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BRIEF COMMUNICATION

A Method for X-Irradiating Selected Brain Regions in Infant Rats[1]

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BAYER, S. A. AND P. J. PETERS. *A method for X-irradiating selected brain regions in infant rats.* BRAIN RES. BULL. 2(2) 153–156, 1977. – A technique for immobilizing rat pups and irradiating selected regions of the head is described. As an example, measurements are given for the focal irradiation of the hippocampus. If animals of nearly the same weight are selected, several pups of the same age can be irradiated simultaneously.

Rats Rat pups Developing rat brain Focal X-irradiation Hippocampus

REPEATED low-level X-irradiation (150-200R) of various regions in the rat brain kills proliferating and migrating cells without harming differentiated cells. Since germinal matrices can recover after partial destruction, repeated exposures are necessary to prevent this. If a particular brain region is exposed to X-rays at a time when either neurons or glia are forming, these cells can be selectively eliminated. Early postnatal irradiation with repeated exposures has been shown to significantly reduce granule cells in the olfactory bulb, hippocampus and cerebellum [5]. This technique has been used successfully to study behavioral [1,3], anatomical [2, 4, 5], neurophysiological [8] and biochemical [6,7] changes which occur after postnatal X-irradiation of the cerebellum and the hippocampus.

Altman *et al.* [2] described a technique to immobilize pups during irradiation exposure. During the past three to four years, an improved procedure for immobilization was developed and is now used exclusively. The method is relatively simple, gives consistent results and is non-traumatic to the pups. This description of the procedure should enable other laboratories to prepare animals and study these preparations. As an example, we give details on how the hippocampus is irradiated; other brain structures can be exposed in a similar manner.

Method

Animal ages and head measurements. Long-Evans or Purdue-Wistar rats are culled to a litter size of 6 on Day 1 of life (the day of birth is Day 0). Constant weight is very important to minimize variability in the location of specific brain structures. In our laboratory, only animals weighing between 6.4 and 8 g on Day 1 and between 7 and 9 g on Day 2 are selected for irradiation. By using pups of the same size, we can irradiate several animals at once using the same measurements for the location of a chosen brain structure.

In order to establish the location of a brain structure, several pups for each of the ages at which we plan to irradiate are killed by an overdose of ether. Sagittal slices of the head are made with a single-edge razor blade about 1–2 mm lateral to the midline. The distance from the tip of the snout to both the anterior and posterior measurements of the structure is recorded. Usually, the variability between animals of the same age and nearly the same weight is within 0.5 mm. Then a chart is constructed listing the location of the structure at each age in terms of its anterior and posterior distance from the snout and its width: 0.5 mm is added to both anterior and posterior ends to allow for animal variability (Table 1). To be sure that the measurements are correct, it is necessary to check each one on another group of animals and look for cells killed by irradiation in that structure. Using the immobilization and shielding procedures to be described below, animals are given one exposure to 200R on the day the measurements

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TABLE 1

HIPPOCAMPAL MEASUREMENTS*

Day	Width (mm)	Ant. Meas. (mm)	Post. Meas. (mm)
2	4.0	10.5	14.5
3	4.5	11.0	15.5
5	4.5	13.5	18.0
7	5.0	15.0	20.0
9	5.0	16.5	21.5
11	5.0	17.5	23.0
13	5.5	19.5	25.0
15	6.0	21.0	27.0
17	6.5	22.0	28.5
19	7.5	22.5	30.0
21	7.5	22.5	30.0

*These measurements are based on Purdue-Wistar rat pups.

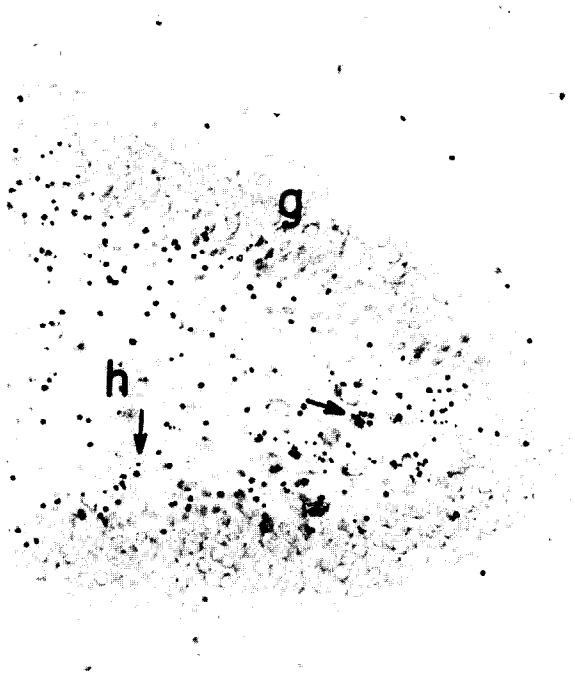

FIG. 1. The dentate gyrus in an 11 day old rat brain 6 hr after a single exposure to 200 R. Most of the pyknotic debris (arrows) is scattered among the undifferentiated cells in the hilus (h) rather than in the zone of differentiated granule cells (g). Cresyl violet, 192X.

are to be checked and are killed six hr later. (Phagocytosis will remove much debris after 6 hr.) The track of the beam of X-rays can be traced through the brain by looking for small round particles of cellular debris (pyknotic cells) remaining after irradiation death (Fig. 1). If the pyknotic material extends beyond or is not located throughout the structure, the measurements are revised accordingly and checked again.

Holding animals immobile during exposure. The animal holders are made of lucite blocks (10 cm long x 4 cm wide x 5 cm high; Fig. 2A). The rat pup is grasped firmly by the loose skin at the nape of the neck with the right hand and the head is eased between the sponge-lined securing plates. With the left hand, these plates are pushed snugly against the head and tightened with the locking screws. A gauze-lined lid is placed over the body and is secured with the elastic band. For pups up to seven days of age, this is sufficient. Older animals are more active during the exposure and must be fastened more securely with masking tape. A narrow strip is placed across the head between the eyes and nose to prevent forward movement; to prevent backward movement, the lid is pushed forward to rest firmly against the back of the head and is then fastened to the base with a wide strip of tape. Pups from 3 hr to 21 days of age have been successfully placed in these holders. For younger animals a thicker sponge pillow is used and more gauze lines the lid. Pups from 13 days of age on should have a thinner sponge pillow (the level of the sponge should be about ¼in. below the lucite surround) and no gauze lines the lid.

We consistently have good results with these holders. The animals can breathe freely while the head is kept immobile; anesthesia is not necessary. The few animals that have died in the holders have the front feet caught up under the throat on the inclined head support. When the animals are placed into the holders, care should be taken to put the front legs either on the outside of the metal plates around the head (young pups) or down in front of the sponge pillow (older pups).

Lead Shielding. The animal holders are now placed under lead shielding so that only the area of the head that is to be irradiated will be exposed. The shielding block (Fig. 2C) has a flat lucite base (60.8 cm x 40.4 cm; not shown in Fig. 2C) to which is mounted two vertical pieces of heavy lucite ("o" in Fig. 2C, 22.8 cm x 6.8 cm x 2 cm) which support the lead strips. There are two wide stationary lead strips ("l" in Fig. 2C, 30.3 cm x 5.3 cm x 1.6cm) each across from a narrow movable lead strip ("m" in Fig. 2C, 30.3 cm x 2.6 cm x 1.6 cm). The animal holders are first positioned in lucite trays (Fig. 2B) so that the tip of the snout is directly under the two threads (to eliminate parallax) strung tightly across the front of the tray ("i" in Fig. 2B). The tray is now slid under the lead shield which also accommodates another tray facing it. Six to eight animals can be irradiated at once. Using the ruler on each side of the tray, the tray is positioned so that the posterior measurement of the brain structure to be irradiated is at the anterior edge of the stationary strip. The locking screws are loosened on the movable strip, and it is positioned so that the posterior edge rests on the anterior measurment of the brain structure.

X-ray levels and dosimetry. A general Electric Maxitron 300 unit is used at the 300 kVp level which delivers approximately 46 R/min with the added filtration of 1.5 mm copper. The half-valve layer (HVL), determined with a Capintec exposure rate meter (Model 192) with a Baldwin-Farmer chamber probe, is 2.4 mm copper. The homogeneity coefficient (h) between the first and second half-value layers is 0.65. For dosimetry, a Victoreen probe, calibrated for 250 R (Model No. 154) is placed in an animal holder and positioned on a tray beneath the sheilding block. The slit is set wide enough to expose the entire sensitive tip of the probe. The probe is then exposed to the

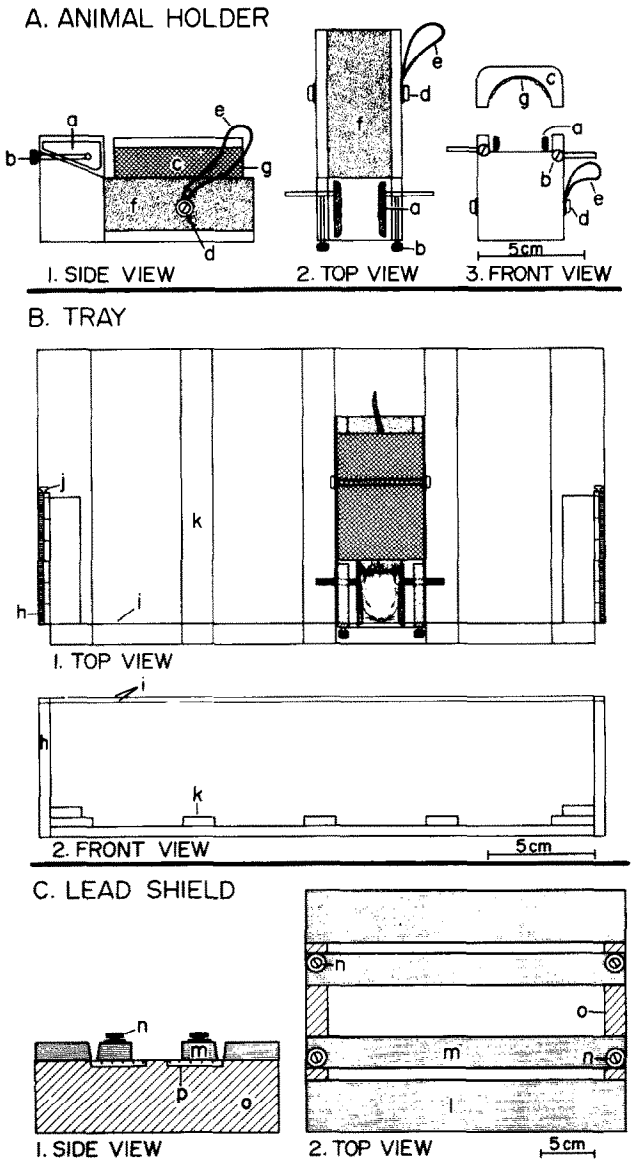

FIG. 2. The immobilization apparatus and lead shield for focal X-irradiation of narrow regions of the rat head. A. Animal holder used to immobilize pups during exposure: a, sponge-lined head securing plates; b, locking screw; c, lid; d, anchor screw for the elastic band, e; f, sponge pillow in lucite holder; g, gauze lining for lid. In these holders, rat pups can breathe freely while the head is kept immobile. B. Tray which will hold up to four animal holders: h, lucite uprights, upon which is mounted a metric ruler; i, double thread (surgical silk) stretched tightly across the front of the tray and secured at the screw, j; k, spacer bar between holders. The top view (1) shows a pup in an animal holder properly positioned under the double thread. The metric ruler across the top of each upright (h) can now be used to accurately locate the anterior and posterior borders (measuring from the tip of the snout) of a chosen brain structure. C. Lead shield which will accommodate two trays facing each other. 1, stationary lead strip; m, movable lead strip. n, locking screw for movable lead strip. o, heavy lucite uprights which support both sets of lead strips. Not shown, base of lucite upon which the shielding block is mounted (60.8 cm X 40.4 cm). The trays, containing animal holders properly positioned, are slid under the lead strips. The anterior and posterior measurements of the irradiated area (slit between each set of lead strips) can be set up using the rulers on each side of the tray.

FIG. 3. Dorsal hippocampus of a normal 2 month old Wistar rat. Hematoxylin and eosin, 20X.

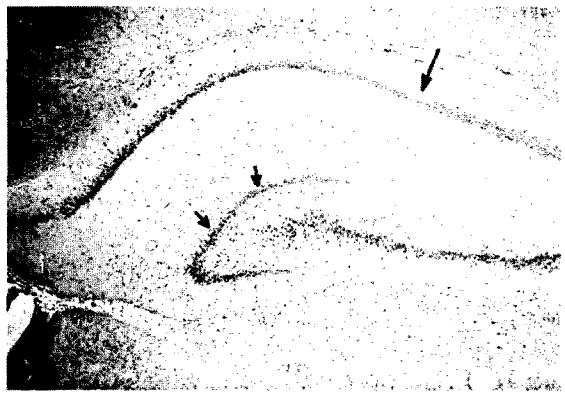

FIG. 4. Dorsal hippocampus of a 2 month old Wistar rat after focal irradiation of the brain in the region of the hippocampus on Days 2, 3 (200 R), 5, 7, 9, 11, 13, and 15 (150 R). The measurements listed in Table 1 were used. Note the selective reduction in the dentate granular layer (small arrows) and the apparent lack of reduction in the Ammon's Horn pyramidal cell layer (large arrow). Hematoxylin and eosin, 20X.

beam for varying lengths of time, and the Roentgens delivered are read in a Victoreen R-meter 570 (Serial No. 350). The time of the exposure is adjusted to deliver exactly either 150 R or 200 R, depending on the exposure scheduled for that day. The X-ray beam at this energy level and filtration is sufficently hard to penetrate the rat head completely; dosimeter readings taken beneath a rat head drop only by 1–2R. It is advisable to use two successive daily doses (200R) when irradiation is started to insure nearly total eradication of the germinal matrices. Thereafter, doses (150R) on alternate days is sufficient to retard recovery. Since the formation time of neurons and gila are regulated, many interesting results can be obtained when various schedules are used. Starting on different days will influence which cell types are reduced; stopping after one or two exposures can give information on the dynamics of recovery [5].

An example of irradiation effects. Figure 3 shows the dorsal hippocampus in a normal animal and Fig. 4 shows the same in an animal irradiated eight times between days 2–15 according to the measurements given in Table 1. The dentate granular layer is selectively reduced. The irradiated animals display behavioral deficits similar to that observed after surgical removal of the hippocampus [3].

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