Studied	Number of Litters Sampled
Impact of Inter-Strain Fostering		
C57-AKR	11	8
C57-C3H	14	10
DBA-AKR	11	8
DBA-C3H	13	9
Impact of Intra-Strain Fostering		
C57-bio	16	12
C57-cross	14	13
DBA-bio	11	7
DBA-cross	11	8

Bio, mice raised by their biological mother; cross, mice raised by a mother of the same strain.

Bromodeoxyuridine Injections and Brain Preparation

To determine lasting effects of the maternal environment on different phases of adult neurogenesis, 11-month-old animals from each of the eight experimental groups (mice raised by their biological mother [bio], by a mother of the same strain [cross], by a AKR mother, or by a C3H mother) received daily injection with bromodeoxyuridine (BrdU) (50 mg/kg/10 mL saline) for 12 days and were killed 24 hours after the last injection (Figure 1B). Brains were cut on a vibratome (30-μm-thick sections), and sections were collected in 10 series.

Immunohistochemistry/Stereological Analysis

For each staining, sections were processed according to a standard immunohistochemical procedure, and the number of immunoreactive (IR) cells was counted under a 100× microscope objective (Supplement 1) (15).

Morphological Analysis

Doublecortin (DCX)-IR cells were subcategorized according to their dendritic morphology (16,17): dendritic processes of DCX-IR cells were traced with NeuroLucida (MicroBrightField, Williston, Vermont) and a Sholl analysis was conducted on the reconstructed neurons.

Statistical Analysis

Statistical analysis was performed with Statistica 8.0 (Statsoft, Tulsa, Oklahoma). Differences between groups were analyzed with two-way analysis of variance, with pup strain and mother strain as main effects, followed by a post hoc comparison with the least significant difference test whenever appropriate. For newborn neurons, morphological analysis unpaired *t* test was used.

Results

Impact of Inter-Strain Fostering on Maternal Behavior

We first analyzed the maternal behavior displayed by AKR and C3H mothers toward the adopted C57 and DBA pups. As expected, AKR and C3H dams were very different in the maternal care they provided, but this difference was irrespective of the pup strain (see Table 2 for complete statistical analysis). Thus C3H mothers exhibited more pup-oriented behavior with a positive valence such as"pup licking" (Figure 2A) and "arched-back nursing posture" (Figure 2B) and less pup-oriented behavior with a negative valence such as "nest scattering" (Figure 2C). They also engaged in less non-pup-oriented behavior such as "passive nest presence" (Figure 2D) and "self-grooming in the nest" (Figure 2E) than AKR mothers,