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Rat Brain Binds Adrenal Steroid Hormone: Radioautography of Hippocampus with Corticosterone

Abstract. *Tritiated corticosterone injected subcutaneously into adrenalectomized male rats 1 hour before killing produced intense labeling of the hippocampus in radioautograms prepared by a method that reduced or prevented diffusion of the radioactive material. The pyramidal neurons of the cornu ammonis and the granule neurons of the gyrus dentatus contained more radioactivity than did other regions of the brain; however, the intensity of labeling varied among adjacent neurons. The nuclei of many neurons were clearly labeled but radioactivity was relatively sparse in the cytoplasm, in the axons where they branch from cell bodies, and in adjacent neuropil.*

Corticosterone, the principal adrenal glucocorticoid in the albino rat (*1*), enters the brain of this species from the blood and attaches to highly specific, limited-capacity binding sites (*2*). Biochemical studies of tissue obtained by gross brain dissection showed that the hippocampus contains the highest concentration of these binding sites (*3*). By subcellular fractionation of brain tissue, it was shown that substantial binding occurs in cell nuclei isolated from the hippocampus and other brain regions (*2*, *4*).

In this report we present evidence from a radioautographic study (*5*) that demonstrates binding of radioactive corticosterone in the hippocampus. We have confirmed and extended observations of sensitivity of the hippocampus to adrenocorticoids—observations made by the use of biochemical techniques (*2–4*), electrical recording of single-unit neuronal activity (*6*), and corticoid implantation (*7*). A radioautographic method that was designed to reduce or prevent artifacts from the translocation of labeled substances (*8*) permitted us to determine the distribution of radioactivity in the hippocampus with a high degree of spatial resolution (*9*). The resolution was adequate to detect a heterogeneity of labeling among adjacent cells of the hippocampus, to identify the labeled cells as neurons, and to observe heavy labeling of cell nuclei.

We employed a modification of the radioautographic technique described by Anderson and Greenwald (*8*), and analyzed radioautograms from four brains. To eliminate competition for binding sites in the brain by endogenous corticosterone released during the stress of injecting [^{3}H]corticosterone, we removed the adrenal glands of male rats of the Sprague-Dawley strain. The rats were given free access to standard rat chow and a solution of 0.8 percent sodium chloride. Between days 9 and 23 after adrenalectomy, each animal received a single subcutaneous injection of 200 to 300 μc of [1,2-^{3}H]corticosterone (*10*) dissolved in 0.1 ml of ethanol. The rats were decapitated 1 hour after the injections, the time at which binding reaches its peak in cell nuclei isolated from the hippocampus (*2*).

The brains were quickly removed and divided longitudinally at the midline, and the left hemisphere was used for sagittal sectioning and the right hemisphere for coronal sectioning. Each piece of brain was mounted onto a sandwich consisting of a thin copper disk between two wafers of fine stainless steel mesh. The mounted tissue was rapidly frozen by partial submersion in liquid nitrogen. Tissues were stored in a liquid nitrogen refrigerator until they were sectioned. Frozen sections 4 μm thick and spaced 100 μm apart were cut in a cryostat at −20°C. In darkness, individual sections were picked up from the cold knife by barely touching them with warmer glass slides, which had been coated with Kodak NTB-3 liquid emulsion (*11*) and desiccated before use. The radioautograms were exposed for 180 to 320 days at 4°C in sealed, double-depth, black plastic slide boxes (*12*) that were half-filled with Drierite and stored in a large box lined with lead. To control for artifacts due to negative chemography, some radioautograms from each brain were thoroughly air-dried and exposed uniformly to light before storage. The brain from an uninjected rat was also processed to control for artifacts due to positive chemography (*13*).

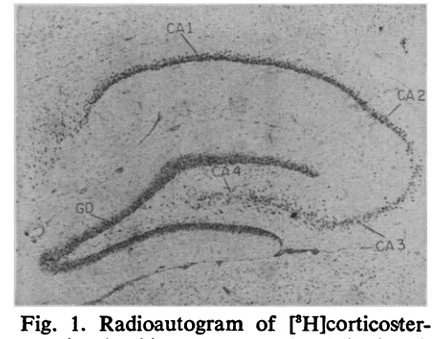

Fig. 1. Radioautogram of [^{3}H]corticosterone in the hippocampus of rat brain. A sagittal frozen section was exposed 180 days and stained lightly with Darrow red and light green. Shown are the longitudinal fields *CA1* to *CA4* of the pyramidal neuron layer in the cornu ammonis, and the granule neuron layer in the gyrus dentatus (*GD*) (×17).

After radioautographic exposure, the slides were warmed to room temperature and breathed on gently several times to insure their subsequent adherence to the emulsion. The radioautograms were then processed at 18°C in Kodak Dektol developer for 2 minutes, in a stop bath with Kodak Liquid Hardener for 3 minutes (*11*), in a tap water rinse for 30 minutes, in Carnoy's solution (a histological fixer) for 5 minutes, and in a second water rinse for 5 minutes. The wet sections were next stained with either Darrow red and light green (*14*), methyl green–pyronin Y, or cresyl violet acetate. When dry they were cleared overnight in terpineol-xylene (1 : 3 by volume) (*15*), rinsed in pure xylene, and then mounted with Permount.

The observations presented here focus on the hippocampus, a sagittal section of which is presented at low magnification in Fig. 1. Radioautograms of brains from each rat injected with [^{3}H]corticosterone showed dramatically that the hippocampus was the most heavily labeled structure and that most neurons throughout the hippocampus incorporated relatively high amounts of radio-

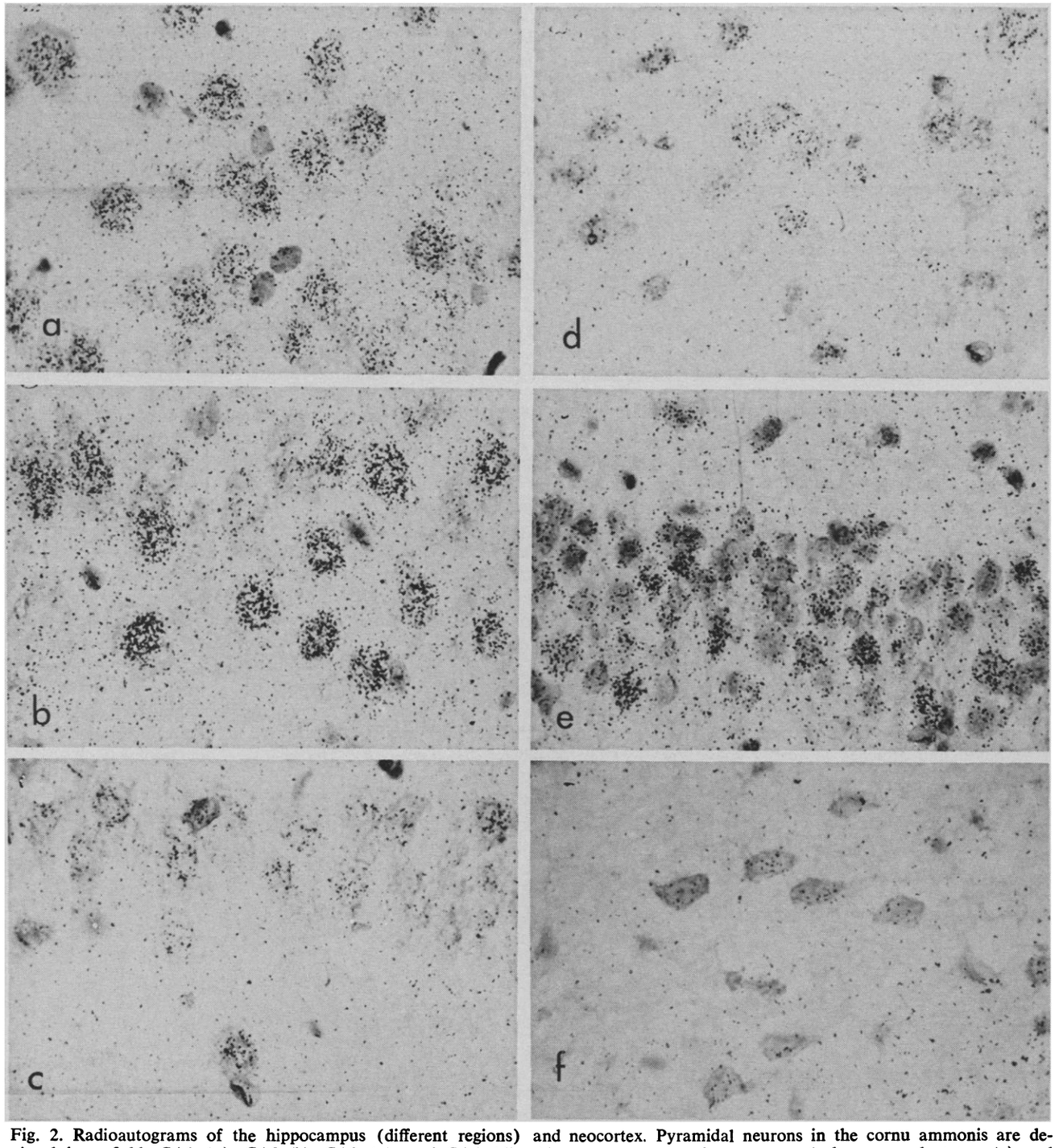

Fig. 2. Radioautograms of the hippocampus (different regions) and neocortex. Pyramidal neurons in the cornu ammonis are depicted from fields CA1 (a), CA2 (b), CA3 (c), and CA4 (d). Also seen are granule neurons of the gyrus dentatus (e) and neocortical neurons (f). The average intensity of labeling in the cornu ammonis is greater in fields CA1 and 2 than in fields CA3 and 4. Neocortical neurons are not labeled as heavily as hippocampal neurons. In each region the extent of labeling varies among adjacent neurons (×520).

activity. At higher magnification this labeling of the hippocampus is very marked (Fig. 2, a–e) and distinct from that of other regions, such as the neocortex (Fig. 2f). At low magnification (Fig. 1), individual silver grains cannot be seen, but the contrast between the appearance of pyramidal neurons of the cornu ammonis and granule neurons of the gyrus dentatus (*16*) and that of surrounding neuroglia and neuropil is due partly to the intensity of labeling in the pyramidal and granule neurons. This is particularly true of a cluster of cells in region CA2 (*17, 18*).

The ability of neurons in field CA2 to bind especially high concentrations of the hormone is clearly illustrated in Fig. 2b. Generally, the intensity of labeling of individual CA2 neurons in such a cluster was as great as or greater than that in the rest of the cornu ammonis or the gyrus dentatus (compare Fig. 2b with Fig. 2, a and c–e). Because these heavily labeled neurons are in a group, this part of field CA2 stands out visually from other labeled regions. In other parts of the pyramidal layer of the cornu ammonis (Fig. 2, a, c, and d), as well as in the granule layer of the gyrus dentatus (Fig. 2e), heavily labeled neurons are adjacent to less heavily labeled neurons. This heterogeneity of labeling among cells may indicate that not all neurons of each structure can concentrate corticosterone or, if they can, that not all respond at the same time. The average intensity of labeling in CA1 and CA2 was greater than in CA3 and CA4.

In many labeled neurons the location of the silver grains indicates that cell nuclei concentrated the radioactive material. This nuclear binding is particularly clear in the pyramidal neurons in Fig. 3a. Relatively few silver grains appeared beneath the cytoplasm of cell bodies or the axons where they branch from cell bodies, or beneath adjacent neuropil. The heavily labeled cells of the hippocampus are in the pyramidal layer of the cornu ammonis and the granule layer of the gyrus dentatus, which contain neurons exclusively. Nuclear labeling is especially striking, since the cytoplasm generally produced fewer grains than did the surrounding neuropil. Neuronal nuclei in the neocortex also concentrated some radioactivity (Fig. 3b), but less than did neurons in the hippocampus. Our observations verify results from cell fractionation experiments that showed less

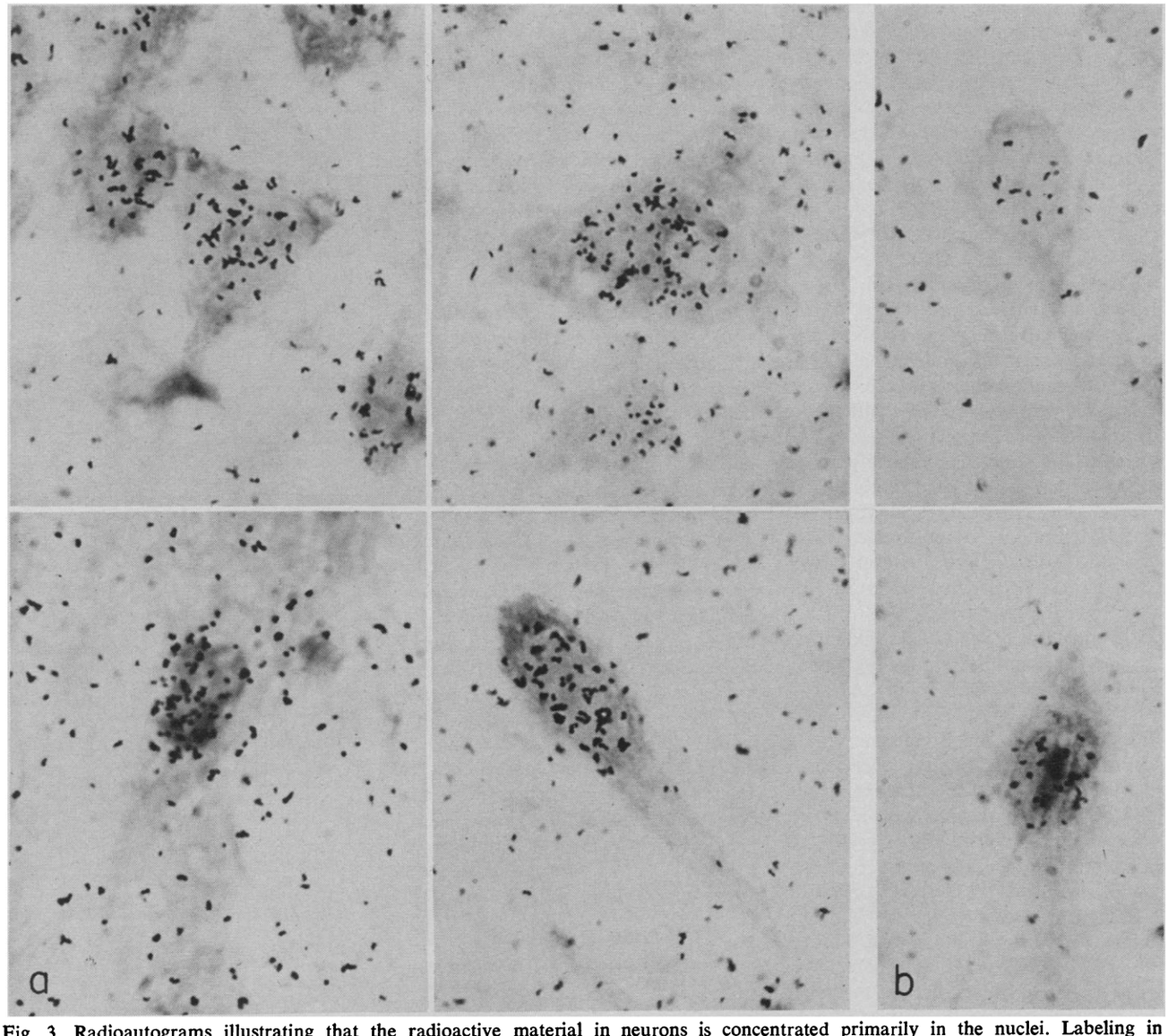

Fig. 3. Radioautograms illustrating that the radioactive material in neurons is concentrated primarily in the nuclei. Labeling in pyramidal cell nuclei (a) is more intense than in neocortical cell nuclei (b) (×1975).

binding of [^{3}H]corticosterone to nuclei from the neocortex than to those from the hippocampus (*2*).

We attribute the reduction of silver grains by radioactivity in the hippocampus to unmetabolized [^{3}H]corticosterone, since previous experiments showed that at 1 hour after the injection of [^{3}H]corticosterone, unchanged hormone accounts for 70 to 80 percent of radioactivity in the hippocampus (*2, 3*) and more than 90 percent of radioactivity in cell nuclei in the hippocampus (*2*).

The sharpness of nuclear boundaries, caused by the absence of gradients of labeling (Fig. 3a), indicates that the technique prevented translocation of the injected hormone to any degree significant within the resolution of light microscopy. However, the mobility of radioactive material may vary among different neural compartments, depending, for example, on the affinities for binding within compartments. Similarly, Anderson and Greenwald (*8*) found this technique satisfactory for localizing the uptake of [^{3}H]estradiol in brains of rats and produced results comparable to those of Stumpf, who used a dry-mount method designed to eliminate all artifacts of diffusion (*19*).

The primary differentiation of afferent and efferent projections of the hippocampus occurs among the longitudinally organized architectonic fields of the cornu ammonis (CA1 to 4) and the gyrus dentatus (*16, 17*). Differences in the efferent projections exist also between the dorsal and ventral portions of individual fields, particularly within field CA1 (*18*). Although slightly greater concentrations of corticosterone in the dorsal than in the ventral hippocampus had been detected earlier (*2*), our radioautograms show the primary differentiation of corticoid binding to be varying intensities of labeling among cells within individual fields CA1 to 4 and within the gyrus dentatus. In addition, fields CA1 and 2 contained more neurons that were intensely labeled than did fields CA3 and 4. Because the degree of labeling in all of these fields was high compared to that in other regions of the brain, the significance of the greater uptake by particular clusters of neurons within field CA2 remains to be determined. However, among the limited observations of corticosterone's influence on neural events that are mediated by the hippocampus is that of Kawakami *et al.* (*20*), who found that corticosterone implanted into the dorsal CA2 field of the hippocampus of rabbits potentiated the secretion of adrenal corticosterone. This observation is consistent with other reports that whereas the hippocampus appears to tonically inhibit the secretion of adrenocorticotropic hormone (ACTH) (*21*), implants of corticosteroids in the hippocampus counteract this inhibition and potentiate the secretion of ACTH under both basal and stressful conditions (*7*). Pfaff *et al.* (*6*) recorded electrophysiological correlates of corticosteroid influence in the hippocampus. In rats injected systematically with corticosterone, they found decreased unit activity in the pyramidal layer of the cornu ammonis and the granule layer of the gyrus dentatus of the dorsal hippocampus. This response occurred 30 minutes after injecting the hormone and persisted more than 3 hours, a latency and duration consistent with the possibility that the hormonal action depends upon intracellular binding and activation of cellular metabolism (*4*).

This is the first radioautographic study of rat brain which demonstrates the localization of corticosterone, the adrenal glucocorticoid that is characteristic of rats. The capacity of neuronal nuclei in the hippocampus to concentrate high quantities of [^{3}H]corticosterone contrasts with the relative inability of the same cells to concentrate [^{3}H]estradiol; however, cell nuclei of the preoptic region and hypothalamus have a strong capacity to concentrate [^{3}H]estradiol (*19, 22*). When present in these last two regions of the brains of female rats, estradiol facilitates sexual behavior and regulates the secretion of gonadotrophin (*23*). This preferential accumulation of steroid hormones by neuronal nuclei in the brain suggests that, as in nonneuronal endocrine target tissues such as uterus, prostate, kidney, thymus, and liver (*24, 25*), these hormones may influence genomic activity (*4*). The regional differentiation of hormone-binding capacity of cell nuclei in the brain opens new possibilities for making neurochemical correlations with neural and behavioral events.

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