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STRESS INHIBITS THE PROLIFERATION OF GRANULE CELL PRECURSORS IN THE DEVELOPING DENTATE GYRUS

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Abstract—The granule cell population of the dentate gyrus is produced predominantly during the postnatal period in rats. Previous studies have shown that experimental increases in the levels of adrenal steroids suppress the proliferation of granule cell precursors during the first postnatal week, the time of maximal neurogenesis in the dentate gyrus. These findings raise the possibility that stressful experiences that elevate adrenal steroid levels may inhibit the production of granule neurons, and thus alter the development of the dentate gyrus. To test this possibility, we exposed naive rat pups to the odors of a known predator, adult male rats, and examined both plasma corticosterone levels and the number of ^{3}H-thymidine labeled cells in the dentate gyrus. A single exposure of rat pups to adult male rat odor elevated corticosterone levels immediately and diminished the number of ^{3}H-thymidine labeled cells in the granule cell layer by 24 h later. These results suggest that stressful experiences suppress the production of granule neurons in the developing dentate gyrus. © 1998 ISDN. Published by Elsevier Science Ltd

Key words: dentate gyrus, stress, neurogenesis, granule neuron, development.

The granule cell population of the dentate gyrus is produced during an extended period that begins in gestation and continues well into adulthood in a variety of mammalian species, from rodents to primates.[1,2,5,6,8,9] In the rat, most granule neurons are produced during the first two postnatal weeks of life.[11] During the postnatal period, granule neurons arise from precursor cells that exist within the dentate gyrus. These precursor cells divide and produce daughter cells that migrate to the granule cell layer and differentiate into granule neurons.[1] The postnatal period of neurogenesis in this system presents the possibility for experience-dependent modifications in granule cell production.

In the rat, the time of maximal neurogenesis in the dentate gyrus coincides with the stress hyporesponsive period. The first two postnatal weeks of life in the rat, termed the stress hyporesponsive period, are characterized by low basal levels of glucocorticoids and diminished response of the adrenal gland to stress.[10] During this time, injections of adrenal steroids suppress the proliferation of granule cell precursors.[4] Because certain stressors can elevate adrenal steroids during the stress hyporesponsive period,[10,16] it is possible that such experiences would inhibit the production of granule neurons during development. To determine whether this is the case, we exposed rat pups to a potentially stressful experience and determined the level of circulating glucocorticoids and the number of proliferating cells in the dentate gyrus. Because rats rely heavily on olfactory cues, we selected a potentially stressful olfactory stimulus, the odor of adult male rats which are known predators of rat pups.[15]

EXPERIMENTAL PROCEDURES

Animal care and treatment

Timed pregnant (15d) Sprague-Dawley rats were obtained from Charles River. On the day after birth, postnatal day (P) 1, the rat pups were pooled, their sex was determined and five male and five female rats were randomly distributed to each dam. The animals were housed in a room without adult male rats. On the day of maximal neurogenesis, P5, rat pups from five litters were removed from the nest and placed in a novel cage with either clean bedding or the soiled bedding of an unfamiliar adult male rat. After 30 min in the novel cage, the animals were injected with ^{3}H-

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thymidine. ^{3}H-thymidine is incorporated into cells during S phase and is a marker of proliferating cells and their progeny. These animals remained in the novel cage for an additional 30 min and were then returned to the nest.

Twenty four hours after ^{3}H-thymidine injection, six male rat pups from each group (control and odor-exposed) were anesthetized with metofane and transcardially perfused using 4.0% paraformaldehyde in 0.1 M phosphate buffer. The brains were dissected from the skulls and postfixed overnight in 4.0% paraformaldehyde. After cryoprotection in 30% sucrose in PBS, the brains were frozen and the entire dentate gyrus was cut on a cryostat. Sections (16 μm thick) were thaw-mounted on to gelatinized glass slides, dried and dipped in NTB-2 photographic emulsion (Eastman Kodak). The slides were stored in the dark for 4 weeks and then developed in Dektol, rinsed in water, fixed in Polymax T, rinsed in water and counterstained for Nissl using cresyl violet.

Data analysis

The slides were coded prior to quantitative analysis and the code was not broken until the analysis was complete. For each section, the number of ^{3}H-thymidine labeled cells in the granule cell layer (suprapyramidal and infrapyramidal blades combined) was counted. A cell was considered to be labeled if it had a minimum of seven grains over its nucleus. This value is greater than 20 times the background level. ^{3}H-thymidine labeled cells in the hilus and those in the molecular layer were excluded from the analysis because they are likely to represent a higher proportion of glial precursors. The cross-sectional area of the granule cell layer was determined from each analyzed section using Image Pro software on a video camera-projected image. The total cross-sectional area was analyzed, the total number of ^{3}H-thymidine labeled cells was determined and the data were expressed as a number of ^{3}H-thymidine labeled cells/mm^{2}. Because ^{3}H only penetrates the top 3 μm of a tissue section, no attempt was made to perform stereological estimates, which assume random distribution of labeled profiles throughout a section, of the total number of labeled cells. However, stereological estimates of the volume of the granule cell layer were performed using Cavalieri's principle.[7] Means of these variables were determined and the data were analyzed using unpaired Student's *t*-tests.

Corticosterone radioimmunoassay

The levels of circulating corticosterone were determined from trunk blood of unanesthetized male rat pups treated as described above but decapitated after a 30 min expose to the novel cage ($n = 4$) or novel cage/male rat odor ($n = 4$). Plasma corticosterone levels were measured using the Rat Corticosterone Coat-a-Count kit (Diagnostic Products Corporation, Los Angeles, CA) modified for low expected corticosterone concentration. The sensitivity of the assay was 5 ng/ml. The intra-assay coefficient of variation was 1–9%. The data were analyzed using unpaired Student's *t*-tests.

RESULTS

Light microscope examination of tissue processed for ^{3}H-thymidine autoradiography revealed a high number of heavily labeled cells throughout the dentate gyrus of all animals examined (Fig. 1) Labeled cells were observed throughout the granule cell layer and hilus. Many of the labeled cells had the morphological characteristics of granule cell precursors, round or oval, medium-sized cell bodies. The remaining labeled cells had the morphological characteristics of glial cells, triangular or irregular, small cell bodies.

A single exposure to the odors of an unfamiliar adult male rat resulted in a significant decrease in the number of cells that incorporated ^{3}H-thymidine in the granule cell layer (Figs 1 and 2). Exposure to the unfamiliar adult male rat odor did not result in a significant change in the volume of the granule cell layer indicating that density measures reflect changes in the total number of labeled cells. The density of ^{3}H-thymidine labeled cells in the control animals, i.e. those removed from the nest and placed in a novel cage on clean bedding, was similar to that previously observed in undisturbed animals at this age,[4] suggesting that transfer to a novel cage did not have a noticeable effect on cell proliferation.

Exposure to unfamiliar adult male rat odor resulted in a significant increase in the levels of circulating corticosterone compared to control rat pups (Fig. 3).

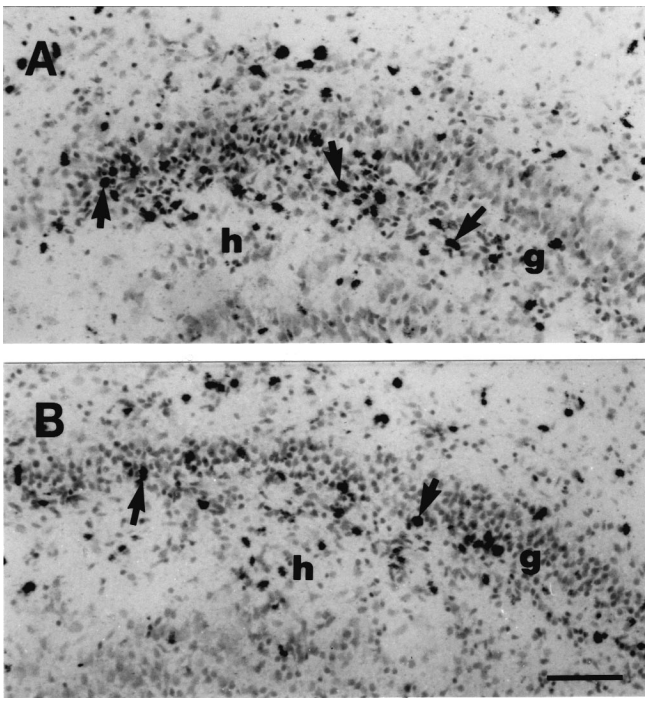

Fig. 1. Representative photomicrographs of ^{3}H-thymidine labeled cells in the granule cell layer of the dentate gyrus on P6. (A) Control rat pups demonstrate many ^{3}H-thymidine labeled cells (arrows) in the suprapyramidal blade of the granule cell label. (B) Rat pups exposed to the odor of unfamiliar adult male rats have fewer ^{3}H-thymidine labeled cells (arrows) in the suprapyramidal blade of the granule cell layer. Scale bar equals 100 μm and applies to both frames.

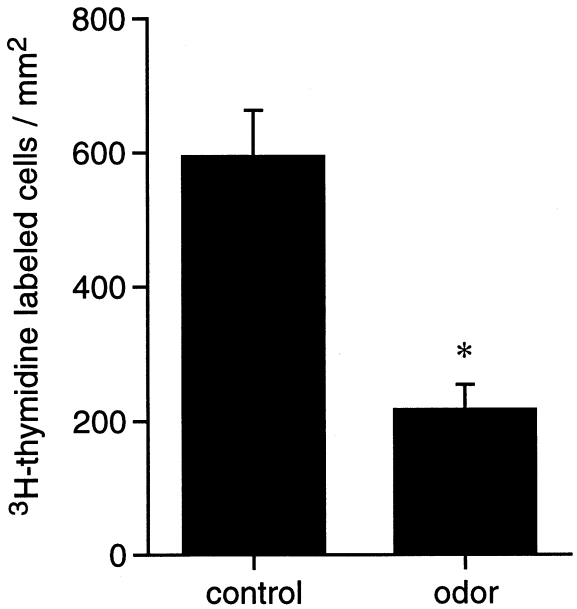

Fig. 2. The density of ^{3}H-thymidine labeled cells in the granule cell layer on P6 is lower following exposure to the odor of unfamiliar adult male rats on P5. These rats were injected with ^{3}H-thymidine 30 min after odor exposure and perfused after a 24 h survival time. Bars represent mean + SEM each obtained from six brains. *Significant difference from control, unpaired Student's t-tests, $P < 0.05$.

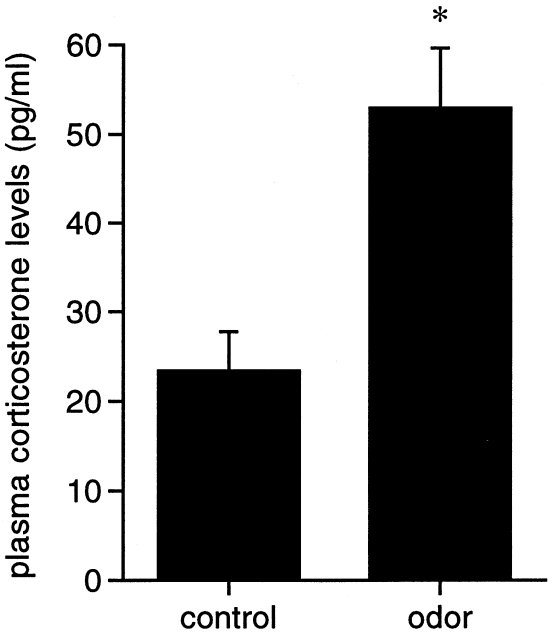

Fig. 3. The level of plasma corticosterone is elevated following exposure to the odor of unfamiliar adult male rats. These animals were decapitated 30 min after placement in a novel cage without odor or a novel cage with adult male rat odor. Bars represent mean + SEM each obtained from four animals. *Significant difference from control, unpaired Student's *t*-tests, $P < 0.05$.

DISCUSSION

These results indicate that male rat pups show a decrease in the density of ^{3}H-thymidine labeled cells in the granule cell layer following acute exposure on P5 to the odor of an unfamiliar adult male rat. The observation that the volume of the granule cell layer did not change with treatment indicates that the change in density reflects a change in the overall number of labeled cells.

Previous studies have shown that injections of corticosterone during the first postnatal week of life in the rat suppresses the proliferation of granule cell precursors in the dentate gyrus.[4] The observations of the current study show similar changes in the production of granule neurons following exposure to a potentially stressful condition, presenting the possibility that odor-induced suppression of granule cell production is mediated at least in part by adrenal steroids. However, P5 is during the stress hyporesponsive period, when basal adrenal steroid levels are low and, perhaps more importantly, stress-induced increases in corticosterone levels are uncommon.[10] Although some stressors, such as maternal deprivation, induce a rise in glucocorticoid levels during early life, many others do not.[10] The results of this study show that exposure to the odors of an unfamiliar adult male rat is a sufficient stressor to elevate circulating levels of corticosterone during the stress hyporesponsive period. Because corticosterone treatment suppresses cell proliferation in the developing dentate gyrus, it is likely that the stress-induced decrease in the number of ^{3}H-thymidine labeled cells observed in the present study is the result, at least in part, of elevated glucocorticoid levels. However, the possibility that other non-hormonal mechanisms underlie this experience-dependent change in granule cell production cannot be discounted. In fact, recent studies have shown that stressful experiences that are insufficient to raise adrenal steroid levels during the stress hyporesponsive period, stimulate expression of immediate early genes in the brain.[12]

A previous study has shown that the dentate gyrus is critical for the emergence of a developmental defensive behavior called behavioral inhibition.[13] When rat pups are exposed to the odors of unfamiliar adult male rats, they engage in behavioral inhibition characterized by complete immobilization and cessation of ultrasonic vocalizations.[14] This response is ecologically relevant because it begins around P14 when rat pups first venture from the nest and are vulnerable to attack by adult male rats.[15] The behavior ends around the time of weaning, when adult male rats are no longer a

predatory threat to juveniles. Lesion of the dentate gyrus prior to the end of the second postnatal week prevents the immobilization component of this behavior.[13] Furthermore, previous work has shown an inverse relationship between the numbers of proliferating cells in the dentate gyrus and the amount of time spent freezing in response to an unfamiliar adult male rat. For example, treatment with corticosterone during the first postnatal week, which is known to suppress the proliferation of granule cell precursors, results in an increase in the amount of time spent freezing in response to an unfamiliar adult male rat on P15.[3] It is possible, therefore, that exposure to unfamiliar adult male rat odor, by suppressing granule cell production, ultimately enhances freezing behavior. By this mechanism, environmental cues could influence granule cell formation in the developing rat and affect the outcome of a behavior dependent on this brain region. Stress-induced suppression of granule cell production might be especially adaptive because rat pups exposed to predator odors during the first postnatal week may be more likely to encounter a predatory threat on venturing from the nest later in development. These pups maybe more likely to engage in freezing behavior and thus avoid detection by potential predators. The extent to which early exposure to stress results in permanent change in the function of the dentate gyrus remains unknown.

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