THE JOURNAL OF COMPARATIVE NEUROLOGY 313:486–493 (1991)

Adrenal Steroids Regulate Postnatal Development of the Rat Dentate Gyrus: II. Effects of Glucocorticoids and Mineralocorticoids on Cell Birth

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ABSTRACT

Unlike the majority of mammalian brain regions, the rat dentate gyrus undergoes maximal cell birth and cell death during the same developmental time period. Granule cell birth and death peak at the end of the first postnatal week. We have found that manipulations of glucocorticoid levels during the stress hyporesponsive period profoundly influence the density of pyknotic cells in the dentate gyrus while apparently not affecting the density of healthy cells. This raises the possibility that glucocorticoids are regulating processes in addition to cell death, i.e., cell birth. In order to determine whether increases in circulating glucocorticoids or mineralocorticoids affect the birth of cells in the developing dentate gyrus, ^{3}H-thymidine autoradiography was performed on brains of rat pups treated with either corticosterone or aldosterone during the first postnatal week. Quantitative analysis of ^{3}H-thymidine-labelled cells revealed significant decreases in the density of labelled cells in the granule cell layers with both corticosterone and aldosterone treatment. In these same brains, significant decreases in the density of pyknotic cells were also observed in the granule cell layers. However, no changes in the numbers of ^{3}H-thymidine-labelled pyknotic cells were observed with any treatment. Increases in circulating corticosterone or aldosterone resulted in significant increases in the density of both ^{3}H-thymidine-labelled and pyknotic cells in the hilus. These results suggest that dentate gyrus cell birth and cell death are related and that these processes are regulated by adrenal steroids.

Key words: corticosterone, aldosterone, granule cell, neurogenesis, pyknotic cell

The vast majority of granule cell birth in the rat occurs postnatally. During the first postnatal week, granule cells of the dentate gyrus arise from precursors located in the hilus and migrate along radial glia to reside in the granule cell layer (Schlessinger et al., '75; Rickmann et al., '87). A substantial number of these newly born granule cells undergo pyknosis during the first postnatal week. The period of maximal cell death occurs between postnatal day (P) 4 and P6 (Gould et al., '91a) and coincides with that of peak granule cell birth (Schlessinger et al., '75; Bayer, '80; Bohn, '80). Such a concurrence is unique to this structure, since in other brain regions neurogenesis generally precedes neuronal death by at least a few days (see Cowan et al., '84; Clarke, '85 for review).

Several lines of evidence suggest that the processes of granule cell birth and death are related and that both are regulated by adrenal steroids. First, the period of maximal cell birth and death in the dentate gyrus occurs during the stress hyporesponsive period when adrenal steroid levels are naturally low (see Sapolsky and Meaney, '86 for review). Second, changes in the levels of circulating glucocorticoids during the first postnatal week are inversely correlated with alterations in the density of pyknotic cells in the granule cell layer (Gould et al., '91b). However, despite dramatic alterations in pyknotic cell density with increasing or decreasing glucocorticoid levels, the density of healthy granule cells does not change (Gould et al., '91b). These findings suggest that changes in granule cell degeneration are accompanied by compensatory alterations in granule cell birth. Third, a previous study has shown that administration of hydrocortisone acetate during the first postnatal week inhibits granule cell neurogenesis (Bohn, '80). It is not known though, whether increases in the endogeneous rat adrenal steroids corticosterone and aldosterone affect gran-

Accepted August 14, 1991.

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ule cell birth and death, or the relationship between these two developmental processes.

In order to determine whether excess adrenal steroids, administered during the stress hyporesponsive period, inhibit dentate gyrus cell division and cell death at their peak, we examined the densities of ^{3}H-thymidine-labelled cells and pyknotic cells in the dentate gyrus of P6 rats which were treated during the first postnatal week with either corticosterone or aldosterone.

MATERIALS AND METHODS

Animal treatment and histology

Timed pregnant Sprague-Dawley (Charles River) rats were housed individually in plastic cages and provided with food and water ad libitum. At P2 (the day following birth), the rat pups were removed from their litters and an approximately equal number of males and females (4–5 each) were distributed among 9 dams. From P2 to P6, the pups received daily subcutaneous injections of one of the following: (1) 5 mg/kg corticosterone in sesame oil, (2) 5 mg/kg aldosterone in sesame oil, or (3) sesame oil alone. P6 was selected because it is the time point of maximal postnatal neurogenesis (Schlessinger et al., '75) as well as maximal postnatal cell death (Gould et al., '91a) in the dentate gyrus. Although pups of both sexes received treatment, only the males were used for this study since a previous report showed no sex differences in the density of pyknotic cells or healthy cells in the developing rat dentate gyrus during this period (Gould et al., '91a). On P5, 24 hours prior to perfusion, male pups received a single injection of 5 μCi/gm body weight ^{3}H-methyl thymidine in sterile water (New England Nuclear, specific activity 80 Ci/mmol). On P6, the male pups were weighed, deeply anesthetized with Metofane, and transcardially perfused with 20 ml of 4.0% paraformaldehyde in 0.1 M phosphate buffer with 1.5% (v/v) picric acid (pH 7.4). The brains were dissected from the cranial cavities and postfixed overnight in a solution having the same composition as the perfusate. Following this, the brains were cryoprotected in 30% sucrose in PBS and frozen. Coronal sections 16 μm thick throughout the entire dentate gyrus were cut on a cryostat. The sections were thaw-mounted onto gelatinized glass slides, dipped in photographic emulsion (NTB-2, Kodak), dried, then stored in a light-tight container in the cold (4°C). After 6 weeks, the slides were developed, fixed, and rinsed in water. Finally, they were stained for Nissl using a cresyl violet stain and coverslipped under Permount.

Data analysis

The slides containing sections chosen for quantitative analysis were coded and the code was not broken until the analysis was complete. For each brain, 4–6 sides of selected sections from each of the following three levels throughout the dentate gyrus were chosen for analysis: (1) the rostral dentate gyrus where the suprapyramidal blade is separate from the infrapyramidal blade, (2) the middle dentate gyrus where the suprapyramidal blade and the infrapyramidal blade are joined at the crest region and the dentate gyrus is oriented horizontally beneath the corpus callosum, and (3) the temporal dentate gyrus where the suprapyramidal and infrapyramidal blades are joined at the crest region and the dentate gyrus is oriented obliquely beneath the corpus callosum (see Gould et al., '91a for examples of these levels). In order to avoid the necessity of correcting for twice counted cells, the selected sections for a given brain were always separated by at least 16 μm. For each side of each selected section, the numbers of ^{3}H-thymidine-labelled cells were counted (400× magnification) in the suprapyramidal blade, the infrapyramidal blade, and the hilus. A cell was considered to be labelled if it showed at least seven silver grains over it; this density represents more than 20 times background level. In addition, the numbers of pyknotic or degenerating cells were counted (1000× magnification) in the suprapyramidal blade, the infrapyramidal blade and the hilus. Pyknotic cells were characterized by condensed, darkly stained chromatin, lack of a nuclear membrane and light or absent cytoplasm (Sengelaub and Finlay, '82). The numbers and distribution of ^{3}H-thymidine-labelled pyknotic cells were also determined at all three levels of the dentate gyrus (1000× magnification).

The areas of the suprapyramidal blade, infrapyramidal blade, and hilus were then determined by the use of the ZIDAS (Zeiss Interactive Digitizing Analysis System) and a camera lucida drawing tube (25× magnification). For each region on each section, the numbers of both ^{3}H-thymidine-labelled cells and pyknotic cells were then expressed per 10^6 μm^2. In order to determine whether cross-sectional cell body areas of granule cells were affected by corticosterone or aldosterone treatment, measurements of 40 representative non-pyknotic cells in the suprapyramidal blade and infrapyramidal blade of rostral, middle and temporal levels were made for the same sections of each brain by use of a camera lucida drawing tube and the ZIDAS. In an effort to assess whether adrenal steroid treatment altered the position of ^{3}H-thymidine-labelled cells within the granule cell layer, the distance from the hilus to labelled cells located in two-thirds of each selected blade was measured using a camera lucida drawing tube and the ZIDAS. Means of these variables were determined for a given region for each animal and the data were subjected to one way analysis of variance with Tukey HSD post hoc comparisons.

RESULTS

Effects of adrenal steroid treatment on ^{3}H-thymidine-labelled cells in the dentate gyrus

Light microscopic examination of brains from P6 control rats revealed large numbers of ^{3}H-thymidine-labelled cells throughout the suprapyramidal blade, infrapyramidal blade, and hilus at all levels examined in the dentate gyrus. Most of the ^{3}H-thymidine-labelled cells were observed deep in the suprapyramidal blade and infrapyramidal blade, bordering on the hilus, with fewer being located superficially within the granule cell layers. In addition, a number of mitotic figures, some showing a low to moderate number of silver grains, were observed throughout the dentate gyrus of control and treated rat brains.

Postnatal treatment with corticosterone or aldosterone resulted in significant changes in the numbers or distribution of ^{3}H-thymidine-labelled cells in the dentate gyrus. Although no significant differences were observed in the rostral region of the dentate gyrus (Fig. 1), in the middle and temporal suprapyramidal blades as well as in the middle infrapyramidal blade, increases in the levels of circulating corticosterone or aldosterone from P2 until P6 resulted in significant decreases in the density of ^{3}H-thymidine labelled cells (Figs. 2–4). In the temporal infrapyramidal blade, a trend toward a decrease in the density of

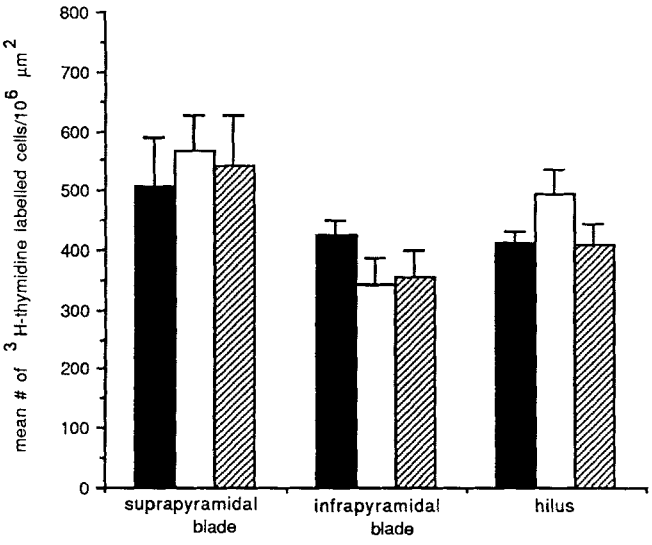

Fig. 1. The density of ^{3}H-thymidine-labelled cells in the rostral dentate gyrus of sham-injected (solid bar), corticosterone-injected (open bar), and aldosterone-injected (diagonal lines) P6 rat pups. Bars represent mean + SEM each obtained from at least four brains. No significant differences were detected.

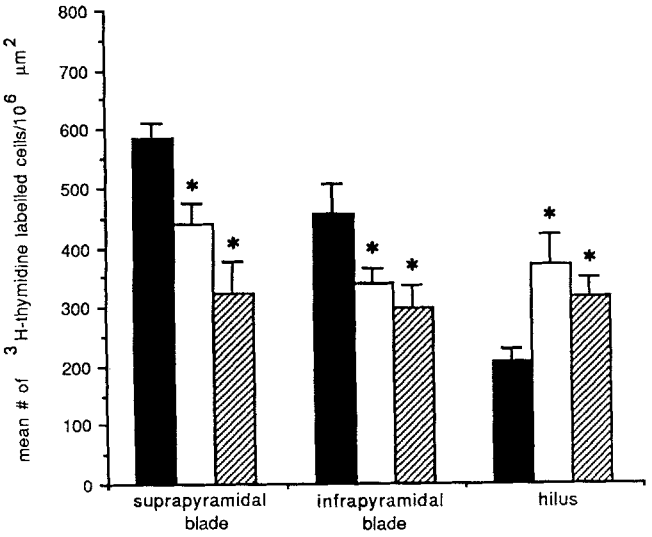

Fig. 2. The density of ^{3}H-thymidine-labelled cells in the middle dentate gyrus of sham-injected (solid bar), corticosterone-injected (open bar), and aldosterone-injected (diagonal lines) P6 rat pups. Bars represent mean + SEM each obtained from at least four brains. Asterisks represent significant difference from sham ($p < 0.05$).

^{3}H-thymidine-labelled cells was noted with both corticosterone and aldosterone treatment ($p < 0.06$), although a significant decrease was not detected (Fig. 3).

In contrast, in the hilus at the middle level, treatment with corticosterone or aldosterone resulted in a significant increase in the density of ^{3}H-thymidine-labelled cells (Figs. 2, 5). No significant differences in the density of ^{3}H-thymidine-labelled cells were observed at the rostral level in either granule cell blade or in the hilus (Fig. 1). Likewise, no significant changes were observed at the temporal level in

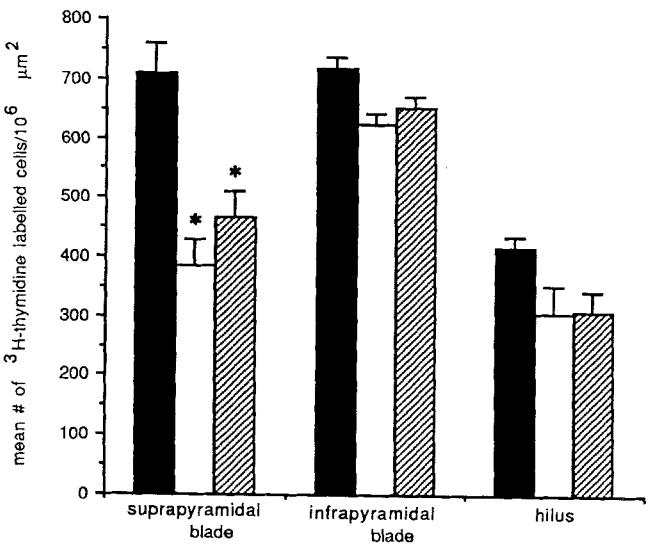

Fig. 3. The density of ^{3}H-thymidine-labelled cells in the temporal dentate gyrus of sham-injected (solid bar), corticosterone-injected (open bar), and aldosterone-injected (diagonal lines) P6 rat pups. Bars represent mean + SEM each obtained from at least four brains. Asterisks represent significant difference from sham ($p < 0.05$).

the hilus (Fig. 3). No differences in the cross-sectional area of the suprapyramidal blade, infrapyramidal blade, or hilus were observed at any level across treatment groups ($p > 0.1$ for all comparisons). In addition, no changes in the cross-sectional cell body area of granule cells in the suprapyramidal blade or infrapyramidal blade at rostral, middle or temporal levels were detected with any treatment ($p > 0.1$ for all comparisons).

Within the granule cell layers, corticosterone or aldosterone treatment did not appear to alter the location of ^{3}H-thymidine-labelled cells in comparison to controls. That is, the mean distance from the hilus to the location of a ^{3}H-thymidine-labelled cell in either the suprapyramidal or infrapyramidal blade was unaffected by treatment with adrenal steroids.

Effects of adrenal steroids on the density of pyknotic cells in the dentate gyrus

In the suprapyramidal and infrapyramidal blades at both middle and temporal levels, increased levels of circulating corticosterone or aldosterone resulted in significant decreases in the density of pyknotic cells (Table 1, Fig. 6). In contrast, a significant increase in the density of pyknotic cells was observed in the hilus at middle levels with corticosterone or aldosterone treatment (Table 1). Treatment with either corticosterone or aldosterone resulted in no changes in the density of pyknotic cells in any of the three areas at the rostral level and no changes in the hilus at temporal levels (Table 1). Low numbers of pyknotic cells labelled with ^{3}H-thymidine were detected throughout the dentate gyrus of all brains examined with no noticeable differences in the distribution or number of these cells observed in any part of the dentate gyrus with treatment (Fig. 5). Although ^{3}H-thymidine-labelled pyknotic cells were observed in both the suprapyramidal and infrapyramidal blades of all brains, the highest numbers of these cells were always observed in the hilus (Fig. 5).

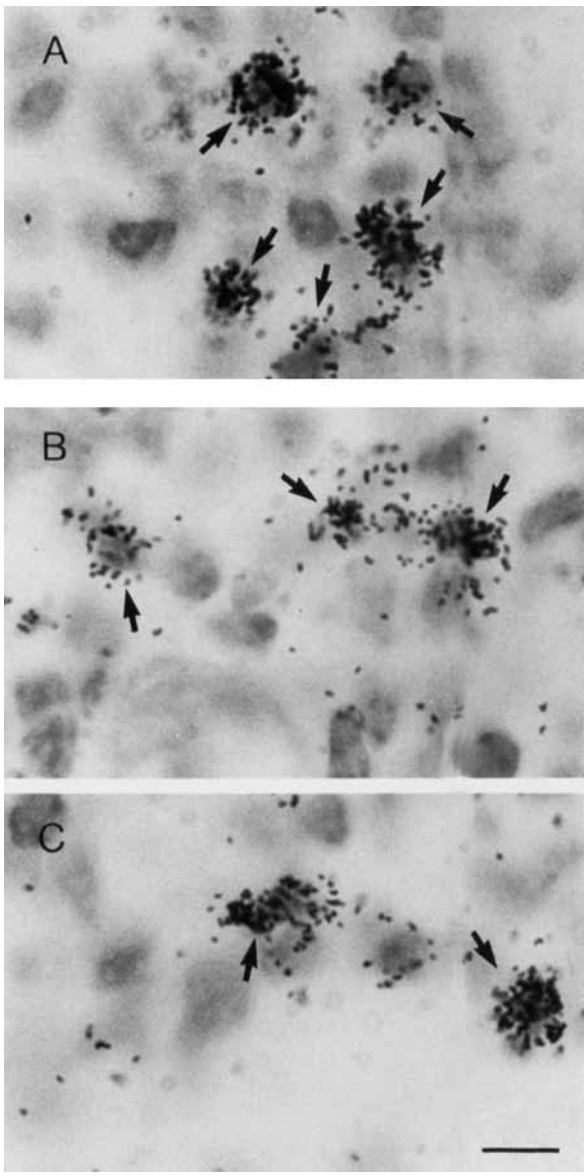

Fig. 4. Representative ^{3}H-thymidine-labelled cells (arrows) in the middle suprapyramidal blade of sham-injected (**A**), corticosterone-injected (**B**), and aldosterone-injected (**C**) P6 rats. Note the decrease in these profiles with adrenal steroid treatment (B, C) compared to the control (A). Scale bar = 10 μm and applies to all frames.

DISCUSSION

The results of this study show that increases in the circulating levels of corticosterone and aldosterone during the stress hyporesponsive period, when levels of these adrenal steroids are natually low, substantially alter the density of ^{3}H-thymidine-labelled cells throughout the dentate gyrus. Treatment of neonatal pups with either corticosterone or aldosterone resulted in decreases in the density of ^{3}H-thymidine-labelled cells in the granule cell blades at middle and temporal levels compared to sham-injected pups. In contrast, increases in the density of ^{3}H-thymidine-labelled cells were detected in the hilus with either corticosterone or aldosterone treatment compared to controls.

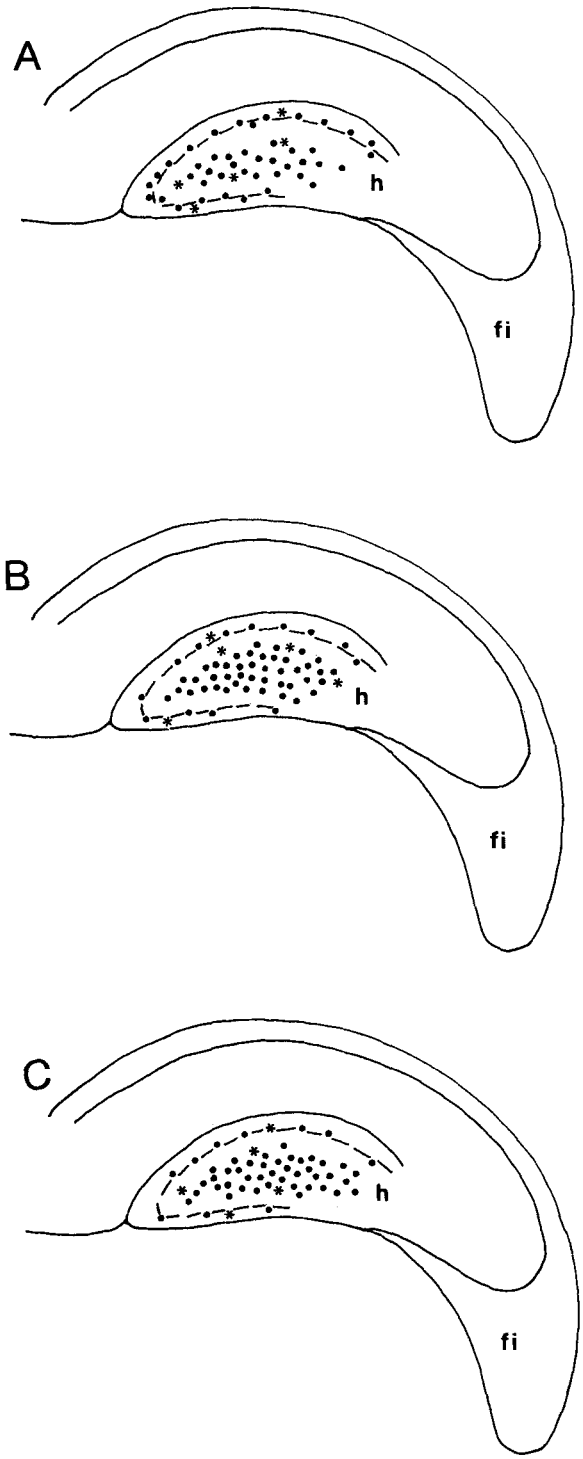

Fig. 5. Templates mapping the distribution of ^{3}H-thymidine-labelled cells (solid dots, 1 dot represents 3 cells) and ^{3}H-thymidine-labelled pyknotic cells (asterisks) in the middle dentate gyrus of sham-injected (**A**), corticosterone-injected (**B**), and aldosterone-injected (**C**) P6 rat pups. Observe the decrease in density of ^{3}H-thymidine-labelled cells in the granule cell layers and the increase in ^{3}H-thymidine-labelled cells in the hilus with adrenal steroid treatment. Note also the lack of a change in the density of ^{3}H-thymidine-labelled pyknotic cells with either treatment. h, hilus, fi, fimbria.

TABLE 1. Effects of Corticosterone and Aldosterone on the Density of Pyknotic Cells in the P6 Dentate Gyrus[1]

Region	Sham (pyknotic cells/ 10^6 μm^2)	Corticosterone (pyknotic cells/ 10^6 μm^2)	Aldosterone (pyknotic cells/ 10^6 μm^2)
Rostral:			
Suprapyramidal blade	45.7 ± 10.5	35.8 ± 7.7	37.9 ± 5.5
Infrapyramidal blade	79.0 ± 22.3	82.1 ± 15.3	70.0 ± 20.6
Hilus	33.9 ± 8.7	29.8 ± 6.7	30.9 ± 2.3
Middle:			
Suprapyramidal blade	65.1 ± 21.4	11.9 ± 1.0*	21.2 ± 3.4*
Infrapyramidal blade	51.0 ± 11.7	13.9 ± 4.4*	11.0 ± 0.7*
Hilus	11.9 ± 2.6	24.9 ± 4.1*	26.2 ± 4.2*
Temporal:			
Suprapyramidal blade	47.8 ± 4.7	27.5 ± 4.8*	26.2 ± 6.2*
Infrapyramidal blade	49.7 ± 4.1	17.2 ± 2.2*	19.0 ± 3.0*
Hilus	29.0 ± 3.2	25.7 ± 2.2	24.8 ± 3.8

[1]Values represent mean ± S.E.M. Data were subjected to one way ANOVA with Tukey HSD post hoc comparisons.
*Significant difference from sham ($p < 0.05$).

Elevated levels of adrenal steroids altered the density of pyknotic cells in a parallel manner; corticosterone or aldosterone treatment decreased the density of pyknotic cells in granule cell layers and increased the density of pyknotic cells in the hilus. Since adrenal steroid treatment did not affect the area of the dentate gyrus regions examined or the cross-sectional cell body area of granule cells, it is likely that these changes in densities reflect differences in the actual numbers of ^{3}H-thymidine-labelled cells and pyknotic cells.

Although it is not possible to determine with certainty what proportion of the ^{3}H-thymidine-labelled cells are neurons, it is likely that the majority of those located in the granule cell layers are neurons, as this region of the dentate gyrus contains very few glial cells (Kosaka and Hama, '86). Since the hilus contains both neuroblasts and glioblasts at this developmental stage (Schlessinger et al., '75; Rickmann et al., '87), it is likely that the ^{3}H-thymidine-labelled cells detected in this region represent a heterogeneous population. It is also likely that the ^{3}H-thymidine-labelled neurons in both the granule cell layer and hilus are granule cells or their precursors, because the other neuronal populations located in the dentate gyrus, i.e., basket cells, mossy cells and CA3/CA4 pyramidal cells, are all generated prenatally (Schlessinger et al., '78).

^{3}H-thymidine labelling: methodological considerations

Circulating thymidine is incorporated into cells which are undergoing DNA synthesis prior to division. Intraperitoneally administered ^{3}H-thymidine is available for uptake 1–2 hours following injection, indicating that the labelled cells observed in this study probably fall into one of the following categories: (1) cells which were synthesizing DNA within 1–2 hours of ^{3}H-thymidine injection but did not divide prior to the time of perfusion (24 hours following injection), (2) cells which were synthesizing DNA around the time of injection and divided shortly thereafter, or (3) cells which are the progeny, subsequent to an initial cell division, of those synthesizing DNA around the time of thymidine injection. Presumably, cells which fall into each of the above categories would contain, in descending order respectively, different quantities of ^{3}H-thymidine, showing different numbers of silver grains over their nuclei. It is likely that most, if not all of the labelled cells observed in this study fall into the latter two categories described above since available evidence indicates that granule cell division occurs within a few hours of DNA synthesis (Lewis, '78) and no evidence of polyploid granule cells in the developing or adult brain has yet been found (see Kaplan and Hinds, '77 for commentary).

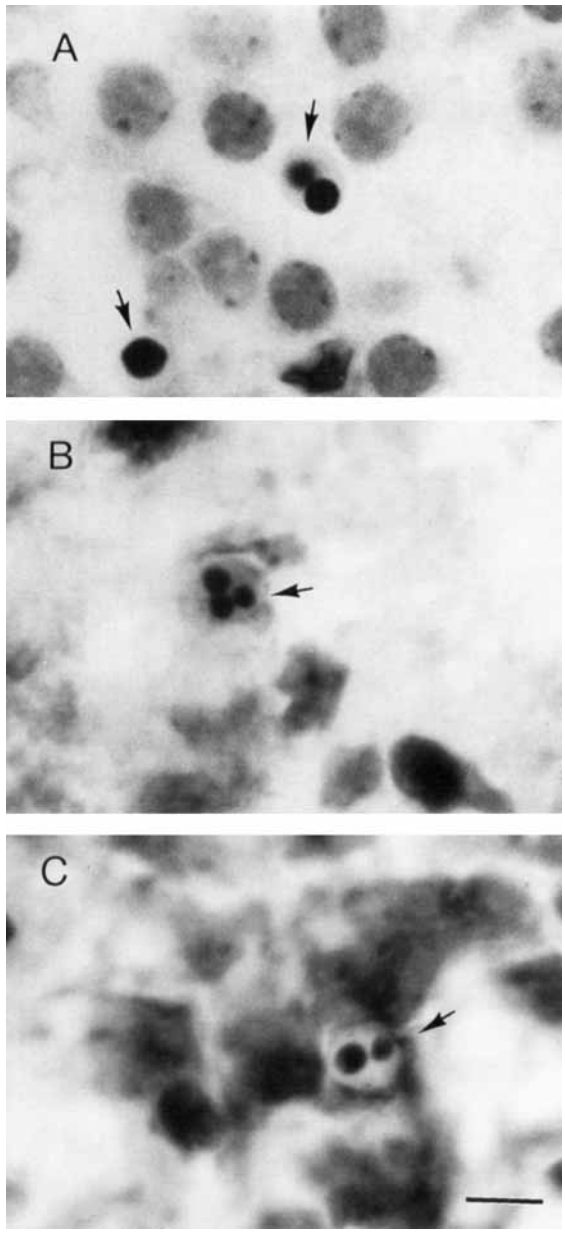

Fig. 6. Representative pyknotic cells (arrows) in the suprapyramidal blade of sham-injected (**A**), corticosterone-injected (**B**) and aldosterone-injected (**C**) P6 rat pups. Observe the decrease in these profiles with adrenal steroid treatment (B, C) compared to the control (A). Scale bar = 10 μm and applies to all frames.

Despite the large numbers of ^{3}H-thymidine-labelled cells observed in the dentate gyrus of all brains included in this study, our data analysis probably underestimates the actual number of cells which divide in the region between P5 and P6 due to the exclusion of three types of cells: (1) cells which underwent multiple mitotic cycles during the 24 hours prior to perfusion, thus diluting the quantity of ^{3}H-thymidine in a given daughter cell to fewer than seven silver grains, (2) cells which divided before the time of

perfusion but after the period when ^{3}H-thymidine was available, and thus did not become labelled, and (3) cells labelled with ^{3}H-thymidine which underwent pyknosis shortly after division and whose debris were rapidly cleared.

It is possible that corticosterone or aldosterone treatment could have decreased the density of ^{3}H-thymidine-labelled cells in the granule cell layer by affecting one of these three types of cells by either enhancing the rate of cell division or enhancing the rate of cell degeneration following division. However, it is unlikely that this is the case for the following reasons: (1) since no obvious changes in the numbers of lightly labelled cells were detected in the dentate gyrus across groups it is improbable that a substantial change in the numbers of dividing cells which escaped detection due to signal dilution occurred; (2) in nonneuronal cells, glucocorticoids have been shown to actually decrease the rate of cell division (Fanger et al., '87); (3) an increase in the number of cells dividing would presumably result in an increase in the density of healthy cells in the region. However, as previously shown (Gould et al., '91b), postnatal glucocorticoid manipulations do not result in changes in the density of healthy cells; and (4) an increase in the death of newly born cells with adrenal steroid treatment would alter the numbers or distribution of ^{3}H-thymidine-labelled pyknotic cells. Our results showed no changes in these profiles across treatment groups.

Regional differences in sensitivity to adrenal steroids

In the middle hilus, postnatal treatment with corticosterone or aldosterone resulted in significant increases in the density of both ^{3}H-thymidine-labelled cells and pyknotic cells. The majority of granule cells that are born during the first postnatal week arise from the hilus (Schlessinger et al., '75), and then migrate from the hilus along radial glial fibers to the granule cell layers (Rickmann et al., '87). It is possible that the increase in ^{3}H-thymidine-labelled cells in the hilus at this level reflects an increase in cell division within the hilus. However, it is unlikely that adrenal steroids solely increase cell division in the hilus because this would result in a greater number of ^{3}H-thymidine-labelled cells as well as an increase in the number of healthy cells in the granule cell layers. The results of this study and those of the previous report (Gould et al., '91b) indicate that this increase does not occur. Alternatively, the increase in the density of ^{3}H-thymidine-labelled cells in the middle hilus observed with adrenal steroid treatment could reflect a decrease in the death of cells in the hilus. This explanation is unlikely, as treatment with corticosterone or aldosterone actually increases the density of pyknotic cells in the hilus at P6. Some indirect evidence suggests that elevated adrenal steroids during postnatal development may inhibit the migration of newly born granule cells from the hilus to the granule cell layers (see Gould et al., '91b for commentary). If cells were incapable of migrating from the hilus to the granule cell layer, it follows that many more ^{3}H-thymidine-labelled cells as well as pyknotic cells would be detected close to their birthplace. Our results suggest that an inhibition of granule cell migration may at least partially explain the adrenal steroid effects we detected at the middle level of the dentate gyrus. This possibility is currently under investigation in our laboratory. However, no increases in either ^{3}H-thymidine-labelled cells or pyknotic cells were detected in the temporal hilus, a level where significant decreases in both of these cell types were seen in the granule cell layer, suggesting that adrenal steroids probably affect a number of different processes.

The lack of any change in the rostral part of the dentate gyrus with adrenal steroid manipulations during the first postnatal week suggests that different factors mediate cell birth and death in this region at this time. Competition for target-derived trophic factors has long been recognized as an important developmental regulator of cell number (Cowan et al., '84; Clarke, '85). Interestingly, granule cells in the rostral dentate gyrus show a much greater degree of convergence onto their target, the CA3 region, than those located more caudally (Gaarskjaer, '78). Because of this greater degree of convergence, it is possible that more competition for target-derived growth factors exists in the rostral compared to the middle and temporal levels of the dentate gyrus. Consequently, the birth and death of cells in the rostral region may be influenced by the availability of target-derived substances for which there is much less competition at more caudal regions.

Role of Type 1 or Type 2 receptors in mediating granule cell birth and death

The observation that the glucocorticoid corticosterone and the mineralocorticoid aldosterone exert a similar influence over dentate gyrus development raises questions as to which adrenal steroid receptor is involved in these processes. In the adult rat, corticosterone activates both Type 1 and Type 2 receptors, however, at low doses it has been shown to occupy primarily Type 1 receptors (Spencer et al., '90). Low doses of corticosterone or aldosterone, both Type 1 receptor agonists, prevent the adrenalectomy-induced degeneration of dentate gyrus granule cells in the adult rat (Sloviter et al., '89; Gould et al., '90; Woolley et al., '91). The observation that Type 1 receptor stimulation, from a low dose of corticosterone or aldosterone, alters the density of both ^{3}H-thymidine-labelled cells and pyknotic cells in the developing dentate gyrus suggests that these effects are also mediated by Type 1 receptors. Numerous studies have examined the development of adrenal steroid receptors in the postnatal hippocampus (Turner, '78; Meaney et al., '85; Rosenfeld et al., '88; '89). Although these studies show that adrenal steroid receptors are expressed in the hippocampus during the first postnatal week, very little information is available concerning the expression of these receptors in developing granule cells. Studies have shown that both Type 1 and Type 2 receptors, as well as Type 1 and Type 2 receptor mRNA are likely to be present in the dentate gyrus during the time of corticosterone and aldosterone treatment in the present study (P2–P6), but it is unknown which cell types express these receptors and whether regional differences within the dentate gyrus exist (Rosenfeld et al., '88; '89; Van Eekelen et al., '91). We are currently investigating the possibility that regional differences in adrenal steroid receptor distribution exist.

Relationship between cell birth and cell death in the dentate gyrus

The results of this and the previous study (Gould et al., '91b) show that manipulations in adrenal steroid levels during the stress hyporesponsive period result in changes in ^{3}H-thymidine-labelled cells which seem to parallel those in pyknotic cells. Excess corticosterone or aldosterone resulted in decreases in the density of both ^{3}H-thymidine-labelled cells and pyknotic cells in the granule cell blades of middle and temporal levels of the dentate gyrus. In con-

trast, significant increases in the density of both ^{3}H-thymidine-labelled and degenerating cells were observed in the middle hilus with adrenal steroid treatment. The lack of a change in the density of healthy cells at any level with glucocorticoid manipulations (Gould et al., '91b), despite the dramatic alterations in the density of ^{3}H-thymidine-labelled cells and pyknotic cells, suggests that a balance exists in the dentate gyrus between the birth and the death of granule cells.

In the normal rat granule cell birth and death occur in three general phases: (1) during embryonic development, granule cell birth peaks on E18 (Schlessinger et al., '75); this is also the gestational time point of maximal pyknotic cell density (unpublished observations). As birth approaches, granule cell genesis and granule cell death are temporarily inhibited (Schlessinger et al., '75; Bohn '80; unpublished observations); (2) during the first postnatal week, the peak of granule cell death coincides with the peak of granule cell birth (Schlessinger et al., '75; Bayer, '80; Gould et al., '91); and (3) from the second postnatal week up to at least one year, cell birth as well as cell death in the dentate gyrus continue at a low but steady rate (Kaplan and Hinds, '77; Bayer, '82; Bayer et al., '82; Kaplan and Bell, '84; Crespo et al., '86; Gould et al., '90). The results of this and our previous report (Gould et al., '91b) show that the maximal granule cell birth and death, which occur during the second phase of dentate gyrus development, are affected by manipulations in circulating adrenal steroids. It is still unknown whether adrenal steroids regulate cell birth and death separately, or whether adrenal steroids regulate one process, and the other is indirectly affected. These possibilities are currently under investigation in our laboratory.

Other possible factors regulating cell birth in the dentate gyrus

A number of other studies performed on developing systems have shown that afferent input is important for neuronal survival (Cunningham, '82; Furber, '87; Crewther et al., '88). Some studies suggest that afferent input is also a major determinant of neurogenesis. In vitro studies have indicated that depolarization of both immature and adult mammalian neurons results in DNA synthesis and mitosis (Stillwell et al., '73; Cone and Cone, '76). In addition, afferent input appears to be a requirement for neurogenesis in at least one invertebrate system (Baptista et al., '90). Neuroanatomical studies performed in the developing rat brain have shown that both neurogenesis and naturally occurring cell death in the dentate gyrus coincide with the arrival of several important excitatory afferents to the region (Schlessinger et al., '75; Gould et al., '91a compare with Loy et al., '77). Perforant path axons arising from the entorhinal cortex enter this region and form synapses with granule cells during the first postnatal week (Crain et al., '73; Loy et al., '77). It is possible that the arrival of these afferents plays a major role in determining both cell birth and cell death among granule cells. The extent to which afferent input regulates cell birth and death in the dentate gyrus, and whether afferent populations are also influenced by adrenal steroids, remains to be determined.

ACKNOWLEDGMENTS

This research was supported by MH 41256 (Bruce S. McEwen). Elizabeth Gould currently holds an individual NRSA postdoctoral fellowship (NS08804).

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