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Research report

The postnatal origin of adult neural stem cells and the effects of glucocorticoids on their genesis

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HIGHLIGHTS

- First postnatal week neural precursors can be traced by dual birth-dating analysis.
- These double-labeled precursors were preferentially dividing at P6.
- DEXA administration at P6 caused a depletion of adult DG precursors.
- Treatment also caused an impairment in adult cognitive and emotional behavior.

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ABSTRACT

The relevance of adult neurogenesis in hippocampal function is well documented, as is the potential impact stress has on the adult neurogenic niche. Adult born neurons are generated from neural precursors in the dentate gyrus (DG), although the point in postnatal development that these cell precursors originate is not known. This is particularly relevant if we consider the effects stress may have on the development of neural precursors, and whether such effects on adult neurogenesis and behavior may persist in the long-term. We have analyzed the proportion of neural precursors in the adult murine hippocampus born on specific days during postnatal development using a dual birth-dating analysis, and we assessed their sensitivity to dexamethasone (DEX) on the peak day of cell generation. We also studied the consequences of postnatal DEX administration on adult hippocampal-dependent behavior. Postnatal day 6 (P6) is a preferred period for proliferating neural stem cells (NSCs) to become the precursors that remain in a proliferative state throughout adulthood. This window is independent of gender, the cell's location in the DG granule cell layer or their rostro-caudal position. DEX administration at P6 reduces the size of the adult NSC pool in the DG, which is correlated with poor learning/memory capacity and increased anxiety-like behavior. These results indicate that aNSCs are generated non-uniformly during postnatal development, with peak generation on day P6, and that stress receptor activation during the key period of postnatal NSC generation has a profound impact on both adult hippocampal neurogenesis and behavior.

1. Introduction

Adult hippocampal neurogenesis involves the *de novo* formation of neurons from progenitor cells than in turn are formed from neural stem cells (NSCs) in the dentate gyrus (DG). This implies that NSCs (or precursor cells) are not only present in the embryo (although the majority can be found in this stage) but also in the postnatal and adult brain. NSCs can generate both neurons and glia, and they are thought to be characterized by the following features [1]: (1) they originate from glial cells; (2) there is a direct relationship between adult and embryonic stem cells (since they are transformed from neuroepithelial cells to radial glia that go on to adopt astroglial characteristics); (3) these cells essentially divide asymmetrically to produce intermediate progenitor cells (IPCs), yet sometimes they divide symmetrically in order to increase their number. By contrast, IPCs always divide symmetrically to amplify the number of proliferating progeny [1]. In the present work, we have adopted the expression aNSCs as the cells from which "progenitor cells" are generated in the adult dentate gyrus neurogenesis.

Abbreviations: NSCs, neural stem cells; aNSCs, adult neural stem cells; DEX, dexamethasone; DG, dentate gyrus; P6, postnatal day 6; IPCs, intermediate progenitor cells; ES, stem cells; GFAP, glial fibrillary acidic protein; LTP, long term potentiation; HPA, hypothalamic pituitary adrenal axis; SGZ, subgranular zone; GCL, granular cell layer; ML, molecular layer.

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NSCs can be found in the nervous system and they can be derived from embryonic stem cells (ES cells) or reprogrammed fibroblasts [2,3]. The main difference between NSCs and IPCs is the rate of proliferation and while adult NSCs (aNSCs) divide slowly, IPCs have a high rate of division. However, there is still some controversy regarding the distinction between NSCs and IPCs, and the markers that can be used to identify them. Nevertheless, some consensus exists regarding many features of aNSCs, such as the expression of glial fibrillary acidic protein (GFAP), which has also changed our perception of glia [1,4]. This was demonstrated when the conditional ablation of $GFAP^+$ cells led to a loss of neurogenesis in the adult mouse brain [5]. Moreover, the labeling of these cells with Sox2 reflects their stem nature [6]. Given the difficulty in labeling aNSCs, as experienced elsewhere, we assumed double labeling with Sox2 and GFAP to be a characteristic of these cells [7]. Moreover, since we were specifically interested in the temporal origin of aNSCs, the cells responsible for adult neurogenesis, we attempted to avoid labeling IPCs in this study, as will be detailed later.

Adult neurogenesis is involved in learning and memory [8,9]. It plays an important role in normal DG function during spatial learning [10–12] and it may also be necessary to maintain the functional integrity of the circuits into which new cells are born. In fact, hippocampal lesions or disrupted hippocampal function impair spatial working memory [13,14]. Moreover, it is known that newborn neurons contribute to synaptic plasticity in the DG (reflected by long term potentiation – LTP) [12,15,16]. Recently, the neurogenic hypothesis of depression was established after the demonstration that serotonin depletion inhibited adult neurogenesis [17], and that chronic but not acute antidepressant treatment increased SGZ proliferation and neurogenesis [18]. In addition, a number of studies demonstrated the relationship between the hippocampus, stress, monoamines and major depressive disorder [19–21]. Consequently, adult neurogenesis has been postulated as a key factor modulating emotional processes like stress, depression or anxiety.

Despite the importance of adult neurogenesis and aNSCs, little is known about the critical periods for aNSCs generation during postnatal development. It was recently shown that aNSCs initially originate in the ventral hippocampus during late gestation and that they then relocate to the dorsal hippocampus [22]. However, we were more interested in the temporal rather than the spatial origin of these cells that later persist and divide in the adult hippocampus. This is also relevant to the long-term impairment in the adult brain that may be caused during development. Moreover, we also evaluated the activity of glucocorticoids as key molecules in the process of stress that may act during this period. Although glucocorticoids are essential for normal brain development, exposure of the fetal brain to an excess of glucocorticoids can also modify fetal brain development and permanently alter the function of the hypothalamic pituitary adrenal axis (HPA) in postnatal life [23–25]. There is evidence suggesting that events during prenatal life have long-lasting effects during postnatal development and in the adult, for example altering the regulation of the HPA, increasing blood pressure and/or impairing glucose tolerance in the brain [26]. Indeed, it is known that prenatal glucocorticoids enhance the vulnerability to environmental toxicants [27] and furthermore, in the fetus glucocorticoids are thought to affect a range of affective and behavioral outcomes in the offspring in later life, as well as producing some somatic outcomes [26].

In summary, in this study we set out to determine the critical time window for the generation of aNSCs, which might influence adult neurogenesis and thereby alter processes such as memory and learning in the adult. The data we obtained suggest that glucocorticoids have a significant effect on the fetal and postnatal brain in terms of aNSC establishment. Thus, we hypothesized that as key molecules in the stress response, glucocorticoids may specifically affect the generation of aNSCs, potentially affecting aspects of adult DG activity related to anxiety and learning/memory.

Table 1
Summary of the different litters/groups used.

Experiment	Litter	Number of animals	Males	Females
A	Litter 1	6	4	2
A	Litter 2	8	5	3
A	Litter 3	5	2	3
A	Litter 4	8	2	6
A	Litter 5	9	5	4
A	Litter 6	7	3	4
A	Litter 7	10	6	4
A	Litter 8	6	6	0
B	Control litter	8	3	5
B	Dexamethasone litter	8	6	2

2. Materials and methods

2.1. Animals

The C57/BL6J mice (males and females) used in this study were obtained from the Cajal Institute Animal House and they were housed under controlled, standard laboratory conditions: temperature (21–23 °C), a light/dark cycle of 12:12 h, *ad libitum* access to food and water (AO4 standard maintenance diet; SAFE, Épinay-sur-Orge, France). The care and handling of animals was in accordance with the European Union Directive (2010/63/EU) and Spanish regulations (RD 1201/2005) for the use of laboratory animals. All the protocols for the animal experiments were approved by the Bioethics Committee of the Cajal Institute and CSIC (approval certificate number BFU2007-60195, issued June 7, 2007). A total of 59 mice were used in experiment A and 18 in experiment B. In experiment A, the mice were divided into eight groups in function of the time of CldU and IdU injection. In experiment B, mice were divided into two groups, one of which received Dexamethasone (DEX) injection and the other received vehicle. Further details about all the groups of mice used are summarized in Table 1.

2.2. Experimental design and injection of thymidine analogs

In experiment A, the mice were distributed into eight groups (1–8) that were injected with 5-chloro-2′-deoxy-uridine (CldU, 57.65 mg/kg body weight, intraperitoneal – i.p. –: Ref. C6891, Sigma–Aldrich, St. Louis, USA) dissolved in saline buffer (0.9%) and 5-iodo-2′-deoxy-uridine (IdU, 42.75 mg/kg body weight, i.p.: Ref. I7125, Sigma–Aldrich, St. Louis, USA) dissolved in phosphate buffer saline (PBS), as summarized in Fig. 1. The doses of the thymidine analogs were based on equimolar doses of 50 mg/kg body weight BrdU [28]. CldU was used to label cells dividing on the 3rd, 6th or 9th postnatal day (P3, P6 or P9) and the IdU injection was used to label cells dividing on P24, P32, P49, P56. One week after IdU injection the animals were sacrificed by intracardiac perfusion (see Fig. 1).

In experiment B, the mice were distributed into two groups (Control and DEX) depending on whether they received the drug or not on P6 (i.p.). Administration of the drug on P6 was chosen based on previous experimental data from our lab that demonstrate greater susceptibility of aNSCs to DEX on P6 than on P3 or P9 (unpublished results). DEX (1 μg/g body weight: Ref. D4902, Sigma–Aldrich, St. Louis, USA) was dissolved in a mix 1:9 of ethanol and saline buffer 0.9%, and the control mice in this experiment were injected with the vehicle alone. Three hours after drug administration the animals received CldU and on P56, the animals were injected with IdU. Between P60–P63 animals performed behavioral tasks (Elevated Plus Maze and Passive Avoidance: see below)