signed-ranks test, $N = 9$) (Table 1). In the final census (August 1980), numbers of frogs in control and experimental plots differed significantly ($P < 0.005$ for adults; $P < 0.05$ for all frogs; t-test). Up to 46 percent of the houses were occupied during the day.

The significant increase in the number of preadult frogs supports the assumption that the increase is intrinsic and is not simply the result of a relocation of frogs from the surrounding forest. The increase in the number of adults in houses is an indicator of the growth of the previous year's young. Movements of ten marked frogs in the River experimental plot were observed throughout one night. The mean maximum distance moved from diurnal retreat sites was 200 cm [standard deviation, 95 cm], indicating that the frogs remained in the plot during their nocturnal activities.

Increasing the number of retreat and nest sites resulted in a significant increase in numbers of preadult and adult coqui and their nests. Most studies of population regulation have emphasized food, predation, or behavioral interactions as regulators of population density (*8*). We have shown experimentally that the number of appropriate retreats and nest sites limits population size of *E. coqui*, a terrestrial ectothermic vertebrate.

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Rate of Synaptic Replacement in Denervated Rat Hippocampus Declines Precipitously from the Juvenile Period to Adulthood

Abstract. *Synaptic contacts per unit area in the rat dentate gyrus reach adult numbers by the end of the first month after birth and remain constant thereafter. This experiment demonstrated that the rate at which synapses were replaced by sprouting after a lesion declined dramatically from 35 to 90 days of age. Thus, the juvenile period of the rat's life is marked by a considerable change in neuronal plasticity. This may be related to age-dependent effects in recovery from brain damage.*

Developing neural systems have great potential to reorganize, including forming aberrant fiber tracts, after lesions in the perinatal animal (*1*). With maturation, lesion-induced neural growth becomes more restricted and arises primarily from a limited formation of new synapses by intact axons in response to adjacent axonal and synaptic degeneration (*2*). Although the rate of synaptic restoration is known to undergo substantial changes during development (*3*), quantitative comparisons of this variable in juvenile and adult animals are lacking. Since sprouting is involved in the behavioral consequences of lesions in the central nervous system (*4*), changes in the speed at which synapses are replaced may help explain certain age-dependent behavioral effects of brain injury [such as acquired aphasia (*5*)]. We now report that reinnervation of the rat dentate gyrus after removal of a major input slows considerably during the juvenile period.

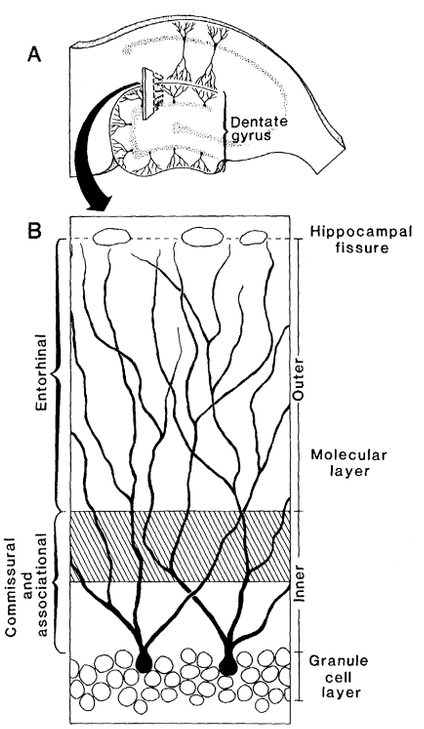

Fig. 1. Diagrams of the region of the rat hippocampal formation sampled for quantitative electron microscopy. (A) Schematic of a section cut at a right angle to the longitudinal axis of the hippocampus. Ultrathin sections were taken from the region of the dentate gyrus shown along the indicated plane. (B) The granule cells are located within a discrete layer, and their dendrites extend outward into a largely cell-free molecular layer. Quantification within the commissural projection to the outer half of the inner molecular layer (hatching) is consistent across animals (*6*); this subfield was used in this experiment.

The dentate gyrus of the rat hippocampal formation contains a population of granule cells organized in a horseshoe-shaped layer. The dendrites of these neurons extend outward and form a homogeneous molecular layer (Fig. 1). The major inputs to the granule cells arise from (i) the entorhinal cortex and (ii) the contralateral (commissural projection) and ipsilateral (associational projection) hippocampus. The hippocampal systems occupy the inner third of the dendritic field, and the entorhinal fibers the outer two-thirds of the molecular layer; the projections do not overlap. The simplicity of these anatomical arrangements has made the dentate gyrus a useful system for anatomical studies of neuronal plasticity.

Commissural axon degeneration, induced by either removal of the contralateral hippocampus (*6–8*) or contralateral cerebral ischemia (*9*), leads to a growth response within the inner molecular layer of the adult rat. Since the commissural

projection generates 35 percent of the synaptic population within the inner molecular layer, synaptic proliferation [presumably from the adjacent associational axons (*6, 10*)] is relatively modest compared with the extensive axonal sprouting required to replace the extremely dense input from the entorhinal cortex (*11*). Commissural lesions, unlike entorhinal lesions (*12*), cause only minor dendritic pathology and no detectable shrinkage of the molecular layer (*6*), and therefore precise electron microscopic descriptions of terminal proliferation can be made. Accordingly, this procedure is well suited for quantitative studies of the time course of axonal sprouting.

The left hippocampus in male rats was removed by aspiration 35, 60, 90, and 180 days after birth (*13*). The animals were killed and the right hippocampus prepared for electron microscopy by conventional procedures 2, 8, and 15 days after the surgery. Our previous studies in adult animals have shown that axonal degeneration is greatest 2 days after the lesion is made, and that synapse replacement begins in the hippocampus approximately 6 to 8 days after deafferentation, progresses steadily for 1 week, and then slows (*7*). Thus, the survival times chosen in this study include one time point before and two time points after the expected onset of synaptic proliferation.

Counts were made of (i) intact boutons forming one or more synapses—synaptic boutons, (ii) intact boutons making two or more synapses—multiple synaptic boutons (*14, 15*), and (iii) degenerating (electron-opaque) synaptic boutons. Quantification (*16*) and measurements of the height of the commissural terminal field and the molecular layer (for possible shrinkage) (*17*) were taken within the same septo-temporal, medio-lateral region in the dentate gyrus of all animals (Fig. 1).

The major results of the experiment are shown in Fig. 2. The density of synaptic boutons in the control group at each age was virtually identical. This result agrees with that of a previous study showing that the dentate gyrus attains an adult complement of synapses in the fourth postnatal week (*18*). The loss of synapses measured 2 days after the commissural lesion was also not detectably different among the four groups of rats. The replacement of lost contacts was progressively and substantially slower with increasing age, however. The synaptic population almost completely recovered to control values by 8 days in 35-day-old rats, while fewer than half of the lost synapses were restored within 15 days in the more mature animals. Our earlier work indicates that in adult rats a normal synaptic density is reached only after 2 months of postoperative recovery (*7*). In this study, the degree of synaptic reinnervation did not differ significantly after lesions at 90 and 180 days of age, thereby suggesting that the rate of sprouting stabilizes in adulthood (*19*).

Table 1. Degenerating synaptic boutons (mean ± standard deviation) in the commissural zone of the dentate gyrus after a commissural lesion at different postnatal ages (*26*).

Postnatal age (days)	Days after lesion		
	2	8	15
35	3.5 ± 0.3	1.2 ± 0.2	0.5 ± 0.1
60	4.7 ± 0.3	1.7 ± 0.3	0.8 ± 0.1
90	6.0 ± 0.4	2.2 ± 0.3	1.2 ± 0.2
180	6.3 ± 0.1	2.2 ± 0.3	1.0 ± 0.2

The age-dependent changes are probably due to the rate of synapse proliferation rather than to the time of its initiation. This hypothesis is suggested by entorhinal lesion studies, which have established that growth and synaptic reinnervation begin no sooner than 5 or 6 days after lesions in 21-day-old and adult rats (*20*). If so, sprouting was nearly four times as fast at 35 days as at 90 days during the initial period of synaptic reinnervation (eight synapses per 100 μm^2 added by day 8 after the lesion in the younger rats and two synapses in the older groups).

The rate of sprouting may be linked to the rate of degeneration removal (*7, 15, 21*). Table 1 shows that degenerating endings were indeed eliminated more rapidly in the younger animals (*3, 22*). However, while the number of degenerating endings 15 days after the lesion in the 90- and 180-day-old groups was equivalent to that found 8 days after the lesion in the 35-day-old rats, the density of intact synaptic contacts was considerably greater in the younger rats. Thus, changes in the rate of degeneration removal alone cannot account for the decline in the rate of reinnervation. Nevertheless, the inverse relationship between the persistence of degenerating terminals and the speed at which new endings were generated by intact axons suggests that these variables reflect the same neuronal properties. Possibly some fundamental aspect of the neuron slowly changes between the second postnatal week of life and young adulthood and, in doing so, alters both the degenerative and growth responses of axons (*23*).

Our results demonstrate that a substantial decline in the rate of sprouting occurs during the juvenile period in the dentate gyrus of the rat. Previous work has focused on neural reorganization in immature or adult tissue, where major differences exist. When axons are growing toward their final destination during development, it is not surprising that they respond more to a lesion than they do in the adult, in which the axons have reached their targets. As this study points out, however, even within a neural system in which axonal ingrowth has ceased and a stable density of synaptic input has been reached, a progressive loss in the rate of synaptic recovery takes place with increasing age (*24*).

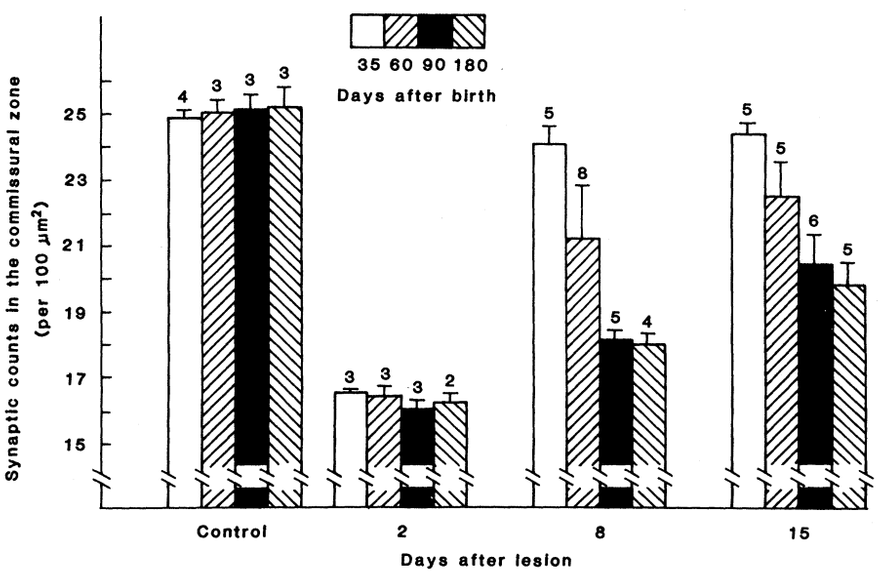

Fig. 2. The effect of a commissural lesion at different postnatal ages on mean number of intact synaptic boutons. Standard deviations and number of rats in each group are shown with each bar.

The nature of the cellular changes responsible for this loss is unclear. Possibly synaptic turnover (the coordinated production and removal of synapses) declines across the juvenile period, leaving the older rats with less ongoing growth to produce a sprouting response. However, there is no necessary reason to assume that sprouting represents an exaggeration of normal synaptogenesis; it may require the activation of novel or usually quiescent processes. The sudden onset of sprouting 5 or 6 days after the lesion would not be predicted from the continuous growth hypothesis, but can be accounted for by a modified version of that idea—factors are present that prevent the expression of growth until 5 days after the lesion.

Whatever the underlying mechanism may be, the sharp decline in growth responses provides a possible explanation for age-related changes in recovery from brain damage (*5, 25*). It will be of interest then to assess the rate and degree of behavioral recovery after commissural lesions in rats of the ages used in this study.

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13. Sprague-Dawley (ARS, Madison, Wis.) rats were reared in groups of three to five to a cage from 30 days after birth; food was available at all times. Rats in all groups were randomly selected from two or three different litters.
14. The number of multiple synaptic boutons increased by 25 to 30 percent between the control animals and the 15-day group (from approximately 1.6 to 2.1 per 100 μm^2). This increase after axonal sprouting has been reported elsewhere and its significance discussed (*6, 7, 15*).
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16. Approximately 40 photomicrographs were taken randomly throughout the outer 50 percent of the dendritic field containing the commissural projection for each animal at a magnification of ×10,000 (later enlarged twofold). Total area analyzed for each animal was generally 2500 μm^2. Two-tailed *t*-tests were used for statistical analysis. At 8 days, both the 35- and 60-day-old and the 60- and 90-day-old rats differed significantly ($P < 0.005$). At 15 days, the 35- and 60-day-old rats differed ($P < 0.005$).
17. Measurements of the height of the inner and entire molecular layer were calculated from tissue embedded in epoxy viewed in a light microscope (*6*). No significant changes in height were detected for either the inner or entire molecular layer at any of the ages tested.
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23. It is also possible that the astrocytes which phagocytize the degenerating axons and terminals show age-dependent changes in reactivity and may thus be partly responsible for the decreasing rates of degeneration removal observed with increasing age.
24. Although the rate of sprouting is faster in the younger rats, the final recovery state (synaptic density) is similar. However, since the synaptic contacts are reestablished within only a few days for the 35-day-old animals as opposed to nearly 2 months in the adult rats, a qualitatively different impact on the final neural circuitry and behavior may take place for these two types of sprouting responses.
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26. Except for two degenerating synaptic boutons seen within the commissural zone in the 35-day-old rats (more than 10,000 μm^2 of tissue analyzed), no degenerating boutons were detected in any of the control (intact) animals in all the tissue analyzed.
27. Supported by a grant from the National Institute of Aging (AG00538-06). J.R.McW. is a National Institute of Communicative Disorders and Stroke postdoctoral fellow (5 F32 NS06821).

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Sex Change in a Coral-Reef Fish: Dependence of Stimulation and Inhibition on Relative Size

Abstract. *The removal of a single dominant individual has been shown to trigger a sex change in some coral-reef fish. In the saddleback wrasse (*Thalassoma duperrey*), however, female-to-male sex change requires visual stimulation from smaller conspecifics. This change is not dependent on the sex or color of the stimulus fish and can be inhibited by larger conspecifics. On the reef, a female probably changes sex when the relative numbers of larger and smaller conspecifics change within her home range.*

Social control of sex change in fishes has been demonstrated experimentally only among harem-living species or those with a rigid dominance hierarchy (*1–3*). In such cases sex change usually occurs as a simple one-to-one replacement: loss of the dominant male or female induces sex change in the dominant fish of the opposite sex. In nonharem species with less rigid social and mating systems, one would predict socially mediated sex change to be under the control of a more flexible mechanism. We report that such a mechanism does indeed control sex change in *Thalassoma duperrey*, a reef-dwelling wrasse abundant throughout the Hawaiian archipelago. This species exhibits protogynous (female to male) hermaphroditism, lives in sexually integrated, overlapping home ranges, and mates promiscuously rather than in a harem (*4*). Sex change in this species is not a function of paired replacement of dominant individuals. Rather, it is a function of the relative sizes of conspecifics in the social group. Their relative numbers on the reef may also be important. Experiments suggest that some threshold value of the proportion of larger or smaller fish within the home range probably triggers the initiation of sex change in individual females.

Fish were taken from coral reefs in Kaneohe Bay, Oahu, Hawaii, and brought immediately to the laboratory where they were held collectively in seawater tables for up to 2 days. During this period fish were sexed, weighed, measured, and placed individually in isolated seawater containers for 1 to 3 days before assignment and transfer to experimental pens. Pens, made of 12.7-mm (half-inch) wire mesh, measured 1 m on each side (*5*) and were submerged at fixed positions in a protected lagoon. There were no resident *T. duperrey* in the lagoon, which was an inappropriate habitat for these fish.

One to four adult wrasses were placed in each pen, with or without a barrier to separate individuals (Table 1). Small fish were 66 to 100 mm, standard length, and large fish, 101 to 135 mm. In the experiment with three fish, the entire size