Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9327–9330, December 1988
Neurobiology

Suppression and induction of epileptic activity by neuronal grafts

(hippocampal graft/locus coeruleus graft/interictal spike/behavioral seizure)

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Communicated by Theodore H. Bullock, August 12, 1988 (received for review July 13, 1988)

ABSTRACT **Fetal rat brain cell suspensions prepared from either the locus coeruleus region or hippocampus were implanted bilaterally into the subcortically denervated seizure-prone hippocampus of adult rats. Animals with locus coeruleus grafts were protected against picrotoxin-induced behavioral seizures and had significantly fewer interictal spikes. In contrast, in rats with fetal hippocampal grafts the incidence of interictal spikes was significantly higher than in lesion-only controls, and spontaneous behavioral seizures occurred in almost half of the animals. We suggest that neuronal grafting offers an alternative method for studying the mechanisms and control of epileptic brain activity.**

The two hallmarks of the human epileptic syndrome, electroencephalogram spikes and clinical convulsions, have been most often studied in experimental animal models (1–3). Transient hypersynchronous activation of a large population of neurons in one focal area, termed interictal spike, is a reliable diagnostic indicator of the epileptic disorder (4, 5). In temporal lobe epilepsies the interictal spikes are thought to represent simultaneous depolarization of a large number of pyramidal cells of the hippocampal formation (6–10). These "mass" depolarizations may be triggered by reduced inhibition and/or increased local excitation (1, 11–16). Following surgical removal of the subcortical inhibitory inputs to the hippocampus the electrical stability of the structure is dramatically reduced, resulting in chronic interictal spikes and an increased susceptibility to seizures (17).

In this study we asked whether partial replacement of the lost subcortical inhibition, by means of neuronal transplantation directly to the denervated hippocampus, may ameliorate the epileptic manifestations. We have chosen noradrenergic cells for grafting because previous studies have established that (*i*) noradrenaline exerts an inhibitory action on hippocampal pyramidal neurons and granule cells (18, 19), (*ii*) the ascending noradrenergic system, originating in the locus coeruleus, may have a seizure-suppressant action in other epilepsy models (20–23), (*iii*) intrahippocampal grafts of fetal locus coeruleus neurons have been shown to establish anatomical and physiologically active connections with the host cells (24, 25), and (*iv*) cografting of fetal locus coeruleus and hippocampus in the anterior chamber of the eye increased the threshold of stimulation-induced epileptic discharges (26, 27). Fetal hippocampal cells were chosen as control grafts to avoid the introduction of transmitters not present in the subcortically denervated hippocampus.

METHODS

Subcortical deafferentation and neuronal grafting were conducted in two separate surgical sessions. The lesion was made by aspirating the medial portion of the parietal cortex and cingulate cortex, the cingulate bundle, the supracallosal stria, the corpus callosum, the dorsal fornix, the fimbria, and the ventral hippocampal commissure. The lesion removed the cholinergic and GABAergic (GABA, γ-aminobutyric acid) afferents from the septal area (28, 29), noradrenergic fibers from the locus coeruleus (30), serotoninergic afferents from the median raphe (31), several minor pathways from >20 other subcortical nuclei (32), the commissural pathways, as well as the subcortical efferent projection of the hippocampal formation. One week to 10 days later subgroups of rats were grafted with locus coeruleus (LC; $n = 6$) and hippocampal (HPC; $n = 10$) cell suspensions obtained from 14- and 15-day-old rat fetuses, respectively. The locus coeruleus area was dissected (≈1 mm^3) with microscissors from above the pontine flexure bilaterally, as described in ref. 25. The hippocampus was dissected in one piece. The tissue from 8–12 fetuses was collected in 0.6% ice-cold glucose/saline. The cells from the locus coeruleus regions were dissociated by repeated pipetting. Hippocampal cells were first incubated in 0.1% trypsin (Sigma type IX) for 30 min at 37°C, washed, and then dissociated. The dilution was one dissected tissue piece per 10 μl. Two 3-μl suspensions were injected into the hippocampus bilaterally using the following coordinates: (*i*) 3.0 mm posterior to bregma, 2.0 mm lateral to midline, and 3.0 mm ventral to dura; (*ii*) anterior–posterior = −5.5, lateral = 3.5, and ventral = 3.0 mm (bregma and lambda in the horizontal plane). Fourteen lesion-only [fimbria-fornix cut (FF)] and 12 intact rats served as nongrafted controls.

Five months after grafting the rats were injected with the GABA antagonist drug picrotoxin (1 mg/kg intraperitoneally) and the latency to the first epileptic behavioral symptoms (myoclonus of the forelimbs or rearing and trunk clonus) was measured (12). Cut-off time was 60 min. One week after the picrotoxin-induced seizure the rats were equipped with recording electrodes in the hippocampus (CA1 region and hilus) and stimulating electrodes in the angular bundle to activate the perforant path input to the hippocampus. Interictal spikes were detected by a waveform discriminator software program. Transients five times larger than the background electroencephalogram were counted on the polygraph charts during immobility periods. A magnet and coil type stabilimeter, fixed to the recording chamber, helped monitor behavioral immobility. Since interictal spikes occurred asynchronously in the left and right hippocampi, counts in the two hemispheres were regarded as independent data. Interictal spikes were counted again 24 hr after the last (sixth) seizure session (see below) to determine the kindling effect of repeated stimulations on the interictal spikes. Seizure threshold was determined by delivering stimulus trains of increasing intensity to the perforant path. Stimulus trains of 5 Hz (6.0 sec in duration) at steps of 100 μA varying from 0.2 to 1.2 mA (0.1-msec pulse width) were given in two separate sessions to each hemisphere. Two additional stimulations at seizure threshold intensity were given to each

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Abbreviations: HPC, hippocampal graft; LC, locus coeruleus graft; FF, fimbria-fornix cut; GABA, γ-aminobutyric acid.

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hemisphere in two subsequent sessions. Upon termination of the experiments the brains of the rats were processed histologically.

RESULTS

Injection of picrotoxin had a differential effect on the animals of the four groups. The latency to the first epileptic behavioral symptom was significantly different in the various groups ($F = 4.16$; $P < 0.01$). Only one rat in the LC group had a behavioral seizure. The LC group differed significantly (at $P < 0.01$) from the FF and HPC groups (Fig. 1).

The incidence of interictal spikes decreased in the HPC, FF, LC, and intact groups order. The occurrence of interictal spikes was 10–30 times less frequent in the LC group than in the FF or HPC animals (Fig. 2). Repeated seizures (see below) increased the incidence of interictal spikes in all groups. The frequency of seizure-induced interictal spikes, however, was substantially less in the LC group than in either the FF or the HPC groups.

The threshold intensity to elicit afterdischarges by perforant path stimulation was significantly lower in all lesioned groups relative to intact rats ($F = 20.40$, $P < 0.001$), but rats in the FF, LC, and HPC groups were not different from each other. In intact and LC rats the only behavioral manifestations of the electroencephalographic seizure were grooming and "wet-dog" shakes. Stimulation of 3 of 20 hemispheres (15%) in the FF group induced behavioral seizures, including rearing and falling, following the very first hippocampal afterdischarges. Stimulation-induced behavioral seizures occurred in 10 of the 17 hemispheres tested (58.8%) in the HPC group.

In 4 of the 10 rats in the HPC group, at least one spontaneous behavioral seizure, including rearing and falling, was observed in the home cage. In two of the four HPC rats, behavioral seizures were detected before the injection of picrotoxin and electrode implantation, indicating that induction of clonic seizures was not a consequence of these manipulations. In another animal of the HPC group, spontaneous electrical seizure was seen without behavioral manifestations. None of the rats of the other groups observed displayed spontaneous behavioral or electrical seizures.

The average volume of the HPCs was three to five times larger than the LCs. Nests of pyramidal and granule cells and scattered GABA-immunoreactive neurons (33) were present in all HPCs (Fig. 3 *a* and *b*). All rats in the HPC group with behavioral seizures had large grafts. It is unlikely, however, that the size rather than the type of cells in the graft was the cause of the epileptic manifestations, since HPCs and LCs of

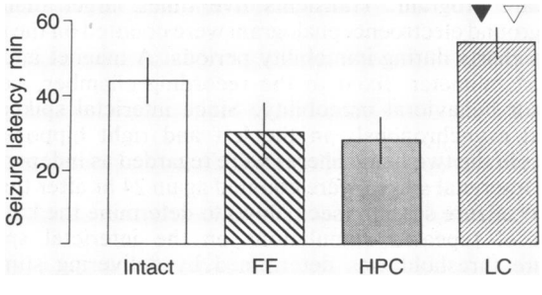

FIG. 1. Latency of behavioral seizures induced by intraperitoneal injection of picrotoxin (1 mg/kg) in the intact and experimental groups. FF, FF only; HPC, FF and intrahippocampal injection of fetal hippocampal cell suspension; LC, FF and intrahippocampal injection of neuronal suspension prepared from fetal locus coeruleus region. Picrotoxin was administered 5 months after grafting. Vertical bars indicate standard errors of the means. Filled and open triangles indicate significant differences from FF and HPC groups, respectively.

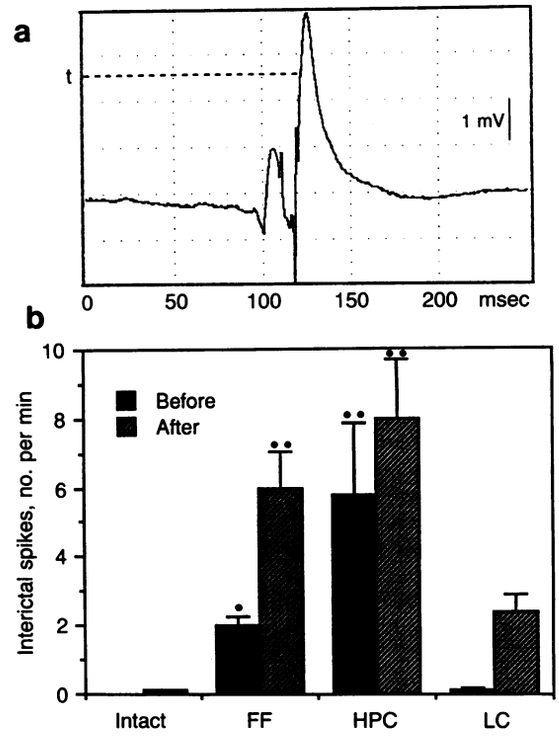

FIG. 2. Frequency of interictal spikes in the hippocampus in the different groups. (*A*) A representative interictal spike recorded from the hilus of the dentate gyrus in a FF rat. t, Amplitude discrimination threshold. (*b*) Frequency of interictal spikes 1 day before (black columns) and 1 day after (striped columns) six hippocampal seizures (1 seizure per day) induced by electrical stimulation of the perforant path. Vertical bars indicate standard errors of the means. Analysis of variance on the FF, HPC, and LC groups indicated a significant group effect ($F = 8.07$; $P < 0.001$), a significant effect of daily seizures ($F = 42.79$; $P < 0.001$), and a nonsignificant group X treatment interaction ($F = 1.88$; $P < 0.005$). Black dots indicate significant differences from intact and LC groups ($P < 0.02$; $P < 0.01$; post-analysis of variance Fisher tests).

equal volume showed opposite changes in the incidence of interictal spikes. The presence of catecholaminergic cells in LCs was detected with tyrosine hydroxylase immunoreactivity (34). Tyrosine hydroxylase positive cell bodies were found in tight groups within the transplant, and their processes were seen to extend several millimeters from the graft into the host hippocampus (Fig. 3).

DISCUSSION

The findings of this study indicate that the increased seizure susceptibility of the subcortically denervated hippocampus can be at least partially ameliorated by intrahippocampal grafts derived from the locus coeruleus region of the fetal brainstem. Rats with LCs had (*i*) significantly fewer interictal spikes, (*ii*) were more resistant to picrotoxin-induced behavioral seizures, and (*iii*) never displayed spontaneous or stimulation-triggered epileptic myoclonus. Suspension grafts of fetal hippocampal cells, on the other hand, increased the incidence of interictal spikes and induced spontaneous and stimulation-triggered behavioral seizures.

Although the brainstem grafts may have contained neurons other than those from the locus coeruleus, the following considerations suggest that the antiepileptic effects of the transplants may have been conveyed mainly through catecholaminergic cells. (*i*) Noradrenaline has been shown to inhibit spontaneous discharges of pyramidal and granule cells and to facilitate the activity of inhibitory interneurons in the

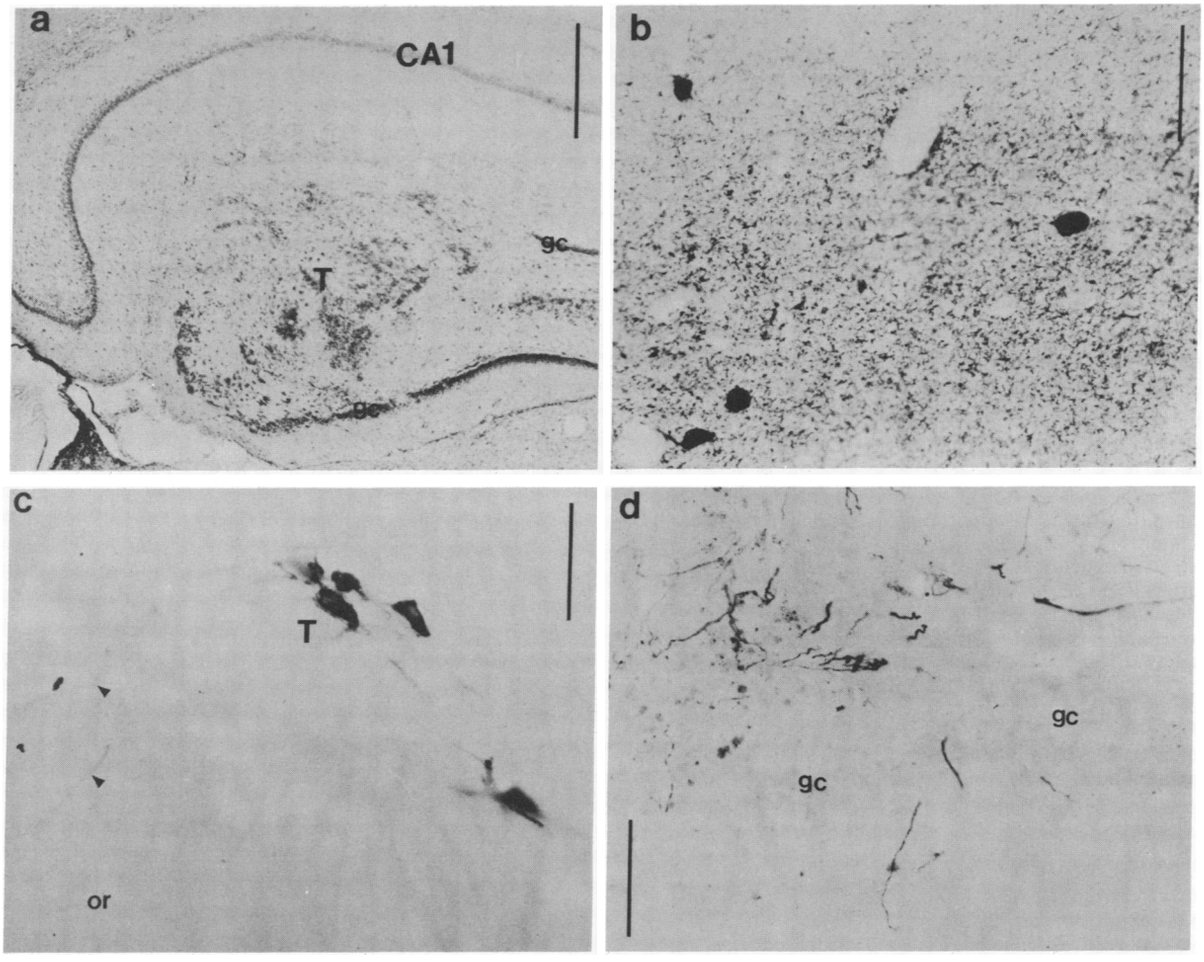

FIG. 3. Photomicrographs of HPC (*a* and *b*) and LC suspension grafts (*c*). (*a*) The medium-size HPC (T) in the dentate gyrus disrupts the continuity of the granule cell layer (gc). CA1, pyramidal layer Nissl stain. This HPC produced spontaneous electrical seizures in the host hippocampus. Dark neurons in *b* are GABA-immunoreactive cells (17). (*c* and *d*) Tyrosine hydroxylase (18) immunoreactive cells and processes in LCs. (*c*) Groups of tyrosine hydroxylase-immunoreactive neurons in an LC located on the ventricular surface of the hippocampus. Arrowheads indicate graft-derived fibers passing through the stratum oriens (or) and the pyramidal layer of CA1. (*d*) LH-immunoreactive fibers in the host dentate gyrus just posterior to an LC (18). gc, Granule cell layer. (*a*, 0.25 mm; *b*, 75 μm; *c* and *d*, 150 μm.)

hippocampus (18, 19). (*ii*) Destruction of the ascending noradrenergic projection facilitates the progression of stimulation-induced seizures in the kindling model of epilepsy (20–23). (*iii*) LCs gave rise to dense catecholaminergic reinnervation of the host hippocampus and the new fibers established physiologically active connections with the host neurons (24), and the grafts restored noradrenaline turnover and release in the subcortically deafferented hippocampus (35). (*iv*) In a recent study locus coeruleus transplants retarded the development of stimulation-induced seizures in animals with previous chemical lesions of the catecholamine system (36). The presence of noradrenergic fibers derived from brainstem cograft dampened the stimulus-elicited seizures of the hippocampus grafted into the anterior chamber of the eye (26, 27). The mechanism of the seizure-suppressant action of the locus coeruleus transplant remains to be elucidated. It was suggested that the seizure-prone nature of the subcortically denervated hippocampus is due to (*i*) the augmentation of after-hyperpolarizations in pyramidal cells which favors the emergence of population synchrony and/or (*ii*) increased collateral excitation due to sprouting of the axon collaterals of pyramidal neurons (17). Noradrenergic cells of the graft may reduce the afterhyperpolarizations in pyramidal cells (37, 38), thereby preventing population synchrony. Alternatively, axons of grafted neurons may compete for postsynaptic sites with the sprouting axons of the host cells and excessive collateral excitation might therefore be reduced.

Based on previous histological and tissue culture experiments and the importance of GABA in the control of epileptic activity (11–16), the epileptogenic nature of the HPCs was unexpected. Developmental and tissue culture investigations reported that the relative proportion of GABAergic cells is higher in the hippocampus at embryonic day 15 than in the adult (39, 40). Recent studies as well as the present experiment confirmed the presence of inhibitory GABAergic neurons in the HPCs (41). In our previous electrophysiological experiments we observed, however, that solid HPCs, placed into the lesion cavity, displayed highly synchronous population bursts and concurrent large-amplitude electroencephalographic spikes. Occasional seizures were also seen in the graft which spread to the host hippocampus (42–44). Subsequent electron microscopic examination of the HPCs revealed the absence of a strategically important inhibitory neuronal type, the chandelier or axo-axonic cell (45). In addition, several asymmetric non-GABAergic, presumably excitatory, synapses were found on the somata of pyramidal cells. Such excitatory synapses are not present on the cell bodies of pyramidal cells in the normal hippocampus (46, 47). Consequently, the epileptic nature of the grafted hippocampus may be explained by the excessive mutual excitation of pyramidal cells by means of their axon collaterals and the

reduction of GABAergic inhibition at a specific site of the pyramidal cells.

In summary, the present investigation demonstrates the potential use of neuronal transplants to control epileptic activity. Grafts of fetal brainstem origin, containing noradrenergic neurons, were able to attenuate epileptic activity in the seizure-prone hippocampus. The specificity of the seizure-suppressant effects of locus coeruleus transplants is demonstrated by the finding that HPCs enhanced the seizure tendency and thus may have caused further deterioration of brain function.

The antibody for GABA was a present from P. Somogyi. We thank Donna Chin and Steven Forbes for excellent technical help and Sheryl Christenson for typing the manuscript. This research was supported by the J. D. French Foundation, the California State Department of Health, National Institute on Aging Grant AGO 6088, Office of Naval Research, and the Margaret and Herbert Hoover Foundation.

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