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ADULT NEUROGENESIS IS REGULATED BY ADRENAL STEROIDS IN THE DENTATE GYRUS

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Abstract—The dentate gyrus of the rat produces new granule neurons well into adulthood. In the adult, newly born granule neurons migrate from the hilus to the granule cell layer, receive synaptic input, extend axons into the mossy fiber pathway, and express a neuronal marker. No previous studies have identified factors that regulate neuronal birth in the adult dentate gyrus. In order to determine whether glucocorticoids control neurogenesis in the adult dentate gyrus, the effects of adrenal steroid manipulations on neuronal birth were assessed using [^{3}H]thymidine autoradiography and immunohistochemistry for the neuronal marker neuron specific enolase. Acute treatment with corticosterone produced a significant decrease in the density of [^{3}H]thymidine-labeled cells in the hilus of the dentate gyrus. In contrast, removal of endogenous adrenal steroids stimulated increased neuronal birth; adrenalectomy resulted in a significant increase in the number of neuron specific enolase-immunoreactive [^{3}H]thymidine labeled cells in the granule cell layer compared to sham operation. Replacement of corticosterone to adrenalectomized rats after [^{3}H]thymidine injection did not substantially alter the increase in neurogenesis observed following adrenalectomy, even though this replacement protects cells from adrenalectomy-induced cell death. These results indicate that the rate of neurogenesis in the dentate gyrus of the adult rat is dependent upon the levels of circulating adrenal steroids.

In most mammalian brain regions, neurogenesis occurs during a discrete embryonic period, after which neurons differentiate and lose the ability to divide. In contrast, a strong body of evidence indicates that precursors in the dentate gyrus of the rat continue to produce neurons throughout adulthood. First, [^{3}H]thymidine-labeled cells in the adult dentate gyrus have been identified as neurons on the basis of structural characteristics at both the light[2,4] and electron microscopic level, including the presence of synapses on their cell bodies and dendrites.[16,17] Second, cells in the dentate gyrus that take up [^{3}H]thymidine have been shown to extend axons into the mossy fiber pathway.[34] Third, newly born cells in the adult dentate gyrus migrate from the hilus into the granule cell layer, and the majority of these cells express the neuronal marker neuron specific enolase (NSE) three weeks after mitosis.[7] However, although these studies have provided solid evidence for the persistence of neurogenesis in the dentate gyrus, none have determined the factors that regulate this process during adulthood.

Several lines of evidence suggest that adrenal steroids naturally inhibit neurogenesis in the dentate gyrus. First, the endogenous levels of circulating adrenal steroids correlate negatively with the rates of neuronal birth in the dentate gyrus throughout the life of the animal. During the late embryonic period, the rate of neuronal birth is high while adrenal steroid levels are relatively low. As parturition approaches, the levels of adrenal steroids rise and neurogenesis is transiently inhibited.[5] After birth, adrenal steroid levels diminish rapidly and remain low during the first two postnatal weeks.[27] This developmental phase, termed the stress hyporesponsive period, is characterized by maximal neurogenesis in the dentate gyrus.[30] As adrenal steroid levels begin to rise during the second postnatal week,[27] the rate of neuronal birth declines.[30] In adulthood, adrenal steroid levels are relatively high[27] and neuronal birth continues at a slow rate[7] at least until 11 months of age,[16] the latest time point studied to date. Second, administration of the glucocorticoid corticosterone or the mineralocorticoid aldosterone during the stress hyporesponsive period inhibits cell birth in the dentate gyrus,[12] whereas adrenalectomy toward the end of the stress hyporesponsive period increases cell division in the hippocampus.[38] Since the dentate gyrus of the rat retains many developmental features throughout adulthood, e.g. neuronal birth and migration, it is possible that the factors which regulate neurogenesis during development continue to do so in adulthood. In a previous study, we have shown that adrenalectomy increases the rate of glial cell birth as well as the birth of cells that do not stain for glial markers in the adult rat.[13] However, no direct evidence exists to indicate whether the birth of neurons is inhibited by

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Abbreviations: NSE, neuron specific enolase; PBS, phosphate-buffered saline.

adrenal steroids in adulthood or whether developmental levels of this process can be reinstated with hormonal manipulations. Many studies have found that adrenalectomy also produces massive granule cell death,[11,13,20,24,32,37] but it is currently unknown whether adrenalectomy-induced cell death affects newly born cells in the dentate gyrus. In order to determine whether adrenal steroids inhibit neurogenesis in the adult rat dentate gyrus and whether adrenalectomy-induced cell death in this region affects newly born cells, we examined the density of newly born neurons using [^{3}H]thymidine autoradiography and immunohistochemistry for NSE in the dentate gyrus of rats subjected to adrenal steroid manipulations in adulthood.

EXPERIMENTAL PROCEDURES

Animal treatments

Adult (three to five months old) male Sprague–Dawley rats (Charles River) were used for the following three experiments ($n = 5$ in each group). The rats were group-housed and provided with unlimited access to food and water. All surgery was performed using aseptic procedures under Metofane anesthesia.

Short-term corticosterone treatment. This experiment was designed to determine whether high levels of corticosterone can further suppress the low levels of neurogenesis normally seen in the adult dentate gyrus.

Rats received subcutaneous injections of corticosterone (40 mg/kg) in sesame oil daily for two successive days, sham injections of sesame oil alone daily for two successive days, or no treatments at any time. This dose of corticosterone is adequate to elevate blood levels of corticosterone for 24 h[28] at which time the rats received a second injection. Since the cell cycle of granule neurons is approximately 16 h,[21] this treatment regimen results in elevated corticosterone levels throughout at least one cell cycle. Rats in all three groups received a single intraperitoneal injection of 5.0 μCi [^{3}H]thymidine/g body weight (New England Nuclear, specific activity 80 Ci/mmol) 1 h after the time of the second corticosterone injection and were perfused 1 h later. The 1 h survival time following [^{3}H]thymidine injection was selected because it is adequate for the uptake of [^{3}H]thymidine by cells synthesizing DNA[19,22] but not sufficient for the completion of mitosis[21] or migration,[7] thus identifying the progenitor population at the birthplace. The brains were processed for autoradiography combined with Nissl staining as described below.

Adrenalectomy. This experiment was designed to positively identify cells born in the adult dentate gyrus after adrenalectomy as neurons.

Rats were subjected to one of the following treatments: sham operation or bilateral adrenalectomy. Adrenalectomized rats were provided with 0.9% NaCl in the drinking water to maintain salt balance. One week after surgery, these rats received an intraperitoneal injection of 5.0 μCi/g body weight [^{3}H]thymidine in water (New England Nuclear, specific activity 80 Ci/mmol) and were perfused three weeks later. This survival interval between [^{3}H]thymidine injection and perfusion provides enough time for newly born neurons to migrate to the granule cell layer and express NSE.[7] The brains were processed for autoradiography, immunohistochemistry for NSE, and Nissl staining as described below.

Adrenalectomy with corticosterone replacement after [^{3}H]thymidine injection. This experiment was designed to investigate the possibility that many newly born neurons degenerate following chronic adrenalectomy, thus diminishing the observed increase in [^{3}H]thymidine-labeled NSE immunoreactive cells, because massive death of granule cells occurs when corticosterone levels are very low.[11,13,32] That is, the increase in newly born neurons observed in the adrenalectomy experiment (see above) may have underrepresented the actual magnitude of cell division. In this experiment, [^{3}H]thymidine was given when corticosterone levels were very low (due to adrenalectomy), and corticosterone was replaced after dividing cells were labeled with [^{3}H]thymidine in order to protect these cells from adrenalectomy-induced cell death.

Rats were subjected to one of the following treatments: sham operation or bilateral adrenalectomy. Adrenalectomized rats were provided with 0.9% NaCl in the drinking water to maintain salt balance. One week after surgery, these rats received an intraperitoneal injection of 5.0 μCi/g body weight [^{3}H]thymidine in water (New England Nuclear, specific activity 80 Ci/mmol). Adrenalectomized rats were given corticosterone replacement in the drinking water (25 μg/ml in 0.9% NaCl with 0.15% (v/v) ethanol) following [^{3}H]thymidine injection. This dose results in blood levels of corticosterone which are within the lower range of normal levels.[11] The rats were perfused four weeks following [^{3}H]thymidine injection. This survival interval provides enough time for the newly born neurons to express NSE and to become morphologically indistinguishable from surrounding neurons.[7] The brains were processed for autoradiography, immunohistochemistry for NSE and Nissl staining as described below.

Histological procedures

The rats were deeply anesthetized with Metofane and transcardially perfused with 120 ml 4.0% paraformaldehyde in 0.1 M phosphate buffer with 1.5% (v/v) picric acid. The brains were dissected from the skulls and postfixed overnight in the perfusion solution.

For autoradiography alone, brains were cryoprotected in 30% sucrose in phosphate-buffered saline (PBS) following postfixation and then frozen on dry ice. Coronal sections (16 μm) were cut on a cryostat. The sections were mounted onto gelatinized slides, dried, dipped in photographic emulsion (NTB-2, Kodak), and stored in the dark at 4°C for four weeks. The slides were then developed in Dektol (Kodak), rinsed in water, fixed in Ektaflo (Kodak), rinsed in water, Nissl stained in Cresyl Violet, dehydrated, cleared in Amer-iclear, and coverslipped under Permount.

For combined immunohistochemistry and autoradiography, 40-μm coronal sections were cut on a Vibratome into a bath of PBS. Sections were immunohistochemically stained for NSE as described previously.[13] Briefly, the sections were incubated overnight in a solution of rabbit polyclonal antibodies to NSE (Polysciences, diluted 1:2000 in PBS). The sections were then rinsed in PBS and incubated in a solution of anti-rabbit secondary antibodies (Vector laboratories, diluted 1:50 in PBS with goat normal serum) for 2 h. The sections were again rinsed in PBS and incubated for 2 h in avidin–biotin–horseradish peroxidase (Vector laboratories, diluted 1:50 in PBS). Following this, the sections were rinsed in PBS and reacted in a solution containing diaminobenzidine, hydrogen peroxide, and PBS for 15 min. As immunohistochemical controls, the above procedures were performed with omission of the primary antibody incubation. Examination of this control tissue revealed no staining of the secondary antibodies. In an effort to maximize the reliability of comparisons within and between animals, immunohistochemistry was performed simultaneously on all sections from both treatment groups. After mounting onto gelatinized slides, the reacted sections were dried, rinsed in distilled water, and dipped in photographic emulsion (NTB-2, Kodak). The slides were stored in the dark at 4°C for four weeks. The slides were then developed in Dektol (Kodak), rinsed in water, fixed in Ektaflo (Kodak), rinsed in water, counterstained for Nissl

using Cresyl Violet, dehydrated, cleared in Americlear, and coverslipped under Permount.

Data analysis

The slides were coded prior to quantitative analysis and the code was not broken until the analysis was completed. For the first experiment (short-term corticosterone treatment), the sections selected for analysis were at least 32 μm apart in order to avoid the necessity of correcting for twice counted cells. Sections were selected from the middle portion of the dentate gyrus. At this level, the dentate gyrus is located horizontally beneath the corpus callosum, and the suprapyramidal and infrapyramidal blades are joined at the crest. For each section, the [^{3}H]thymidine labeled cells in the granule cell layer and in the hilus were counted using a light microscope (1000 ×). A cell was considered labeled if it had greater than 20 × the background number of silver grains over its nucleus; this value was always ≥ 5 silver grains. These cells were categorized as immunoreactive or nonimmunoreactive for the second and third experiments (adrenalectomy and adrenalectomy with corticosterone replacement). The granule cell layer and the hilus were then traced using a camera lucida drawing tube (25 ×), and the cross-sectional area of each was determined with a Zeiss Interactive Digitizing Analysis System (ZIDAS). The data were expressed as numbers of cells of each type per 10^6 μm^2. Means of these densities were determined for each animal from at least four sections per brain. In order to achieve homogeneity of variance, some values in the first and second experiments were log transformed. The data were subjected to one-way analysis of variance followed by Tukey HSD *post hoc* comparisons in the first experiment or two-tailed Student's *t*-tests in the second and third experiments.

RESULTS

Corticosterone decreases the number of cells born in the hilus of the adult rat

In the dentate gyrus of untreated adult rats, a small number of dividing cells, i.e. cells that were [^{3}H]thymidine labeled following a 1 h survival between injection and perfusion, were observed in the granule cell layer and hilus (Fig. 1). Following corticosterone injection, the density of [^{3}H]thymidine-labeled cells in the hilus decreased significantly (Table 1). The density of [^{3}H]thymidine-labeled cells in the sham-injected group was intermediate between that of the corticosterone injected group and the untreated group (Table 1). In contrast, no change in the density of [^{3}H]thymidine-labeled cells was observed in the granule cell layer following corticosterone treatment (Table 1). No changes in cross-sectional area of the dentate gyrus were observed with corticosterone or sham injection.

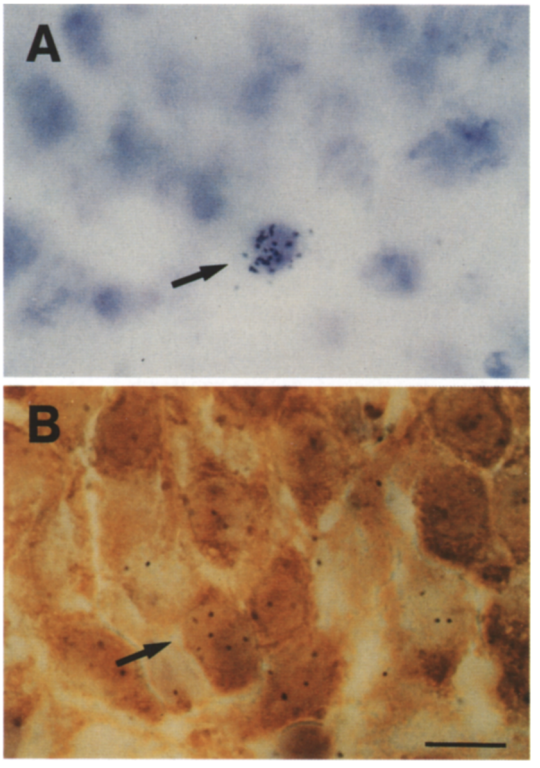

Fig. 1. (A) Photomicrograph of a [^{3}H]thymidine-labeled Nissl-stained cell (arrow) in the dentate gyrus of an untreated rat following a 1 h post-[^{3}H]thymidine injection survival period. (B) Photomicrograph of a [^{3}H]thymidine-labeled NSE-immunoreactive neuron (arrow) in the dentate gyrus of an adrenalectomized rat given corticosterone replacement during the four week post-[^{3}H]thymidine injection survival period. Note the granule neuron morphology of both [^{3}H]thymidine-labeled cells. Scale bar = 25 μm and applies to both panels.

Adrenalectomy increases the number of neurons born in the dentate gyrus of the adult rat

In the dentate gyrus of adult rats subjected to sham operation and perfused four weeks later, low numbers of [^{3}H]thymidine-labeled cells that were immunoreactive for the neuronal marker NSE were observed. Most of these [^{3}H]thymidine-labeled NSE-immunoreactive cells were located in the granule cell layer and possessed the morphologic characteristics of neurons (see Fig. 1 for example). Following adrenalectomy, a significant increase in the density of [^{3}H]thymidine-labeled NSE-immunoreactive cells

Table 1. Effects of corticosterone injection on the density of newly born cells in the dentate gyrus[1]

	Untreated control	Sham injection	Corticosterone injection
Hilus	0.74 ± 0.24	0.44 ± 0.17	0.18 ± 0.05*
Granule cell layer	1.48 ± 0.59	1.28 ± 0.52	0.57 ± 0.16

[1]Values indicate mean number of [^{3}H]thymidine-labeled cells/10^6 μm^2 ± S.E.M. each obtained from five animals. These data were log transformed to achieve homogeneity of variance and were subjected to one-way analysis of variance with Tukey HSD *post hoc* tests. *Represents significant difference from control ($P < 0.05$).

Table 2. Effects of adrenalectomy on the density of newly born cells in the dentate gyrus[1]

		Adrenalectomy alone		Adrenalectomy with corticosterone replacement after [^{3}H]thymidine injection	
		Sham	ADX	Sham	ADX
Granule cell layer	NSE+	1.51 ± 0.25	6.60 ± 1.73*	0.98 ± 0.31	4.28 ± 0.40*
	NSE−	0.52 ± 0.20	0.56 ± 0.16	0.30 ± 0.14	0.91 ± 0.28
Hilus	NSE+	0.03 ± 0.03	0.10 ± 0.05	0.00 ± 0.00	0.10 ± 0.07
	NSE−	0.16 ± 0.08	0.41 ± 0.16	0.13 ± 0.06	0.24 ± 0.11

[1]Values indicate mean number of [^{3}H]thymidine-labeled cells/10^6 μm^2 ± S.E.M. each obtained from five animals. These data were subjected to two-tailed Student's t-tests. In order to achieve homogeneity of variance, NSE+ granule cell layer values in the adrenalectomy alone experiment were log transformed. ADX, adrenalectomy; NSE+, neuron specific enolase immunoreactive; NSE−, neuron specific enolase nonimmunoreactive. *Represents significant difference from sham ($P < 0.05$).

(four times control value) was observed in the granule cell layer (Table 2). There were very few [^{3}H]thymidine-labeled NSE-immunoreactive cells in the hilus in either group (Table 2). The densities of NSE-nonimmunoreactive [^{3}H]thymidine-labeled cells in both regions were not significantly changed by long-term adrenalectomy (Table 2). As previously reported, adrenalectomy resulted in a large increase in the number of pyknotic (degenerating) cells in the granule cell layer.[11,31,32] These cells had darkly stained condensed chromatin, no nuclear membrane and pale or absent cytoplasm (see Refs 11 and 33 for examples). No [^{3}H]thymidine-labeled pyknotic cells were observed. A small but significant change in the cross-sectional area of the granule cell layer was observed with adrenalectomy (from 0.51 ± 0.02 μm^2 to 0.40 ± 0.04 μm^2, $P < 0.05$). Since the magnitude of this change is small (≈20%), it cannot account for the large increase in the density of newly born neurons (≈340%).

Replacement of corticosterone after [^{3}H]thymidine injection does not alter the adrenalectomy-induced increase in neuronal birth

Light microscopic examination of brains from sham operated rats revealed small numbers of [^{3}H]thymidine-labeled NSE-immunoreactive cells in the dentate gyrus. The density of [^{3}H]thymidine-labeled NSE-immunoreactive cells in the granule cell layer was significantly increased by adrenalectomy with corticosterone replacement during the post-[^{3}H]thymidine injection survival interval (Fig. 1, Table 2). No change in the density of NSE-non-immunoreactive [^{3}H]thymidine-labeled cells was observed (Table 2). The percentage increase in [^{3}H]thymidine-labeled NSE-immunoreactive cells with adrenalectomy was the same with or without corticosterone replacement (four-fold increase in both experiments). The distribution of [^{3}H]thymidine-labeled cells and the proportion that were NSE-immunoreactive following adrenalectomy with corticosterone replacement were also similar to those seen with adrenalectomy alone. Unlike long-term adrenalectomy without corticosterone replacement, very few pyknotic cells were observed in the dentate gyrus of rats subjected to adrenalectomy with corticosterone replacement. No [^{3}H]thymidine-labeled pyknotic cells were observed. No significant change in the cross-sectional area of the dentate gyrus was observed following adrenalectomy with corticosterone replacement.

DISCUSSION

The results of this study indicate that precursor cells located in the dentate gyrus are sensitive to manipulations of the levels of adrenal steroids; acute administration of corticosterone significantly decreased the number of [^{3}H]thymidine-labeled cells, whereas adrenalectomy significantly increased the number of [^{3}H]thymidine-labeled neurons. There are two possibilities to account for these results: (i) a subpopulation of progenitor cells either starts or stops dividing in response to low or high levels of glucocorticoids respectively, and/or (ii) the cell cycle of progenitors in the dentate gyrus is altered by changing levels of glucocorticoids such that the rate of cell division changes. These two possibilities are not mutually exclusive and could reflect different degrees of the same process. With regard to the first possibility, it is relevant that glucocorticoids have been shown to reversibly halt progression of the cell cycle in a non-neuronal *in vitro* system.[26] If glucocorticoids naturally act to prevent the division of some precursor cells in the adult dentate gyrus, then removal of this inhibitory factor by adrenalectomy would allow these cells to begin dividing. With regard to the second possibility, it is interesting that previous studies have shown that glucocorticoids lengthen the cell cycle in non-neuronal cells *in vitro* by prolonging the G1 phase[15,35] or G2/M phases.[10] If glucocorticoids exert a similar influence over proliferating cells in the dentate gyrus, then corticosterone injection would increase the total length of the cell cycle, causing new neurons to be produced more slowly, while adrenalectomy would shorten the cell cycle, thus permitting new neurons to be generated more rapidly.

It is unlikely that these results can be attributed to

changes in the length of the DNA synthetic phase without alterations in the length of the entire cell cycle (thus altering the number of cells incorporating [^{3}H]thymidine at a given time without changing the number of new cells produced) for the following reasons. First, we observed differences in the numbers of [^{3}H]thymidine-labeled cells that are too large to arise from changes in the length of the S phase. The entire granule neuron cell cycle is estimated to be approximately 16 h with the S phase encompassing approximately 8 hours.[21] Following adrenalectomy, a four-fold increase in the number of [^{3}H]thymidine-labeled NSE-immunoreactive cells was observed. Even if the length of the S phase was doubled following adrenalectomy such that DNA synthesis occurred throughout the entire cell cycle, an unlikely if not impossible change, only twice as many [^{3}H]thymidine cells would result. Second, the DNA synthetic phase has been shown to remain constant across species,[8,9] age,[36] cell types[3,9] and following corticosterone manipulations;[10,15,35] differences in the length of the cell cycle have been shown to be due to differences in the length of G1 or of G2/M in all of these cases. Third, following adrenalectomy, the total number of granule neurons barely diminishes despite massive granule neuron death,[13] indicating that an increase in newly generated granule neurons partially offsets the degeneration.

Fate of newly born neurons in the dentate gyrus of adrenalectomized rats

It has previously been shown that the majority of granule neurons born in the adult express NSE three weeks following mitosis and that the vast majority of NSE-immunoreactive cells are located within the granule cell layer at this time point.[7] Although it is not yet known whether the neurons produced in response to adrenalectomy receive appropriate synaptic input or extend axons into the proper target sites, these cells express NSE and survive for at least four weeks, like those born in control animals.

Unlike most other brain regions, the dentate gyrus maintains a significant population of radial glia throughout adulthood.[13] These cells are thought to participate in the migration of newly born neurons from the hilus to the granule cell layer. Developmental studies have suggested that newly born neurons are most likely to migrate along young radial glia.[23] In this regard, it is interesting that adrenalectomy has also been shown to increase the birth of radial glia in the dentate gyrus.[13] These newly born cells may provide the increased number of radial glia necessary to accommodate the increased number of neurons generated following adrenalectomy.

Direct versus indirect actions of adrenal steroids on cell death

The results of this report suggest that adrenal steroids regulate neurogenesis in the adult dentate gyrus. However, we have recently shown that the majority of dividing cells in this region are not immunoreactive for adrenal steroid receptors in the adult.[6] This suggests that either adrenal steroids act directly on the small population of dividing cells that express adrenal steroid receptors or adrenal steroids influence cell birth indirectly through another factor. Blockade of *N*-methyl-D-aspartate receptors by treatment with competitive or noncompetitive antagonists has been shown to increase the rate of neuronal birth in the developing dentate gyrus.[14] Since the dentate gyrus of the rat maintains several developmental features into adulthood, including sensitivity to adrenal steroids, it is possible that *N*-methyl-D-aspartate receptor activation continues to mediate neurogenesis in adulthood as well and that adrenal steroids affect granule cell genesis through changes in *N*-methyl-D-aspartate receptor activation.

The relationship between cell death and cell birth in the dentate gyrus

It is important to emphasize that removal of adrenal steroids, in addition to increasing cell birth, also produces massive granule cell death. Cell death that shows the characteristics of apoptosis occurs in the granule cell layer just days after adrenalectomy.[11,33] However, despite massive death of granule neurons seven days after adrenalectomy, the total number of granule cells is barely diminished.[13] The results of the present report indicate that granule neuron death does not substantially affect the total number of granule neurons because of a coincident increase in neuronal birth. This paradoxical effect, i.e. that cell birth and cell death are both increased by adrenalectomy, raises the issue of whether the increases in birth and death are occurring in the same population of granule neurons, i.e. shortening the life span of newly born neurons, or whether two distinct subpopulations of cells are being affected differently. Because so many [^{3}H]thymidine-labeled neurons were seen after a three week survival time, and no [^{3}H]thymidine-labeled pyknotic cells were seen following adrenalectomy, it is clear that many of the newly born neurons survive for at least several weeks despite ongoing cell death in the region. Additionally, the observation that replacing low levels of corticosterone in adrenalectomized rats during the post-[^{3}H]thymidine injection survival period, which prevents the massive cell death in this region,[11] produces no additional increase in the number of surviving newly born neurons indicates that the dividing population is likely to be distinct from the dying population.

In this regard, it is interesting to note that recent studies have indicated that a close relationship exists between mitosis and apoptosis in many systems.[25] It has been hypothesized that removal of a trophic factor or application of a toxic substance signals the target cells to re-enter the cell cycle. Cells that are able to complete the cell cycle, probably due to their undifferentiated state, undergo mitosis whereas those

that cannot complete the cell cycle undergo apoptosis.[25] If this model applies to the rat dentate gyrus, then removal of adrenal steroids would be a signal for granule neurons to divide. Undifferentiated neuronal precursors would divide and contribute to the increase in [^{3}H]thymidine-labeled NSE-immunoreactive cells observed in the granule cell layer following adrenalectomy. Differentiated neurons would unsuccessfully attempt to divide and undergo apoptosis instead. This possibility might also explain the observation that long-term adrenalectomy (three to four months) occasionally results in almost complete destruction of the granule cell layer.[32] In the time interval between adrenalectomy and perfusion, many cells born due to adrenalectomy could mature enough to undergo apoptosis. Because there are more mature neurons than neuronal precursors, cell death may predominate over cell birth, causing the dentate gyrus to diminish. However, it should be noted that destruction of the majority of the granule cell layer has only been reported to occur in a very small minority of adrenalectomized rats ($<5\%$[32]) indicating that individual differences in the proportion of differentiated and undifferentiated granule neurons are likely to exist.

The role of adrenal steroids in cell death and cell survival throughout life

The present results indicate that adrenal steroids inhibit neurogenesis in the dentate gyrus throughout life. During development and in adulthood, the rate of neurogenesis can be manipulated by altering the levels of adrenal steroids. Corticosterone injection during the postnatal period[12] and in adulthood (present report) decrease the rate of cell birth. However, the natural decrease in cell birth brought about by increased levels of adrenal steroids late in development is not permanent; adrenalectomy in adulthood reinstates levels of neurogenesis which reflect an earlier stage in the animal's life.

Collectively, these studies indicate that adrenal steroids are a natural signal for the termination of high levels of neurogenesis in the dentate gyrus and that this region retains its sensitivity to changing levels in glucocorticoids in adulthood. This raises the possibility that physiological changes in the levels of adrenal steroids, which occur diurnally,[1] with aging,[18,29] and with stress,[29] affect the production of new neurons in the dentate gyrus. In this regard, it is relevant that the number of [^{3}H]thymidine-labeled cells in the hilus of sham-injected rats was intermediate between that of untreated rats and corticosterone-injected rats. It is likely that this result reflects an endogenous rise in corticosterone due to the stress of injection, supporting the contention that cell birth in the dentate gyrus is regulated naturally by physiologic changes in adrenal steroid levels.

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