Behavioural Effects of Interference with the Postnatal Acquisition of Hippocampal Granule Cells

MULTIPLYING cells of the nervous system, unlike differentiating and mature cells, are extremely radiosensitive[1-3], which allows selective elimination of the postnatally forming basket, stellate and granule cells in the cerebellar cortex[4,5]. The proliferating precursors of these cells are killed by one or two doses of 150–200 r X-irradiation, but the cerebellum must be exposed to up to 8 repeated doses during early infancy to prevent regeneration[6,7]. Such schedules of low-level irradiation do not produce pathological changes in the prenatally-formed, differentiating Purkinje cells, as shown by electron microscopy[8]. As most granule cells of the dentate gyrus of the hippocampus originate postnatally in altricial species[9-12], irradiation of the infant rat hippocampus using a schedule derived from previous cerebellar autoradiographic and irradiation studies[6,7] should reduce the population of granule cells without directly harming the prenatally-formed pyramidal cells of Ammon's horn.

Purdue-Wistar male rat pups, cross fostered and raised eight to a litter, were used. To determine the location of the hippocampus, pups of two to eighteen days were decapitated, sagittal slices were made through the head 2 mm from the midline and distances from the snout to the anterior and posterior borders of the hippocampus were measured. A curve was constructed to serve as a guide for establishing hippocampal position as a function of age allowing a 2 mm error margin. Pups from twelve litters were wrapped in plastic tubes as described previously[13] and placed in a lead-shielded lucite block holder with a slit allowing only that portion of the head containing the hippocampus to be exposed to X-rays from a 'Maxitron' 300 kV unit. At the rate of 50 r min, 200 r was delivered on days 2 and 3, followed by 150 r on days 5, 7, 9, 11, 13 and 15. Pups from 14 control litters were wrapped in the same manner as the irradiated group, pups from 7 nonwrapped control litters were left undisturbed.

The 15 irradiated and 15 wrapped control animals on which we report quantitative histology were killed at 30, 60 and 90 days by cardiac perfusion with 10% buffered formalin, and the brains were postfixed in Bouin's solution. As there were behavioural differences between the wrapped and nonwrapped control groups, 5 nonwrapped control animals were included in the 90-day study. The brains were embedded in 'Paraplast'

and 6μm serial coronal sections cut through the entire hippocampal region. The total sagittal hippocampal length and the number of granule and pyramidal cells in matched anatomical sections were determined. Every 30th coronal section from the anterior to the posterior poles of the hippocampus was mounted and stained with haematoxylin and eosin. The sagittal length of the hippocampus was calculated from the number of sections which included the stratum pyramidale of Ammon's horn. Dorsal hippocampal dentate granule cells were counted in that section where the stratum pyramidale of dorsal and ventral Ammon's horn intersect. The Ammonic pyramidal cells were counted in the section which first contained a definite dentate crest.

The total length of the hippocampus (Fig. 1a) increased as a function of age in both wrapped control and irradiated animals ($P < 0.01$; Newman-Keuls Sequential Range Test). The length of the hippocampus in the irradiated group, however, was below control levels at all ages ($P < 0.01$). There was no significant effect of age in the number of granule cells in either group (Fig. 1b); in the irradiated group this was 17% of control levels at 30 days, 15% at 60 days and 18% at 90 days. The missing cells may represent the entire population of postnatally formed granule cells[9-11]. The number of pyramidal cells in matched sections decreased as a function of age in all groups (30 to 60 days, $P < 0.01$; 60 to 90 days, $P < 0.05$). This may not represent a true reduction in the cell population, but could be attributed to the volumetric expansion of the hippocampus from 30 to 90 days (Fig. 1a). There was no significant difference in the numbers of prenatally-formed pyramidal cells between the groups at any age, in agreement with earlier findings of no reduction in the prenatally-formed Purkinje cells[14], after a similar schedule of cerebellar cortex irradiation.

The drastic reduction in the number of granule cells in the dentate gyrus is shown in Fig. 2. Preliminary examination of Golgi-impregnated sections showed no distinct morphological differences in granule and pyramidal cells. The overlying cortex and underlying thalamus and hypothalamus appeared normal in irradiated animals, and no damage is expected in the prenatally-formed septum situated anteriorly. Subtle changes may, of course, be detected in the future in all of these regions.

Four behavioural situations, which have repeatedly been found to be sensitive to surgical destruction of the hippocampus, were selected. Rats with hippocampal lesions display a high level of locomotor activity during exploration of an open field or maze[15-17]. Starting at 40 days of age, groups of 15 irradiated, wrapped and nonwrapped rats were exposed for 300 s to a brightly lit open field on five consecutive days. The number of squares traversed, rearing responses and faecal

Fig. 1 a, Sagittal length (μm) measured in a series of coronal sections irradiated (X), wrapped (black) and nonwrapped (white) groups ($n=5$ for each group). b, Dentate granule cells at the level where the stratum pyramidale of the dorsal and ventral Ammon's horn are joining. c, Ammon's horn pyramidal cells at the most anterior level containing a dentate crest.
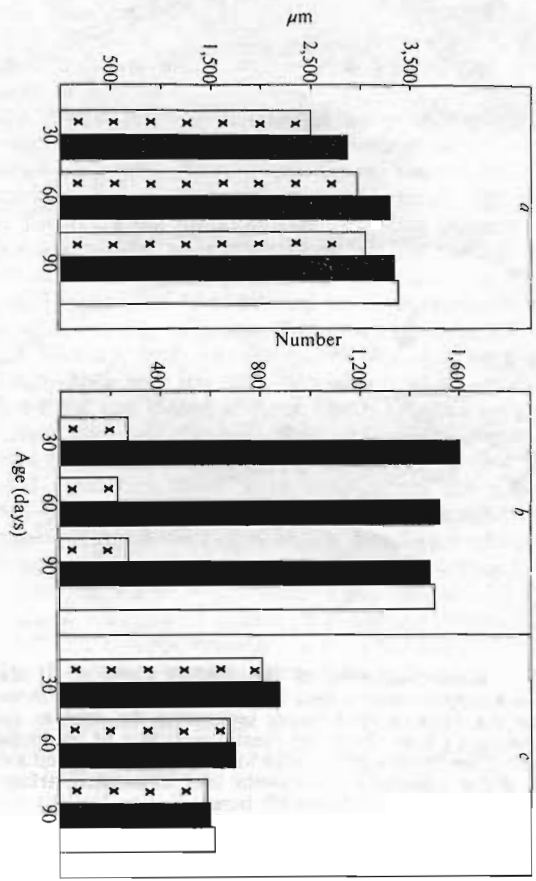
boluses were recorded. The irradiated group, had a higher activity level than the control groups, which is consistent with known effects of hippocampal lesions (Figs. 3a and 3b). An analysis of variance showed that the enhanced activity was reflected both in squares traversed ($P < 0.001$) and in rearing
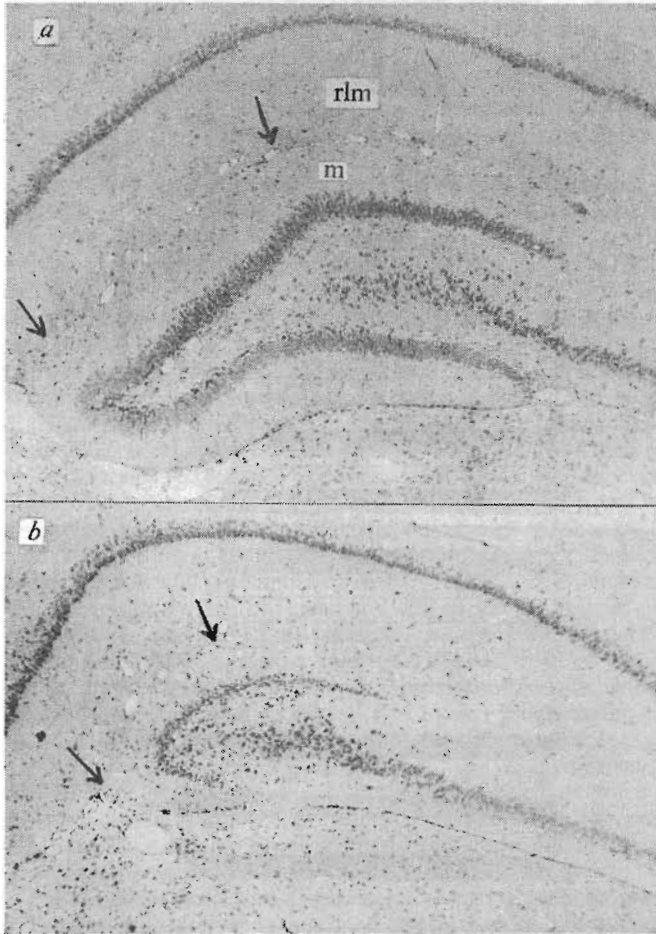

Fig. 2 Photomicrographs of the dentate gyrus at 21 days, (a) in a wrapped control rat; (b) in an irradiated rat. Arrows indicate the hippocampal fissure separating the dentate gyrus from Ammon's horn. Note the drastic reduction of the molecular layer of the dentate gyrus (m) and the much smaller reduction in the strata radiatum, lacunosum and moleculare (rlm) of Ammon's horn.

responses ($P < 0.001$). In addition, irradiated rats had a surprisingly low defaecation rate (irradiated group $=0.08$ boluses/rat/day; wrapped group $=1.5$; nonwrapped $=1.9$; $P < 0.001$). All trials and interaction effects were not significant; the somewhat higher activity and lower defaecation level of wrapped as compared to nonwrapped controls may reflect an effect of "handling" during infancy[18]. No histological differences were detected between wrapped and nonwrapped controls in any of the parameters studied (Fig. 1a, b, c).

Spontaneous alternation behaviour refers to the tendency to enter opposite goal arms during two successive non-rewarded explorations of a T maze with a probability of between 0.7 and 0.9. Rats with hippocampal lesions repeat or alternate previous goal arm entries on a chance basis[19-21]. Sixty to seventy day old irradiated ($n = 23$), wrapped ($n = 24$) and nonwrapped ($n = 11$) rats were tested for spontaneous alternation tendency in an unpainted wooden T maze (4 in. wide; 18 in. long; 6 in. deep). Control rats displayed a significant alternation tendency (wrapped $\chi^2 = 13.5$, $P < 0.001$; nonwrapped $\chi^2 = 9.2$, $P < 0.01$) whereas irradiated rats alternated at a chance level ($\chi^2 = 0.09$) as shown in Fig. 3c.

In the third experiment 70-day-old rats of each group ($n = 18$) were tested for the acquisition and retention of a passive avoidance response. Passive avoidance, the withholding of responses that lead to punishment, is severely disturbed by lesions involving the hippocampus[22-27]. Rats reduced to 85% *ad lib* body weight were trained to run for wet mash in a straight alley (6 in. wide; 25 in. long; 16 in. deep) for five trials (inter-trial interval of 60 s) per day on three consecutive days. Latencies to eating were recorded and rats were allowed to feed for 10 s per trial. On the fourth day, rats were given a single non-shock trial followed by trials on which each contact with the food cup closed a shock circuit (3 mA) between the metal food cup and the grid floor. After receiving a shock, rats were left in the alley for 10 s during which time the cup remained electrified. Rats were replaced in the alley after intervals of 240 s until the food cup was avoided for 120 s. Groups did not differ before shock. Irradiated rats required an average of 1.7 trials to reach the 120 s avoidance criterion during training; both control groups had means of 1.1 trials. These scores were highly reliable and the differences between irradiated and control animals were significant ($P < 0.005$, *t*-test). Fig. 3d shows average approach latencies to the food cup for the single non-shock (0 h) trial prior to the avoidance training phase and for the 24 h post-shock retention test. In the retention test, control groups had significantly longer approach latencies than the irradiated group ($P < 0.001$: Mann-Whitney U test) and did not differ from each other.

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Fig. 3 *a*, Squares traversed in an open field. Average during 5 daily tests of 600 s each ($n=15$). *b*, Average rearing responses in open field during 5 successive daily tests of 600 s each ($n=15$). *c*, Average spontaneous alternation performance in irradiated ($n=23$), wrapped control ($n=24$), and nonwrapped control ($n=11$) rats. *d*, Average food cup approach latencies before shock and 24 h after passive avoidance training. *e*, Percentage of conditioned avoidance responses in a two-way active avoidance (shuttle) task during a single block of 100 trials. X, Irradiated; ■, wrapped control; □, ○, nonwrapped control.
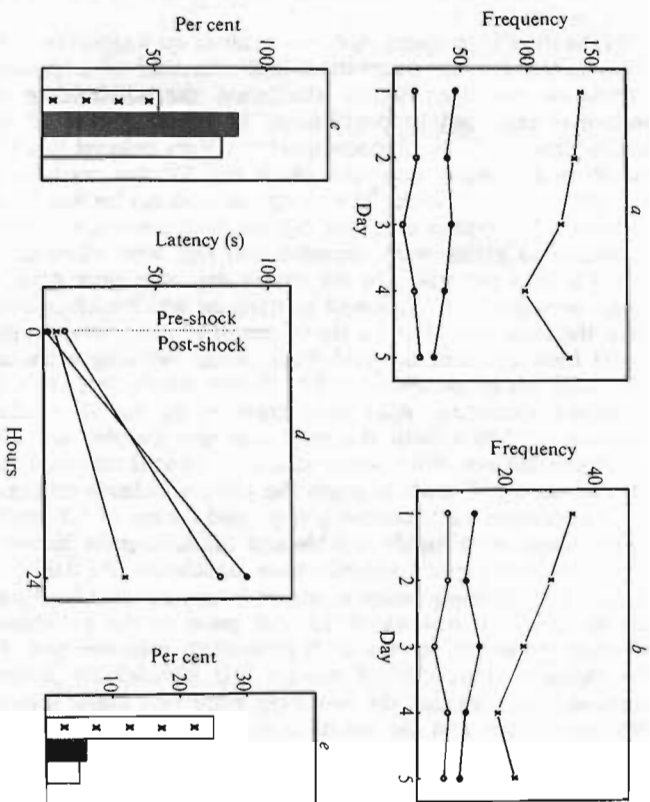
It has been reported that hippocampal ablated rats are superior to controls in acquiring a two-way (shuttle) conditioned avoidance response[28-31]. Finally, we tested irradiated and control rats in a fully automated two-way avoidance apparatus[32]. A motor driven door and house light served as the conditioned stimulus (CS); 5 s following the CS a 2.5 mA scrambled footshock was delivered through the floor and walls until the rat escaped to the other compartment. Five rats of each group were given a single block of 100 trials with an inter-trial interval of 30 s. The irradiated rats made more avoidances (Fig. 3e) and because of no overlap between the control and irradiated rats no statistical test was performed.

Our data show that the selective prevention of the acquisition of dentate granule cells in the hippocampus by irradiating infant rats results in behavioural changes closely resembling those observed following surgical destruction of the hippocampus in adults. They implicate an important role for the granule cells in hippocampal function and point to the lasting deficit produced by early retardation of hippocampal development.

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