Selective Loss of Hippocampal Granule Cells following Adrenalectomy: Implications for Spatial Memory

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We examined the effects of long-term adrenalectomy (ADX) on hippocampal anatomy and behavioral learning in two spatial memory tasks. We assessed damage throughout the hippocampus by stereological analysis of the dentate gyrus and Ammon's horn. Rats were ADX or sham operated, and then tested in the Morris water maze 12 weeks after surgery, followed by testing on an eight-arm, alternating-baited radial maze at 22 weeks postsurgery. Animals were killed 7½ months after surgery.

ADX rats had selective volume reduction in the dentate gyrus with no changes in pyramidal regions CA1, CA2, CA3, or CA4. Dentate gyrus damage in some cases occurred throughout the entire rostrocaudal extent of the hippocampus. Analysis of corticosterone serum levels, serum Na^+/K^+ ratios, and body weight gain suggested that individual differences in dentate gyrus damage appear to be due to incomplete adrenalectomies or remaining ectopic tissue. ADX rats were able to learn in both the Morris water maze and eight-arm radial maze, even when the dentate gyrus was severely damaged (80% volume reduction). However, in the Morris water maze, the ADX rats' learning rate was significantly slower compared to controls. There was no difference between ADX and controls during reversal in either task.

These data indicate that damage to the dentate gyrus following long-term ADX is severe enough to cause learning impairment in selected learning tasks. Such damage is restricted to the dentate gyrus and can occur throughout the rostrocaudal regions of the hippocampus.

[Key words: adrenalectomy, hippocampus, dentate gyrus, Morris water maze, stereology, spatial memory, radial arm maze, corticosteroid, rat]

Glucocorticoids released by the adrenal cortex have the potential to cause hippocampal damage. Stress levels of endogenously or exogenously administered glucocorticoids exacerbate pyramidal cell death due to a variety of metabolic and neurotoxic insults (Aus Der Mühlen and Ockenfels, 1969; Sapolsky, 1985b;

Sapolsky and Pulsinelli, 1985; Morse and Davis, 1990). Prolonged exposure to high levels of glucocorticoids over the life span is thought to contribute to the age-related loss of pyramidal cells in rats (Landfield et al., 1981; Sapolsky et al., 1985, 1986). Such damage can be reduced by adrenalectomy (ADX) (Landfield et al., 1981; Sapolsky, 1985a,b; Sapolsky and Pulsinelli, 1985; Stein and Sapolsky, 1988). However, ADX and the consequent permanent loss of corticosteroids is damaging to another hippocampal region, the granule cells of the dentate gyrus. This cell loss occurs even in the absence of other insults (Sloviter et al., 1989; Gould et al., 1990; Maehlen and Torvik, 1990; Roy et al., 1990; McNeill et al., 1991; Sapolsky et al., 1991; Jaarsma et al., 1992).

The behavioral implications of hippocampal damage following ADX have not been assessed. Selective destruction of the dentate gyrus after colchicine injections leads to spatial task deficits in both radial arm and Morris water mazes (Sutherland et al., 1983; Whishaw, 1987; McNaughton et al., 1989; but cf. Jarrard et al., 1984). However, colchicine injections require cannulation, which causes dorsal hippocampus damage. Because ADX damage is specific for the dentate gyrus, with major loss of the hippocampal granule cells and minor loss of the CA4 pyramidal cells (Sloviter et al., 1989; Sapolsky et al., 1991), the importance of the dentate gyrus in spatial memory can be selectively investigated without disruption of the Ammon's horn.

Since a previous study showed that some ADX rats had nearly complete loss of granule cells (Sloviter et al., 1989), it is of great interest to determine the effects of long-term ADX on spatial memory. A preliminary report by the same laboratory suggested that long-term ADX has no effect on water maze performance (Armstrong et al., 1991). Such an effect would be significant considering the degree of dentate gyrus damage. In the present study, we investigated the effect of long-term ADX on two spatial memory tasks, the Morris water maze and the eight-arm radial maze, and sought to correlate any behavioral deficit with an assessment of dentate gyrus damage.

To determine the histological consequences of ADX, the extent of hippocampal damage was analyzed stereologically. Such a stereological analysis of the dentate gyrus damage due to ADX has not been reported. In the present study, we quantitatively analyzed the volume of granule cell and pyramidal cell regions throughout the entire hippocampus using Cavalieri volume estimations (Gundersen et al., 1988a,b).

Previous studies have reported large individual variations of ADX-induced dentate gyrus damage. Incomplete ADX or ectopic tissue (which can be located anywhere throughout the body, Shire; 1975) was hypothesized to be secreting sufficient amounts of glucocorticoids to prevent dentate gyrus reduction

in some cases, but at levels that were undetectable by radio-immunoassay (RIA) (Sloviter et al., 1989; Roy et al., 1990).

Aldosterone is another steroid secreted by the adrenal cortex. Insufficient amounts of this hormone cause reduced Na^+/K^+ ratios and body weight loss (Martin, 1978). Remaining adrenal tissue would affect not only corticosterone (CORT) levels, but aldosterone, Na^+/K^+ ratios, and body weight gain as well. Because of the insensitivity of RIA for low CORT levels, Na^+/K^+ ratios and postsurgical body weight gain might provide supplementary indices of remaining adrenal tissue and, consequently, dentate gyrus damage.

Materials and Methods

Subjects. Male Long Evans hooded rats were individually housed under reversed 12 hr:12 hr light/dark cycle. Rats had free access to food and water. There were a total of 16 young male rats (5 sham and 11 ADX).

Surgery. The rats were between 29 and 34 d old at the time of surgery. Anesthesia was induced with sodium pentobarbital (50 mg/kg body weight, i.p.) and supplements were given as necessary. ADX was performed by the dorsal approach. All animals were allowed to recover in an incubator (approximately 36°C) for about 1 hr and then returned to their home cages.

After surgery, the ADX rats were given 0.9% sodium chloride in their drinking water throughout the entire course of the experiment (Richter, 1941). Body weights were measured weekly.

Behavioral testing: Morris water maze. At 12 weeks postsurgery, the rats were tested in the Morris water maze. The water maze consisted of a pool 170 cm in diameter. The pool was divided into four quadrants with north, south, east, and west arbitrarily marked. A white, circular platform (25.5 cm high and 15 cm diameter) was placed in the middle of the southwest quadrant. The platform remained in the same quadrant throughout the regular testing period.

The pool was filled 2 cm above the platform with warm water (32–35°C) (Rauch et al., 1989) and powdered milk was added to make the platform's location undetectable. Various extrinsic cues (geometric paper cutouts) were affixed around the room and not moved during the entire experiment. The investigator was visible to the rats but sat in the same location throughout testing.

Rats were given a pretesting trial 1 d before testing. During the pretrial, rats were allowed to swim in the maze for 90 sec without the platform present.

The maze testing began approximately 3–5 hr into the animals' dark cycle. Rats were placed in one of two start positions, north or east. These start locations were chosen pseudorandomly such that by the end of each day, each rat started from both locations the same number of times. All rats followed the same start location patterns. The rat was always placed in the pool with its head facing the edge of the pool. The latency from release to the time the rat reached the platform was recorded. The swim paths were later analyzed by a digitizing program. If the rat did not reach the platform within 90 sec, the rat was guided to the platform. All rats were allowed 10 sec to sit on the platform.

The rats were given eight trials a day for 5 consecutive days (40 trials total). There was a 5 min intertrial interval between the testing trials within each day. Reversal started on the sixth day, whereby the platform was placed in a new location (i.e., the southeast quadrant). The rats were given eight more trials during reversal.

Behavioral testing: radial arm maze. Animals were food deprived until body weights were 85% of the original weight. Although ADX rats had gained significantly less weight than controls at 12 weeks post-ADX, these rats had nevertheless gained a significant amount of weight by 21 weeks (weight gained: ADX, 348 ± 80 gm; sham, 462 ± 43 gm). One ADX rat was excluded from the radial maze experiment because it appeared unhealthy after losing some weight. This was not one of the two rats with the most dentate gyrus volume reduction.

The maze was elevated 92.5 cm off the ground. It was painted brown and did not have sides along the arm edges. The rats were slowly accustomed to the maze in the following way. On first exposure to the maze, Froot Loops were placed throughout and groups of two to three rats were allowed to roam the maze freely for 20 min. On the second day, the maze was run the same as before except rats were run individually (and for the rest of the exposures). On the third day, Fruit Loops were placed all along the arms and not in the center. For day 4 and 5,

the Froot Loops were placed at the end of each arm and were not visible to the rats unless the rats were at the end of the arm. At the end of each session, rats were given a few blocks of rat chow to maintain their body weight.

The radial arm maze testing commenced 22 weeks following surgery and was located in a different room than the Morris water maze. At the end of every other arm (four arms) was placed a piece of Froot Loops cereal. This food was not visible unless the rat was at the end of the arm. The same arms were always baited with one Froot Loop and the empty arms were always without. Various extrinsic cues were visible in the room, including the investigator.

Testing began approximately the same time each day (3–5 hr into the rats' dark cycle). A rat was placed in the center of the maze. Testing ended when all four Froot Loops were found or when 15 min had elapsed. The number of misses were recorded (entries into unbaited arms), errors (reentry into an arm that had contained a Froot Loop), and time to complete the maze. Entries into an arm were determined when all four paws were placed in an arm. At the completion of the trial, the rat was returned to its cage and the maze was wiped with ethanol before the next rat was tested.

Rats were tested four times a week for 6 weeks. Reversal began on the seventh week. Arms that had been baited no longer contained Froot Loops, and arms that never had Froot Loops were baited. Rats were tested in reversal for an additional 3 weeks (four trials/week).

Death. Seven and a half months after ADX, animals were injected with an overdose of sodium pentobarbital. Before intracardial perfusion with 0.9% saline and 10% formaldehyde, blood samples were collected for CORT and Na^+/K^+ assays. The perfused brains were then removed and placed in 10% formaldehyde until processing. The abdominal cavities of the rats were inspected for adrenal tissue.

Corticosterone radioimmunoassay and Na^+/K^+ ratio determination. Corticosterone was assayed using 15 μl samples of unextracted serum, 1,2-^{3}H-corticosterone (Amersham), and charcoal separation of free corticosterone and corticosterone bound to antibody (ICN Biomedicals). The cross-reactivity of the antibody was 33% to progesterone, 5.3% to cortisol, and 6.4% to 20-α-dihydroprogesterone.

Na^+/K^+ levels were determined by Dr. Dorner at the University of Illinois Veterinary Pathology laboratory using ion-specific electrodes.

Hippocampal histology. The fixed brains were placed in 30% sucrose for cryostat sectioning. Frozen brain sections were cut at 30 μm, saving every third section. Any losses were noted so that volume could be accurately estimated. Sections were stained with 1% buffered cresyl violet.

Volume estimation. Bilateral volume determination was estimated using the Cavalieri principle (Gundersen et al., 1988a). Stereology allows for the unbiased estimation of a whole structure of interest. Measuring areas or cell counts of designated regions based upon landmarks can give inconsistent results, especially in this situation where the damage might be unevenly distributed. A section to be stereologically analyzed was randomly picked. After this section was identified measurements were taken at every 1080 μm (every 36th section). The number of sections analyzed varied between five and seven sections per brain.

The designated sections above were coded, and the analysis was performed blind. Bilateral volume determinations were obtained using an Olympus BH-2 microscope (40×) and MEDICOSOFT GRID software. Pyramidal cell regions were determined by landmarks previously described (Lorente de Nó, 1934; Blackstad, 1956).

Statistical analyses. Statistical comparisons were analyzed in the following ways. Correlations were analyzed by simple regression. Group comparisons between corticosterone levels, Na^+/K^+ ratios, body weight gain, hippocampal volumes, and swim distances were measured by one-way ANOVAs and Fisher (protected LSD) post hoc analyses (Keppel, 1982). Graphs from the one-way ANOVA designs were illustrated with means and standard errors. The water maze data were not normally distributed and were analyzed both by a two-way, repeated measures ANOVA following a transformation for skewed data (1/score). In addition, the water maze data were analyzed by a second, nonparametric median test (Neter et al., 1985). The radial maze data were analyzed by a two-way, repeated measures ANOVA. The differences were considered significant when a probability value of 0.05 or less was obtained.

Results

CORT serum levels, body weight gain 12 weeks after ADX, and Na^+/K^+ ratios for all rats were plotted against each other to
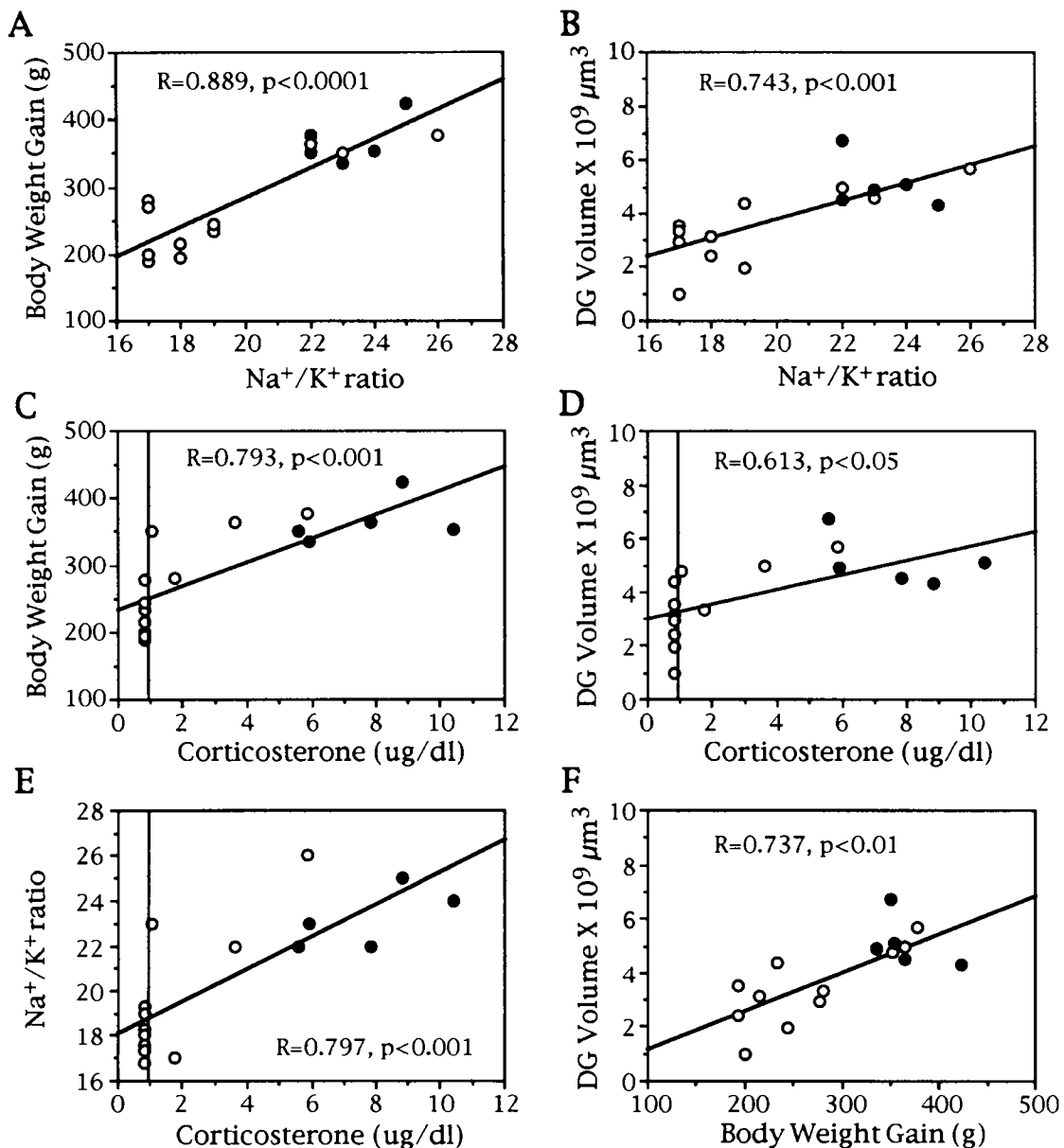
Figure 1. Correlations between Na$^+$/K$^+$ ratios, body weight gain, CORT, and dentate gyrus (*DG*) volume (in μm^3). Each *circle* represents one rat: *open circles*, ADX; *solid circles*, sham. CORT RIA was not sensitive to hormone levels below 0.8 μg/dl (indicated by *vertical solid line*). Rats with CORT levels below RIA detection were designated with 0.8 μg/dl CORT levels. All correlations are highly significant (see each graph), which suggests that rats with low body weight gain, Na$^+$/K$^+$ ratios, and CORT also have reduced dentate gyrus volume.

determine if these measures were correlated. The correlation between Na$^+$/K$^+$ ratios and body weight gain is highly significant ($R = 0.889$, $n = 16$, $p < 0.0001$; Fig. 1A). The correlations for CORT versus body weight gain and CORT versus Na$^+$/K$^+$ ratio were significant as well ($R = 0.793$, $n = 16$, $p < 0.001$ and $R = 0.797$, $n = 16$, $p < 0.001$, respectively; Fig. 1C,E). Rats with low Na$^+$/K$^+$ ratios tended to have low body weight gain after surgery and low serum CORT levels.

CORT serum levels, body weight gain 12 weeks after ADX, and Na$^+$/K$^+$ ratios were plotted against dentate gyrus volume. The highest correlation with dentate gyrus volume was with Na$^+$/K$^+$ ratios (Fig. 1B; $R = 0.743$, $n = 16$, $p < 0.001$), although the other correlations were also significant (CORT, $R = 0.613$, $n = 16$, $p < 0.05$; weight gain, $R = 0.737$, $p < 0.01$; Fig. 1D,F). Rats with low Na$^+$/K$^+$ ratios, low serum CORT, and small

weight gain after surgery also have decreased dentate gyrus volume.

Distributions of dentate gyrus volume, body weight gain, and Na$^+$/K$^+$ ratios were bimodal. All sham rats (solid circles) are grouped together. The ADX rats that are located in the same cluster with the shams in Figure 1A are also the same ADX rats with high dentate gyrus volume. Because we are interested in determining the functional consequences of ADX-induced dentate gyrus damage, we wanted to separate ADX rats with no dentate gyrus damage from those rats with damage. The ADX rats with no dentate damage were designated as partially ADX (pADX) and categorized as a third group in the next analysis. The remaining ADX rats will still be designated as ADX (pADX $n = 3$, ADX $n = 8$).

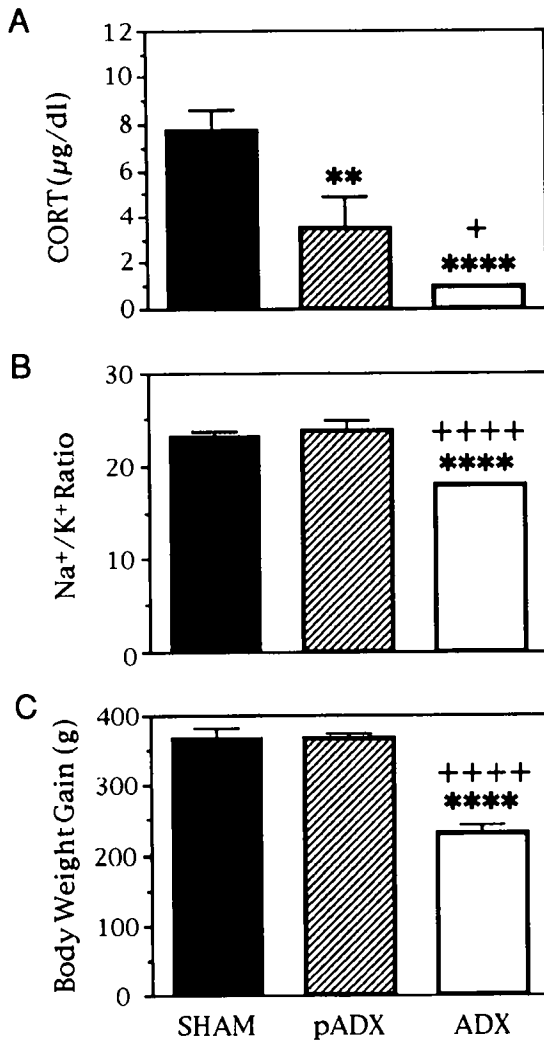The three groups of animals were compared on mean CORT

A

B

C

Figure 2. The means and respective SEs from serum CORT (A), Na⁺/ K⁺ ratios (B), and body weight gain (C) 12 weeks after surgery. Shams (n = 5), ADX rats with adrenal tissue remaining (pADX; n = 3), and completely adrenalectomized rats (ADX; n = 8) were compared. ADX rats were significantly different from sham and pADX rats in all measures investigated. *, Comparison with sham; +, comparison with pADX; ****, $p < 0.0001$; ++++, $p < 0.0001$; **, $p < 0.01$; +, $p < 0.05$.

serum levels, average body weight gain after ADX, and Na⁺/ K⁺ ratios using one-way ANOVAs with Fisher post hoc analysis. ADX rats have significantly reduced CORT levels ($p < 0.0001$; Fig. 2A), Na⁺/K⁺ ratios ($p < 0.0001$; Fig. 2B), and body weight gain ($p < 0.0001$; Fig. 2C) when compared to shams. ADX rats are also significantly different than the pADX rats in all three measures (CORT, $p < 0.05$; Na⁺/K⁺ ratio, $p < 0.0001$; weight gain, $p < 0.0001$). The pADX rats differed from shams in only one measure: they had lower CORT serum levels ($p < 0.01$).

Figure 3 shows the mean dentate gyrus and pyramidal region volumes for each group. An ANOVA and Fisher analysis reveals that ADX rats have significantly reduced dentate gyrus volume when compared to both pADX and sham ($p < 0.01$). The average ADX dentate gyrus volume loss compared to shams was 45%. Sham and pADX are not significantly different from each other. Pyramidal cell volumes for all CA regions are not statistically different.

The degree of damage in the ADX rats is not restricted to the rostral end of the dentate (Sloviter et al., 1989) but appears
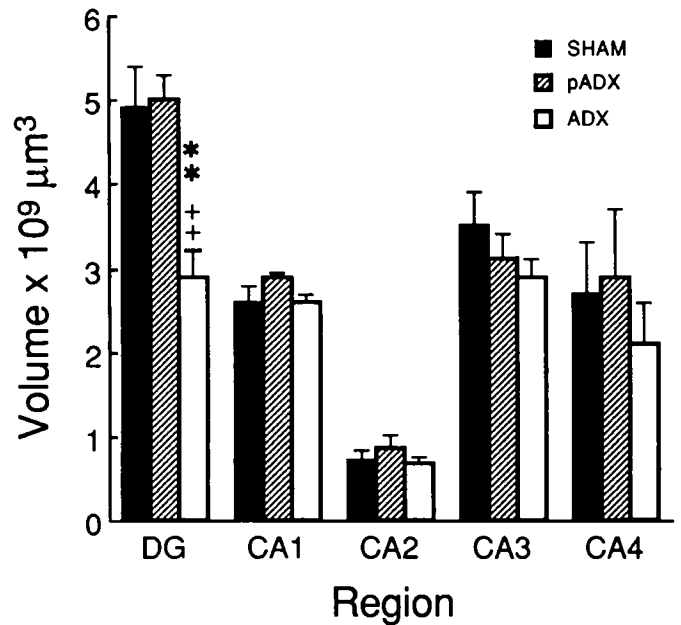

Figure 3. Volumes of designated hippocampal regions. Data are represented by the means and SE. Sham (n = 5), *solid bars;* ADX rats with adrenal tissue remaining (pADX; n = 3), *hatched bars;* completely adrenalectomized rats (ADX; n = 8), *open bars.* Only the dentate gyrus volume of ADX rats was significantly different than both shams and pADX (ADX, 2.84 ± 1.04; pADX, 5.14 ± 0.48; sham, 5.10 ± 0.97 × 10⁹ μm³). **, $p < 0.01$ compared to sham. ++, $p < 0.01$ compared to pADX.

throughout the rostrocaudal plane. An example of one of the worst cases of ADX-induced dentate gyrus damage is illustrated in Figure 4, A, C, E, and G. An example of a typical sham dentate gyrus is illustrated in Figure 4, B, D, F, and H. Loss of granule cells in the extreme case is apparent in all regions from the most rostral (Fig. 4A) to the most caudal (Fig. 4G). This animal had an 80% volume loss compared to the sham mean.

Damage to the dentate does not necessarily begin at the most rostral plane. Figure 5 illustrates a case in which ADX damage was mild. Volume reduction is not evident in the rostral plane (Fig. 5A) or the mid-rostral region (Fig. 5B). However, the lateral end of the dentate gyrus dorsal blade located in the mid-caudal plane shows some signs of degeneration (Fig. 5C).

Behavioral data

Shams and pADX were combined as one control group and compared to the ADX group in the behavioral analyses because (1) there was no difference between sham and pADX groups on dentate gyrus volume, Na⁺/K⁺ ratios, or body weight gain (only serum CORT levels differed), and (2) ADX rats were significantly different from both pADX and sham groups in all measures investigated.

Escape latencies of the two groups in the Morris water maze are plotted in Figure 6. The median escape latencies of the combined control group are shown with solid circles. Individual performances from the ADX rats are plotted with open circles. Each day represents a total of eight trials. Both groups were able to learn the location of the platform [repeated measures ANOVA; $F(4,56) = 20.6, p < 0.0001$]. However, the ADX rats were not able to perform as well as the control [main group effect, $F(1,14) = 19.7, p < 0.001$]. A median test also shows that the performance between days 2 and 4 is significantly different ($p < 0.0001$). The ADX rats' slower latencies in the Morris water
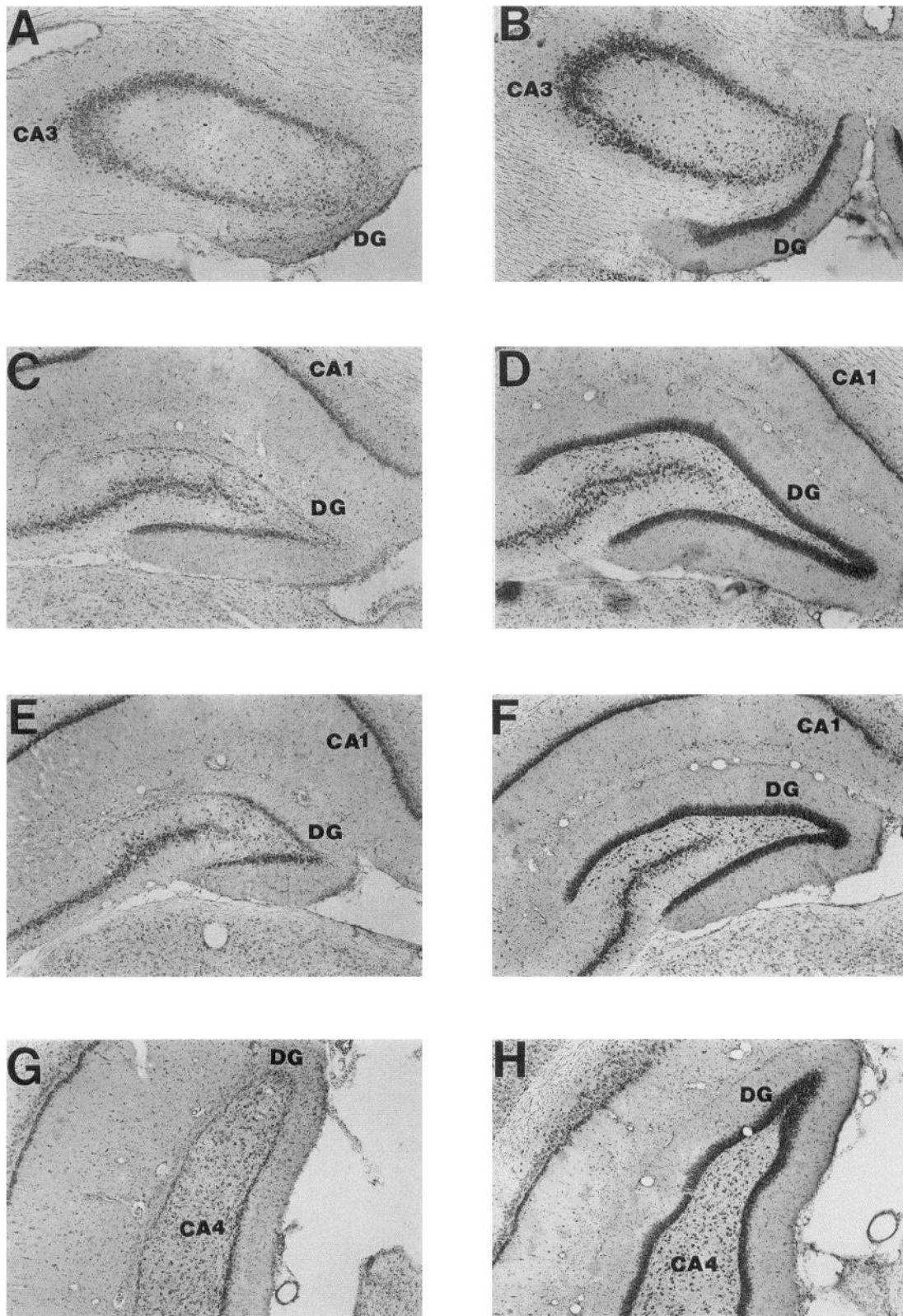
Figure 4. Coronal sections of the hippocampal dentate gyrus. The *left column* is an extreme case of granule cell loss in the dentate gyrus of an ADX rat. The *right column* is a typical control rat (sham). The sections are sequential, with the *top row* (*A, B*) being most rostral and the *bottom row* (*G, H*) most caudal. Note the extensive degree of granule cell loss throughout the dentate gyrus (*DG*) in the ADX rat compared to the sham.
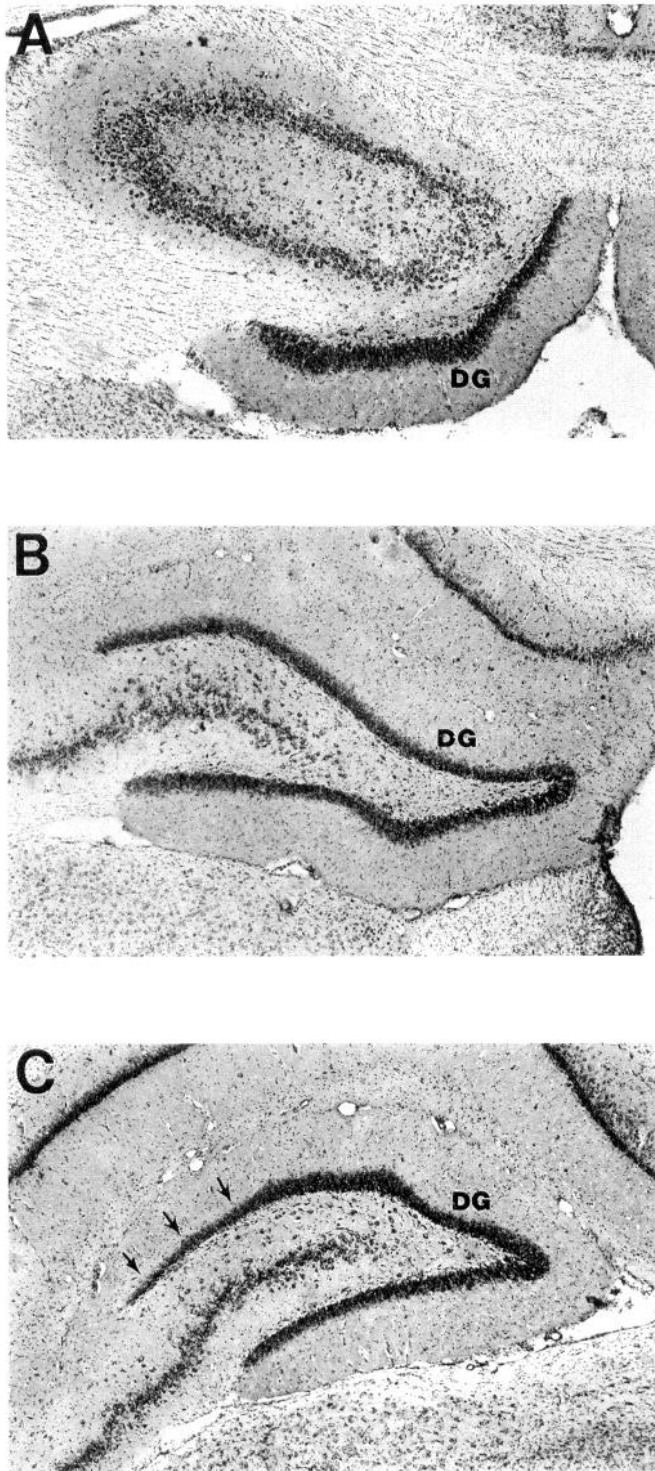
Figure 5. Coronal sections of hippocampal dentate gyrus in a mild case of dentate gyrus degeneration from an ADX rat. *A*, most rostral; *B*, middle-rostral; *C*, middle-caudal. No degeneration is apparent in the first two sections illustrated (*A, B*). The middle-caudal section shows some signs of degeneration in the lateral end of the tip (*arrows*) of the dorsal blade of the dentate gyrus (*DG*).

maze were partially caused by the longer distances swam on days 3 and 4 (Fig. 7).

There was no interaction effect on the Morris water maze escape latencies, nor were there statistical differences in performance between groups during reversal. Both groups were able
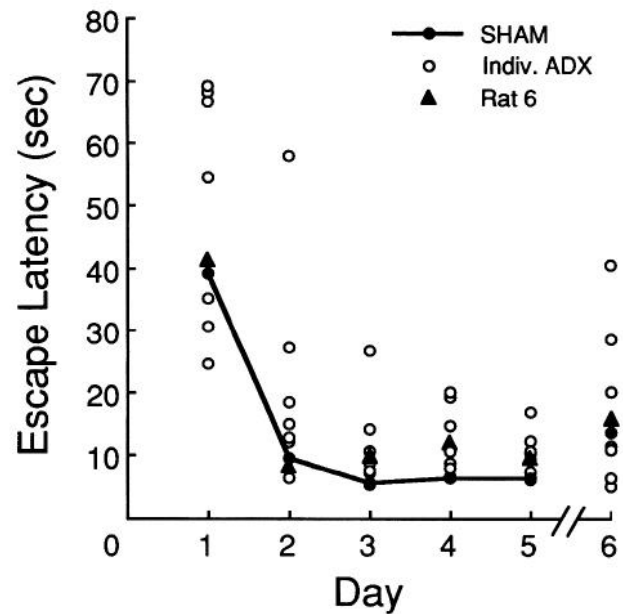

Figure 6. Escape latencies in the Morris water maze. Each day represents eight trials. The platform was in the same location on days 1–5. On day 6 (reversal) the platform was moved to a new location. Sham and pADX were combined as one group, and their median escape latencies are illustrated by the *solid circles*. The median escape latency for each individual ADX rat is illustrated with an *open circle*. The escape latency illustrated with a *triangle* is from rat 6, which is the same ADX rat with severe dentate damage shown in Figure 4, *A, C, E,* and *G*. ADX rats are slower to learn than the controls [group effect, $F(1,14) = 19.7$, $p < 0.001$ and median nonparametric test, $p < 0.0001$]. However, both groups were able to learn the location of the platform [$F(4,56) = 20.6$, $p < 0.0001$]. Analysis of the first trial of reversal showed no statistical differences between ADX and controls in search strategies.

to learn the platform's new location [$F(7,98) = 10.925$, $p < 0.0001$].

The performance of the two groups in the eight-arm radial maze showed no statistical difference (repeated measures, two-way ANOVA) in all the behavioral aspects investigated (Table 1). By the sixth week, both groups showed a decrease in time to complete the maze [$F(5,60) = 20.3$, $p < 0.0001$], a decrease in errors [$F(5,60) = 3.044$, $p < 0.05$], and a decrease in misses [$F(5,60) = 9.0$, $p < 0.0001$]. During reversal (weeks 7–9) there
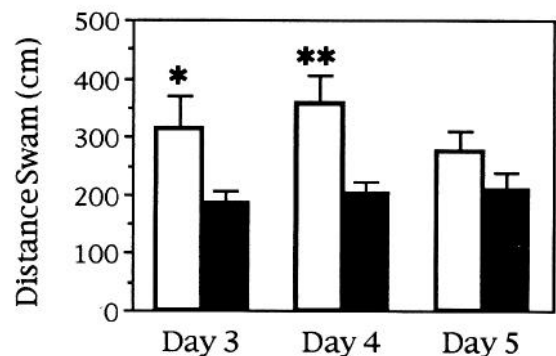

Figure 7. Swim distances in the Morris water maze. Each day represents the average distances swam after eight trials. Means and SEs are graphed for days 3–5. *Solid bars*, controls; *open bars*, ADX. ADX rats swam longer distances than controls on days 3 and 4 [one-way ANOVA, Fisher post hoc analysis: day 3, $F(1,14) = 4.5$, $p = 0.05$; day 4, $F(1,14) = 8.2$, $p = 0.01$].

Table 1. Radial maze performance of ADX and control rats

Initial training

Group	Time (Week 1)	(Week 6)	Error (Week 1)	(Week 6)	Misses (Week 1)	(Week 6)
Control (n = 8)	12.9 ± 2.5	4.9 ± 3.3	1.6 ± 1.8	0.3 ± 0.3	3.3 ± 2.0	0.8 ± 0.6
ADX (n = 7)	13.4 ± 2.9	5.9 ± 4.7	1.3 ± 1.6	0.3 ± 0.4	1.9 ± 2.2	0.5 ± 0.4

Reversal training

Group	(Week 7)	(Week 9)	(Week 7)	(Week 9)	(Week 7)	(Week 9)
Control	44. ± 0.6	4.1 ± 1.0	1.4 ± 0.9	0.4 ± 0.4	3.3 ± 0.8	2.2 ± 0.8
ADX	5.9 ± 2.4	4.5 ± 1.1	2.2 ± 1.4	0.3 ± 0.3	3.3 ± 0.5	2.4 ± 0.3

There was no significant difference in learning between ADX and controls. Both groups were able to learn the maze as evidenced by the decrease in latency, errors, and misses. There was no difference between groups in reversal, and both groups showed decreased errors and misses.

was no group effect. However, errors [$F(2,22) = 18.7, p < 0.0001$] and misses [$F(2,22) = 9.6, p < 0.001$] were significantly reduced by the ninth week. There was no effect of reversal on the time to complete the maze.

Dentate gyrus volume was plotted against mean water maze escape latencies for each day to determine if behavior was correlated with hippocampal anatomy. None of the correlations were significant (data not shown).

Discussion

Spatial memory as tested by the Morris water maze was impaired in ADX rats compared to shams. The slower escape latency of the ADX group cannot be solely attributable to slower swim speeds, as the ADX group had longer swim distances than the controls. The ADX rats were slower than the controls in learning the Morris water maze, but they were all able to learn the task. Interestingly, these same ADX rats were not impaired in the eight-arm radial maze when compared to controls, nor were they impaired in learning reversal in both tasks.

Although many correlational analyses were performed, there was no correlation between dentate gyrus volume and the mean Morris water maze escape latency for each day. Part of the reason for this noncorrelation was that the two rats with the most dentate damage were among the best performers of the ADX group. Another reason why behavior and dentate gyrus volume may not have correlated could be because the rats were not killed immediately after completion of the Morris water maze but after an additional 4 months when the radial maze was completed. Consequently, there is no way to ascertain conclusively how much dentate was present during water maze testing. Although it is not clear how much dentate gyrus was functional at the time of the Morris water maze testing, clearly enough dentate was lost to impair performance.

One might argue that the differences in learning between the ADX and control rats might be attributable to the endogenous corticosteroid in the controls. Besides having a damaged dentate gyrus, the ADX rats do not have endogenous corticosteroid during the behavioral testing. Therefore, the differences in the spatial learning could be due to the actions of corticosteroid in the controls *during* learning as opposed to the long-term effects of corticosteroid removal on the dentate gyrus. However, ongoing experiments on our lab are demonstrating that ADX rats

supplemented with exogenous corticosteroid during behavioral testing (but not during the 12 weeks in between ADX and testing) have impaired learning performances on the water maze task. These results strongly support the idea that the learning difference between ADX and controls is due to neuronal degeneration and not due to acute effects of corticosteroids.

Dentate gyrus damage occurs gradually following ADX (Jaarsma et al., 1992); therefore, more destruction should be observed at 22 weeks than 12 weeks postsurgery. If spatial abilities were solely a function of dentate gyrus cell number, then one would expect spatial tests at 12 weeks to reveal less spatial deficits than tests given at 22 weeks. However, this was not the case in our experiment. ADX rats were impaired in the Morris water maze that was given at 12 weeks postsurgery and not impaired on the radial maze that was started at 22 weeks postsurgery.

Although ADX clearly induced a deficit on learning in the Morris water maze, it is noteworthy that rats with massive dentate loss can still perform two spatial tasks, the water maze with some impairment and the eight-arm radial maze with no impairment. The first trial of reversal in the water maze is analogous to a probe trial, and we analyzed parameters of this trial to determine whether ADX rats used strategies for finding the platform similar to controls' strategies. ADX and control animals did not differ on this trial in measures of start direction or swim distance in the quadrant that previously contained the platform, suggesting that ADX animals used similar spatial strategies. Furthermore, even the most severely impaired rats were able to learn the radial maze and learn reversal as well as controls. Because the rats were killed immediately after completing the radial arm maze, we know that as much as 80% dentate gyrus volume reduction did not impair performance on this task.

The findings that spatial memory impairment is evident in only one of two spatial learning tasks and not observed when destruction would be at the maximum raises a question about the importance of the dentate gyrus in spatial learning. Colchicine lesion studies strongly support the hypothesis that an intact dentate gyrus must be present for normal spatial learning (Sutherland et al., 1983; Whishaw, 1987; McNaughton et al., 1989). Interestingly, Jarrard et al. (1984) reported that colchicine lesions of the dentate gyrus had little effect on the performance

of a previously learned radial maze task. Colchicine destroys nearly all of the dentate gyrus except the basket cells. In our experiment, two ADX rats had near total ablation of the dentate gyrus and the remaining ADX rats had varying degrees of degeneration. One possible explanation of the divergent behavioral patterns is that the dentate gyrus circuitry is redundant. Nearly complete destruction may be required to produce major impairments. Neuronal connection redundancy and the absence of a deficit to a large proportion of neuronal loss are well documented in the nigrostriatal system. As much as 90% of substantia nigra neurons can be lost before any functional deficits are observable (Heikkila et al., 1981; Calne and Zigmond, 1991). In our experiment, it is possible that the few remaining granule cells of the ADX rats might have been sufficient to enable the animals to perform the spatial tasks as well as controls in the radial arm maze.

Another hypothesis is that the dentate connectivity is restructured after long-term ADX. Entorhinal cortex damage has been shown to result in hippocampal reorganization, which includes the dentate gyrus (Hagan et al., 1992; Steward, 1992). Kindling can also cause dentate gyrus mossy fiber reorganization (Cavazos et al., 1991). Such reorganization may be more likely in this and other ADX studies because of the absence of glucocorticoids, which when administered exogenously, can reduce axonal sprouting (Scheff et al., 1980; Scheff and Cotman, 1982; Scheff and DeKosky, 1983; Scheff and DeKosky, 1989). Analysis of dentate gyrus fiber tracts would help resolve this issue.

Finally, it is possible that another brain region can substitute for the function of the dentate gyrus granule cells. Various cortical areas have been shown to be important in spatial memory tasks (Becker et al., 1980; Goodale and Dale, 1981; Kolb et al., 1983; Kolb and Gibb, 1990). A study on single-unit recordings suggests that spatial information transmitted to pyramidal cells may be processed through other routes than by the trisynaptic circuitry such as from the entorhinal cortex (McNaughton et al., 1989). The colchicine studies tested learning a short time after dentate gyrus destruction (within a few weeks). If plasticity could occur, then a few weeks after the insult may not be sufficiently long for recovery of function. In our study, Morris water maze impairment was evident 12 weeks after the initial insult, but not in the radial maze at 22 weeks later. It is possible that there was sufficient time for another brain region(s) to compensate for the function of the dentate gyrus at the time of the radial maze but not for the water maze.

The dentate gyrus volume reduction after ADX supports previous investigations demonstrating that long-term ADX causes granule cell loss in the dentate gyrus. The results show that dentate gyrus damage occurs throughout the hippocampus and is as severe in the caudal regions as it is in the rostral regions. In accordance with Sloviter et al. (1989), we found that remaining dentate gyrus neurons in severe cases appear to be basket cells. Although the average ADX-induced dentate gyrus volume reduction is 45%, estimates of granule cell loss might be more extensive because our volume estimations included basket cells.

This is the first study in which the complete dentate gyrus was systematically analyzed after long-term ADX according to the principles of stereology. Previous studies determined dentate gyrus damage by measuring area of selected regions, counting neurons in selected regions, or using qualitative measures (Sloviter et al., 1989; Roy et al., 1990; McNeill et al., 1991; Sapolsky et al., 1991). However, such measures could be misrepresen-

tative if many animals displayed inconsistent damage like that illustrated in Figure 5. In this case, only a fraction of the dentate gyrus showed damage. If the plane of analysis did not include the damaged part, then the results could misrepresent the destruction.

Our results further suggest that pyramidal cells are not damaged by ADX. We were not able to replicate the findings by Sapolsky et al. (1991) that ADX is damaging to the CA4 cells. Sapolsky counted cells in one plane of the hippocampus, and consequently the numbers might be biased according to the theory of stereology (Gundersen et al., 1988a). On the other hand, the relatively small CA4 reduction that Sapolsky observed (19%) might be hard to detect with volume stereology. Stereological cell counts will give the best estimate of whether ADX is damaging to the pyramidal CA4 region. It is interesting to note that the pyramidal cell region in question, CA4, is sometimes considered to be part of the dentate gyrus (Cowan et al., 1980).

Individual differences of the dentate gyrus degeneration to ADX appear to be due to incomplete ADX or ectopic tissue. The three ADX rats (pADX rats) that did not have a reduction in dentate gyrus volume also had detectable CORT serum levels, normal Na$^+$/K$^+$ ratios, and normal body weight gain. Such variations among the whole ADX population leads one to speculate that the variation within the animals that lost dentate gyrus neurons (ADX rats) might also be reflective of the amounts of serum CORT. However, the CORT RIA was not sensitive enough to detect the low levels of endogenous CORT among the ADX rats with dentate volume reductions.

Body weight gain and Na$^+$/K$^+$ ratios, however, were highly correlated, suggesting that both are affected by a common factor. Aldosterone removal would greatly impact both body weight gain and Na$^+$/K$^+$ ratios. Consequently, measures that reflect aldosterone activity such as Na$^+$/K$^+$ ratios and body weight gain, or even aldosterone measurement might more accurately reflect which animals are thoroughly adrenalectomized.

Dentate gyrus degeneration is hypothesized to be more severe in younger than in mature rats (McEwen and Gould, 1990; McEwen et al., 1990). Our results in conjunction with other published reports are consistent with this hypothesis. Our rats were prepubertal at the time of ADX and the dentate gyrus showed massive amounts of cell loss, as observed in the first report using prepubertal rats (Sloviter et al., 1989). Older rats, 5 months old, demonstrated a significant but much less severe degeneration, 26% granule cell loss (Sapolsky et al., 1991). These studies suggest that massive cell loss would be more likely in younger animals and factors that make granule cells mature would "protect" them from ADX-induced cell death. A caveat is that older rats may be less affected by ADX because their increased body fat would make complete ADX difficult.

In summary, long-term ADX causes an average of 45% dentate gyrus stereological volume reduction and as much as 80% in the extreme case without affecting pyramidal cells. Variations of dentate gyrus damage among individual rats appear to be due to circulating corticosteroids; however, Na$^+$/K$^+$ ratios or body weight gain are better indices of remaining adrenal tissue. Finally, while all ADX rats were able to learn the two mazes, ADX rats had spatial memory impairments in the Morris water maze escape latencies. No detectable impairments in the eight-arm radial maze were found. Given the high degree of granule cell loss, we propose that the spatial learning can still proceed due to redundancy or plasticity in the hippocampal circuitry.

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