The Functional Anatomy of Limbic Status Epilepticus in the Rat.

I. Patterns of ^{14}C -2-Deoxyglucose Uptake and Fos Immunocytochemistry

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Limbic status epilepticus was induced in awake, unrestrained rats by electrically stimulating the olfactory cortex or the basal amygdaloid nucleus for about 40 min. One of four stable forms of status was induced, which were distinguished on the basis of their behavioral and EEG manifestations, and their distinct patterns of ^{14}C -2-deoxyglucose uptake and Fos-like immunoreactivity. Type I status was characterized by sporadic EEG discharges and the activation of the amygdalohippocampal area, but had no overt behavioral manifestation. Type II status involved incessant exploratory behaviors, single EEG discharges, and the additional activation of the basal amygdaloid nucleus, some of its efferent projections, and parts of the olfactory cortex. Type III status included all of these same patterns, plus the episodic development of ictal EEG activity associated with facial and forelimb clonus, and the concurrent recruitment of the entire amygdala, ventral hippocampal formation, prefrontal, insular, and olfactory cortices, and related subcortical structures. Type IV status was characterized by generalized clonus, unremitting ictal EEG discharges, and the additional activation of most of the dorsolateral neocortex, neostriatum, and thalamus. In each case of status type I, II, or III, the same anatomical structures that displayed high levels of ^{14}C -2-deoxyglucose uptake also contained many cells that were immunoreactive for Fos, with the exception of the parataenial and mediodorsal thalamic nuclei and the substantia nigra pars reticularis. Thus, the overall patterns of ^{14}C -2-deoxyglucose uptake and Fos-like immunoreactivity from the same animals displayed a remarkable degree of correspondence. The major results indicate that different levels of status are related to the activation of discrete epileptogenic foci, and the capacity of such foci to interact with a distinct set of interconnected anatomical structures. It is suggested that the behavioral manifestations of limbic status epilepticus may be explained by influences of limbic structures in the ventral forebrain upon lower motor elements

in the brainstem and spinal cord, without the participation of the "pyramidal" motor system.

[Key words: seizure, temporal lobe epilepsy, amygdala, hippocampus, immediate-early gene, epileptogenic focus]

In order to understand the cellular mechanisms that underlie partial seizures, it is important to identify the affected neural structures and the anatomical relationships among them. In patients with temporal lobe epilepsy, EEG, neuropathological, and positron emission tomography studies have all emphasized the primary involvement of the anterior hippocampal formation and the amygdaloid complex in the pathogenesis of most complex partial seizures (Margerison and Corsellis, 1966; Engel et al., 1983; Theodore et al., 1983; Wieser, 1983; Mazziotta and Engel, 1984; Quesney, 1986; Bruton, 1988; So et al., 1989; Goldring et al., 1992). In animals, investigations with the ^{14}C -2-deoxyglucose autoradiographic technique (Sokoloff et al., 1977) have implicated the same structures in a variety of experimental models of epilepsy, and have supplemented the clinical findings with more precise anatomical detail (e.g., Ben-Ari et al., 1981; Lothman and Collins, 1981; Handforth and Ackermann, 1988a).

Among the several models of complex partial seizures, *limbic status epilepticus* induced by electrical stimulation is especially well suited for the anatomical characterization of the neural substrates of seizure activity (Handforth and Ackermann, 1988a,b; McIntyre et al., 1991; VanLandingham and Lothman, 1991a,b). This model features self-sustaining epileptiform discharges that may persist in a relatively stable form for several hours after the termination of the electrical stimulus (McIntyre et al., 1982, 1991; Milgram et al., 1985; Handforth and Ackermann, 1988a,b; Ackermann et al., 1989; Lothman et al., 1989). The major advantage of this model is that ^{14}C -2-deoxyglucose may be administered during status epilepticus to reveal patterns of neural activation associated with the seizure state, without the contaminating effects of an ongoing exogenous inducing agent (i.e., electrical stimulation or a chemical convulsant). Previous studies have found that several levels of status epilepticus may be induced in the rat by electrical stimulation of the deep amygdaloid nuclei (McIntyre et al., 1982, 1991; Handforth and Ackermann, 1988a), or of the ventral hippocampal formation (Lothman et al., 1989). The patterns of ^{14}C -2-deoxyglucose uptake associated with the less severe forms of status activity primarily involve parts of the amygdaloid complex and the ventral hippocampal formation, while the more severe, convulsive levels also involve widespread structures throughout the ventral forebrain (Handforth and Ackermann, 1988a; McIntyre et al., 1991; VanLandingham and Lothman, 1991a).

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Figure 1. Schematic depiction of the surgical preparation (*A*) and the experimental design (*B*). *A*, A concentric bipolar electrode implanted into the olfactory bulb, the anterior olfactory nucleus, the anterior piriform cortex, or the basal amygdaloid nucleus (marked by *) was used for electrical stimulation and subsequent depth EEG recording. Skull screws with wire leads were used for surface EEG recording. *B*, In each rat, stimulation of one of the four sites for 30–60 min evoked focal seizure activity that led to the development of one of four types of limbic status epilepticus. After at least an hour of status, a solution of ^{14}C -2-deoxyglucose was infused intravenously and 45 min later the rat was killed by transcardial perfusion.

In addition to the ^{14}C -2-deoxyglucose autoradiographic method, the induction of immediate-early genes provides a basis for mapping functional activity within the brain (for recent reviews, see Morgan and Curran, 1989, 1991). The *c-fos* gene and other related immediate-early genes are expressed in neurons during strong activation, such as that which occurs during paroxysmal seizure activity. Antibodies raised against the products of these genes, Fos and other related transcriptional factors, can be employed to label cells that participate in seizure activity (Dragunow and Robertson, 1987a,b; Morgan et al., 1987; Le Gal La Salle, 1988; Sagar et al., 1988; Sonnenberg et al., 1989). Such labeling may give a similar but not identical pattern to that seen with ^{14}C -2-deoxyglucose. While increases in ^{14}C -2-deoxyglucose uptake largely represent regions of increased synaptic activity, whether excitatory or inhibitory (Sharp, 1976; Schwartz et al., 1979; Di Rocco et al., 1989), the localization of mRNA for Fos or the Fos protein itself is related to cellular activity. In particular, the acute induction of the *c-fos* gene within neurons has been associated mainly with depolarization (Dragunow and

Robertson, 1987a,b; Le Gal La Salle, 1988; Sonnenberg et al., 1989). By comparing patterns of ^{14}C -2-deoxyglucose uptake and Fos immunoreactivity under the same conditions, it may be possible to distinguish brain regions that are dominated by excitatory synaptic activity from those with substantial inhibitory activity.

The present study was undertaken to characterize the anatomical basis of both subconvulsive and convulsive forms of limbic status epilepticus, with the use of ^{14}C -2-deoxyglucose autoradiography in conjunction with immunocytochemistry for the product of the *c-fos* gene. The results indicate that different levels of status are associated with activity in distinct anatomical structures, and that the same types of status may be induced by the electrical stimulation of disparate sites in the ventral forebrain. The results of this study have formed the basis for experiments aimed at identifying the foci of the epileptiform activity in these levels of status, which are reported in the following companion article (White and Price, 1993).

Materials and Methods

Surgery. Adult Sprague-Dawley rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) or halothane (0.25–0.50%) in a 2:1 mixture of nitrous oxide and oxygen, and prepared for surgery by shaving the surgical fields and wiping with alcohol and activated iodine solutions. A polyethylene catheter (PE-60, Clay Adams), for the delivery of ^{14}C -2-deoxyglucose, was implanted into the left femoral vein and routed subdermally to exit from the back of the neck. The patency of the catheter was maintained by flushing with heparinized (20 U/ml) normal saline. Next, the rat was placed in a stereotaxic apparatus (David Kopf), and a concentric bipolar electrode (0.25 mm shaft diameter, 0.50 mm contact separation; Rhodes Medical Instruments) was inserted into one of the following four sites in the ventral forebrain, guided by stereotaxic coordinates from the atlas of Paxinos and Watson (1986): the olfactory bulb (AP +7.5, ML +1.2, DV_{dura} –2.8), the anterior olfactory nucleus (AP +4.7, ML +2.4, DV_{dura} –4.2), the deep parts of the anterior piriform cortex (AP +2.7, ML +2.8, DV_{dura} –6.5), or the basal nucleus of the amygdala (AP –2.8, ML +4.7, DV_{dura} –8.0). The bipolar electrode was used for both electrical stimulation and depth EEG recording (Fig. 1*A*). In addition, three screws mounted with wire leads (Plastics One) were implanted in the skull over the right frontal, and left and right parietal areas for surface EEG recording. All wire contacts were fitted into a plastic pedestal (Plastics One) and secured to the skull with dental cement.

Electrical stimulation. Following a recovery period of 5–10 d, a multichannel cable (Plastics One) was attached to the pedestal, and the afterdischarge threshold was determined. Electrical stimuli consisting of 2 sec trains of 100 μsec pulses were delivered through the bipolar electrode at a rate of one train per minute, with an intratrain pulse frequency of 60 Hz. The behavior of the rat and the surface EEG were monitored with a six-channel polygraph (Grass Instruments) for evidence of an afterdischarge. The current intensity of the pulses started at 100 μA and increased in 100 μA increments with each successive train, until an afterdischarge was elicited, or until 1500 μA was reached.

After a rest period of about 1 hr, electrical stimulation was applied continuously for 30–60 min via the concentric bipolar electrode, in an attempt to induce limbic status epilepticus. The stimulus consisted of 20 msec trains of ten 100 μsec pulses, with three trains delivered every second (Collins et al., 1988). The current intensity was set initially to equal the afterdischarge threshold for each rat. If a rat did not respond, the current was increased incrementally until obvious seizure activities were induced, or until 1500 μA was reached.

Limbic status epilepticus. After the offset of electrical stimulation, the concentric bipolar electrode was connected to the polygraph, and the surface and depth EEG records and the behavioral activities were observed for evidence of status epilepticus (see Fig. 1*B*). For this study, status epilepticus is operationally defined as a persistent state of epileptiform discharges at a rate of at least one discharge every 5–10 sec. Status developed in 71% of all the animals used in these studies (Table 1). The typical behavioral and EEG patterns that characterize each of the different levels of status were usually apparent within the first 3–5 min after the termination of electrical stimulation. In most experiments,

Table 1. Frequency of induction of four types of status epilepticus by the application of electrical stimulation to different sites in the ventral forebrain (n = number of animals in each group)

	Failure to induce status epilepticus	Status epilepticus			
		Type I	Type II	Type III	Type IV
% of total	29%	5%	24%	41%	2%
Olfactory bulb (n = 25)	17	2	3	2	1
Anterior olfactory nucleus (n = 13)	4	0	2	7	0
Anterior piriform cortex (n = 98)	16	1	24	55	2
Basal amygdaloid nucleus (n = 49)	16	6	16	11	0

after status proceeded for about an hour, a solution of 1- 14 C-2-deoxyglucose (60 μ Ci/kg; American Radiolabeled Chemicals; specific activity, 55 mCi/mmol) in normal saline was administered intravenously through the femoral catheter. About 45 min later, the rat was deeply anesthetized with an overdose of intravenous sodium pentobarbital, and killed by transcardial perfusion with 200 ml of heparinized 0.1 M sodium phosphate buffer (pH 7.3), followed by 600 ml of 4.0% paraformaldehyde buffered in 0.1 M sodium phosphate (pH 7.3). The brain was immediately dissected from the cranial cavity, frozen in isopentane (-50°C), and stored at -70°C until sectioning.

14 C-2-deoxyglucose autoradiography. Each brain was sectioned coronally in a cryostat at -20°C . Three 20 μm sections were taken every 300 μm , melted onto subbed coverslips, and dried on a warm plate at 50°C . The coverslips were mounted onto paper sheets and exposed to x-ray film (Kodak XAR) for 7 d. A set of 14 C-standards (American Radiolabeled Chemicals) was included with each sheet of sections to allow calibration of the autoradiographs in terms of the amount of radioactivity in the sections. The film was processed in a developer solution (Kodak GBX), and the middle section of each triplet was removed from the sheet and stained with thionin.

A quantitative assessment of the patterns of 14 C-2-deoxyglucose uptake within specified brain regions was performed. The goal was to measure the amounts of accumulated 14 C-2-deoxyglucose, rather than to determine glucose consumption. The optical density of specific regions was measured using a computer-based image processor (Drexel University BRAIN Autoradiographic Analysis Software Package). Calibration curves were generated for each sheet of film by plotting the optical density of the 14 C-standards against their isotope content. These curves were then used to calculate values of 14 C-2-deoxyglucose concentration. Each brain region of interest from a single case was measured in triplicate, and a mean value was determined and pooled with corresponding values from other cases within groups. Differences in mean isotope concentration across groups were identified using one-way analysis of variance and the Tukey test for multiple comparisons. For presentation, contrast-enhanced photographs of selected autoradiographic images were prepared with the computer-based image processor.

Fos immunocytochemistry. Series of 40 μm sections were collected from the cryostat in 0.1 M sodium phosphate-buffered saline (0.9% w/v) (pH 7.3) (NaPBS). All incubations and rinses were performed with the sections free-floating on a mechanical shaker at room temperature. The primary anti-Fos antibody that was used in these studies was directed against a synthetic peptide derived from a conserved sequence near the N-terminus of Fos (Microbiological Associates) (van Straaten et al., 1983; de Togni et al., 1988). Vectastain ABC Kits (Vector Laboratories) were used for amplification and visualization of the primary antibody-antigen complex (Hsu et al., 1981). Briefly, sections were preincubated with 10% (v/v) normal horse serum (Colorado Serum), 2% (w/v) bovine serum albumin (fraction V, Sigma), and 0.3% (v/v) Triton X-100 (Sigma) in NaPBS for 1 hr. Sections were then incubated overnight in the primary antibody solution, which contained the anti-Fos antibody at a 1:2000 dilution, 10% (v/v) normal horse serum, 2% bovine serum albumin, and 0.3% Triton X-100 in NaPBS. The following day, the sections were rinsed thoroughly with NaPBS and incubated for 1 hr in the secondary antibody solution, which contained biotinylated horse IgG raised against mouse IgG (1:200), 10% normal horse serum, 2% bovine serum albumin, and 0.3% Triton X-100 in NaPBS. After further rinsing, the sections were incubated with the ABC Reagent [0.1% (v/v) Avidin

DH complexed with 0.1% (v/v) biotinylated horseradish peroxidase in NaPBS (Vector Laboratories)] for 1 hr, followed by thorough rinsing in NaPBS. Finally, sections were reacted briefly in a peroxidase substrate solution containing 0.05% (w/v) diaminobenzidine and 0.01% (v/v) hydrogen peroxide in NaPBS, rinsed in NaPBS, and then mounted onto subbed slides, dehydrated, cleared, and coverslipped. In most immunocytochemical trials, some sections were processed without the primary antibody; these control sections did not show cellular staining.

The distribution of Fos-like immunoreactivity for each case was assessed systematically using bright-field microscopy. In representative cases, stained cells were plotted with an x-y digitizer (Minnesota Datametrics) attached to a microscope stage, and linked through a microcomputer to a pen plotter. For each animal, the pattern of Fos-like immunoreactivity was directly compared with the pattern of 14 C-2-deoxyglucose uptake. The overall patterns of activation for each status group were compared to patterns from implanted but unstimulated cases, and from stimulated animals that failed to develop status epilepticus.

The patterns of 14 C-2-deoxyglucose uptake and Fos-like immunoreactivity were also correlated with the projections of the basal nucleus of the amygdala and the piriform cortex (see Krettek and Price, 1978a,b; Luskin and Price, 1983). In addition to comparisons with published accounts, experimental material from previous studies (e.g., Ray and Price, 1992) was available for reanalysis, in which the anterograde axonal tracer ^3H -leucine was injected into the deep amygdaloid nuclei or the piriform cortex.

Results

Induction of limbic status epilepticus

Electrical stimulation of the basal amygdaloid nucleus or one of several sites within the olfactory cortex produced a pattern of behaviors that was very similar to the manifestations associated with fully kindled limbic-motor seizures (Racine, 1972a,b; Racine et al., 1973). This pattern began with a persistent "motionless-staring" posture, in which the neck was dorsiflexed and one forelimb was extended while the other was flexed. Episodes of convulsive seizures evolved repeatedly from this posture (about once every 3–5 min) throughout the period of stimulation. The convulsive behaviors began as mild facial clonus, followed by chewing movements, excessive salivation, and head nodding. Next, widespread seizure activity ensued as forelimb clonus appeared, followed by an ascent to an upward rearing posture with repeated falling. The same seizure behaviors were observed during the electrical stimulation of each of the different anatomical sites in the ventral forebrain.

In animals that were given 14 C-2-deoxyglucose and killed during the period of stimulation, the patterns of activity associated with stimulus-evoked seizures involved the entire amygdaloid complex, the olfactory cortex, the ventral hippocampal formation, the ventral striatum, the magnocellular basal forebrain region, the substantia nigra pars reticularis, and the prefrontal

cortex and associated structures in the thalamus and hypothalamus (e.g., Carnes and Price, 1987). The stimulation of either the basal nucleus of the amygdala or the olfactory cortex produced identical patterns of ¹⁴C-2-deoxyglucose uptake, consistent with the similarity in seizure-related behaviors across stimulation site groups.

Clinical presentation of four types of status epilepticus

General observations

The sustained seizure activity produced by the electrical stimulation of the ventral forebrain led to the development of one of four levels of status epilepticus. These were distinguished initially on the basis of the associated behavioral manifestations and EEG patterns, and were designated types I–IV, in order of increasing severity. Status types II and III were observed most frequently, followed by type I, and then type IV, which was rarely observed (Table 1). Type II and type III status were induced following the electrical stimulation of the olfactory bulb, the anterior olfactory nucleus, the anterior piriform cortex, or the basal amygdaloid nucleus, and the less common types I and IV were induced from at least two of these sites (Table 1). None of the types of status was uniquely associated with any one site of stimulation.

Each type of status was stable throughout the duration of the experimental period, which usually lasted about 2 hr from the offset of stimulation (see Fig. 1*B*). There were no transitions between the different levels of status. In a few rats, status was allowed to proceed for several more hours. Type I status continued for an 18 hr period, and type III status was observed for more than 7 hr without significant changes in the behavioral or EEG patterns, although these manifestations began to decompose and subside after about 8 hr.

Behavioral and electroencephalographic manifestations

Type I status. This least severe level of status was detectable only in the EEG recordings and was not associated with any marked or consistent behavioral alterations. Shortly after the termination of electrical stimulation, most rats in type I status appeared to be somewhat lethargic with reduced spontaneous activities (e.g., grooming and normal explorations of the cage). After about half an hour of status, the rats generally assumed a resting posture in the corner of their cages, and several appeared to fall asleep. The EEG features of type I status consisted of variable, 50–100 μ V epileptiform discharges that occurred at a rate of about one every 2–10 sec (Fig. 2*B*). These discharges were recorded from the deep bipolar electrodes in the anterior piriform cortex or the basal amygdaloid nucleus, but not from the superficial skull screw leads.

Type II status. Type II status was associated with incessant, but “discontinuous” exploratory behaviors. Specifically, the animals roamed their cages persistently throughout the duration of the experiment, but the locomotion was very briefly arrested about once every second. No manifestations of sustained seizure activity, such as episodes of “motionless staring,” were observed. The animals were unable to maintain normal behaviors, such as grooming or standing on their hindlimbs against the side of the cage. Although such behaviors were initiated occasionally, they were not continued for more than a second or two.

Regular epileptiform discharges at amplitudes of 100–200 μ V and frequencies of about 1 Hz were associated with the exploratory behaviors (Fig. 2*C*). These discharges were recorded from

the bipolar electrode in each of the stimulation sites. Similar but markedly attenuated discharges were observed from the skull screw leads. By listening to the scratch of the pen writer marking the occurrence of an epileptiform discharge, it was apparent that these EEG discharges coincided with the momentary arrests in locomotion. Occasionally, more complex wave forms were seen, but these discharges never developed into sustained ictal patterns.

Type III status. Type III status encompassed all the behavioral and EEG patterns of type II status. Its additional, defining feature was the recurrent development of discrete episodes of convulsive seizure activity. Behaviorally, these episodes ranged in severity from immobility and motionless staring with only mild facial clonus, to severe facial and forelimb clonus, rearing, and loss of balance. Most animals tended to demonstrate the milder of these behavioral patterns, and only infrequently did these episodes progress to manifest the more severe seizure behaviors.

Electroencephalographically, the convulsive episodes were expressed as discrete segments of high-frequency (10–20 Hz) discharges, which usually lasted between 20 and 40 sec, but sometimes exceeded 60 sec (Fig. 2*D*). They tended to recur every 3–5 min. These segments of ictal discharges were associated with very mild behavioral manifestations (e.g., immobility and motionless staring with mild facial clonus), as well as more severe signs (e.g., forelimb clonus with rearing and falling). Consequently, type III status was identified by the periodic emergence of this EEG pattern, rather than by observation of overt convulsive behaviors. In most cases, the behavioral and EEG manifestations of type III status were fully established within 5 min of the offset of electrical stimulation.

Type IV status. Type IV status was characterized by nearly continuous facial and forelimb clonus, and unremitting, high-frequency EEG discharges (Fig. 2*E*). These patterns were established during the period of electrical stimulation and continued unabated following stimulus offset. Generalized clonic seizures frequently evolved from the persistent lower-grade clonus, but no tonic seizure activities were observed. One animal had several “running and bouncing” seizures (e.g., Browning and Nelson, 1986) during intense generalized clonus.

Patterns of ¹⁴C-2-deoxyglucose uptake and Fos-like immunoreactivity associated with the different types of status epilepticus

General observations

In all of the 185 cases studied, each type of status was invariably associated with a distinct anatomical pattern of neural activation, as defined by ¹⁴C-2-deoxyglucose uptake and Fos-like immunoreactivity (Table 2). Furthermore, just as each type of status could be induced following the electrical stimulation of disparate sites within the forebrain, the same distinct patterns were observed irrespective of the site of electrical stimulation. Thus, the patterns of activation were associated with discrete levels of status epilepticus, and not with electrical stimulation of any one anatomical structure.

It is crucial to the interpretations of the patterns of Fos-like immunoreactivity to establish that the labeled cells were activated during status epilepticus, rather than during the period of electrical stimulation or the resting state. Several observations helped to clarify this issue. First, in rats that were killed in the resting, naive (i.e., never-stimulated) state, there were no Fos-like immunoreactive cells in the entire forebrain. This obser-

Figure 2. EEG patterns associated with the resting state and each of four types of status epilepticus. For each state the *upper trace* depicts EEG activity recorded from skull screws and the *lower trace* depicts activity recorded from a concentric bipolar electrode deep in the amygdala or the piriform cortex. *A*, EEG activity in the resting state, prior to the onset of electrical stimulation. *B*, EEG activity associated with type I status. Variable, 50–100 μ V epileptiform discharges occurred at a rate of about one every 2–10 sec in the depth EEG channel, but there were no specific behavioral manifestations. *C*, EEG activity associated with type II status, characterized by a regular pattern of 100–200 μ V epileptiform discharges at a frequency of about 1 Hz. This pattern was associated with “discontinuous” exploratory behavior. *D*, EEG activity associated with type III status. In addition to the pattern associated with type II status, there were discrete periods of sustained fast discharges (*arrows*), which coincided with periods of facial and forelimb clonus. *E*, EEG activity associated with type IV status. This pattern was characterized by unremitting fast discharges and accompanied by continuous facial and forelimb clonus that frequently evolved into generalized clonic seizures. Horizontal calibration, 10 sec; vertical calibration: 100 μ V in *A*, *C*, and *D*; 50 μ V in *B*; and 200 μ V in *E*.

vation is consistent with other findings with similar antibodies directed against the N-terminus of Fos (Dragunow and Faull, 1989). Second, in 14 of 16 rats that were electrically stimulated for 40–60 min but failed to develop status and were killed 1–2

hr later, no Fos-like immunoreactive cells were found (Fig. 3*D*). In the other two cases, only faint cellular staining was observed within the anterior olfactory nucleus and parts of the amygdaloid complex. Third, rats that developed the less severe forms of

Table 2. Quantitative analysis of ¹⁴C-2-deoxyglucose concentrations (mean ± SE, in μCi/gm) in selected brain regions in the resting unstimulated state, and in four types of status epilepticus

Region	Resting state (n = 19)	Status epilepticus			
		Type I (n = 4)	Type II (n = 5)	Type III (n = 13)	Type IV (n = 1)
Limbic structures					
Basal amygdaloid nucleus	0.21 ± 0.02	0.22 ± 0.03	0.71 ± 0.10 ^{*,a,b}	0.80 ± 0.10 ^{*,a,b}	0.71
Amygdalohippocampal area	0.19 ± 0.01	0.48 ± 0.04 [*]	0.69 ± 0.06 ^{*,a}	0.74 ± 0.09 ^{*,a}	0.75
Anterior olfactory nucleus	0.25 ± 0.02	0.24 ± 0.03	0.55 ± 0.07 ^{*,a,b}	0.74 ± 0.06 ^{*,a,b}	0.86
Piriform cortex	0.31 ± 0.02	0.26 ± 0.04	0.29 ± 0.03	0.65 ± 0.06 ^{a,b,d}	0.90
Dorsal endopiriform nucleus	0.23 ± 0.02	0.23 ± 0.03	0.62 ± 0.06 ^{*,a,b}	0.82 ± 0.08 ^{*,a,c}	0.85
Entorhinal cortex	0.20 ± 0.01	0.21 ± 0.02	0.33 ± 0.05	0.68 ± 0.08 ^{*,a,b,d}	0.66
Dentate gyrus (ventral)	0.21 ± 0.02	0.26 ± 0.03	0.23 ± 0.03	0.53 ± 0.07 ^{*,a,b,d}	1.00
Subiculum (ventral)	0.22 ± 0.01	0.26 ± 0.03	0.43 ± 0.07	0.76 ± 0.09 ^{*,a,b,d}	0.89
Nucleus accumbens	0.25 ± 0.02	0.26 ± 0.05	0.48 ± 0.06 [*]	0.74 ± 0.06 ^{*,a,b,d}	0.90
Olfactory tubercle	0.23 ± 0.02	0.24 ± 0.04	0.37 ± 0.04	0.72 ± 0.06 ^{*,a,b,d}	0.83
Extralimbic structures					
Thalamic midline nuclear complex	0.27 ± 0.02	0.29 ± 0.05	0.47 ± 0.04 [*]	0.50 ± 0.06 ^{*,a,c}	0.85
Thalamic ventral basal complex	0.26 ± 0.03	0.27 ± 0.05	0.28 ± 0.03	0.29 ± 0.04	0.90
Substantia nigra pars reticularis	0.18 ± 0.01	0.20 ± 0.02	0.24 ± 0.03	0.33 ± 0.05 ^{*,a}	0.79
Neocortex					
Agranular insular (dorsal)	0.24 ± 0.02	0.23 ± 0.03	0.37 ± 0.03	0.67 ± 0.07 ^{*,a,b,d}	0.84
Anterior cingulate	0.28 ± 0.02	0.26 ± 0.05	0.23 ± 0.02	0.31 ± 0.03	0.90
Motor	0.28 ± 0.03	0.24 ± 0.04	0.20 ± 0.02	0.23 ± 0.02	1.05
Somatosensory	0.29 ± 0.03	0.23 ± 0.03	0.20 ± 0.02	0.23 ± 0.03	0.92

* Data indicate values significantly different from control value ($p < 0.05$).

^a Value is significantly different from control value ($p < 0.01$).

^b Value is significantly different from type I status epilepticus value ($p < 0.01$).

^c Value is significantly different from type I status epilepticus value ($p < 0.05$).

^d Value is significantly different from type II status epilepticus value ($p < 0.01$).

status (i.e., types I and II) exhibited patterns of Fos-like immunoreactivity that matched the concurrent patterns of ¹⁴C-2-deoxyglucose uptake (see below), rather than the more widespread ¹⁴C-2-deoxyglucose patterns seen in other rats that were killed during the period of electrical stimulation. Taken together, these observations indicate that any Fos-like antigens that were produced during the period of electrical stimulation did not persist for more than about an hour.

Limbic status epilepticus

Type I status. During type I status, the only anatomical structure in the forebrain that accumulated significant amounts of ¹⁴C-2-deoxyglucose and displayed many Fos-like immunoreactive cells was the amygdalohippocampal area (Figs. 4G, 5G; Table 2). This structure lies in the posterior-medial region of the amygdaloid complex, just anterior to the hippocampal formation (e.g., Price et al., 1987); it is equivalent to the "posterior nucleus of the amygdala" of Canteras and Swanson (1992). The region of increased metabolic activity usually included the posterior-dorsal division of the medial amygdala nucleus (Figs. 4F, 5F), and the extreme ventral-medial tip of the subiculum. The only other region with elevated ¹⁴C-2-deoxyglucose uptake was the caudomedial part of the bed nucleus of the stria terminalis (including the "principal encapsulated nucleus"; Ju and Swanson, 1989), and the adjacent medial preoptic area (Fig. 4D). This area has been shown to receive a major direct projection from the amygdalohippocampal area (Krettek and Price, 1978b; Canteras and Swanson, 1992). However, no more than a few Fos-like immunoreactive cells were found in this region.

It is especially notable that the site of electrical stimulation (i.e., olfactory bulb, anterior piriform cortex, or basal amygdaloid nucleus) did not display increased ¹⁴C-2-deoxyglucose uptake or many Fos-like immunoreactive cells. Thus, the site of electrical stimulation was not involved in the anatomical substrate of type I status.

Type II status. The pattern of ¹⁴C-2-deoxyglucose uptake that was observed with type II status involved the same structures that were associated with type I status, plus the basal nucleus of the amygdala, some of its efferent projections, and restricted portions of the olfactory cortex (Fig. 6, Table 2). Within the amygdaloid complex, this pattern involved the amygdalohippocampal area, the basal and central nuclei, and the nucleus of the lateral olfactory tract. Within the hippocampus and olfactory cortex, the ventral subiculum and lateral entorhinal area, anterior olfactory nucleus, ventral tenia tecta, and dorsal endopiriform nucleus were involved. The deep layers of the medial prefrontal and agranular insular cortical areas were activated, as were the bed nucleus of the stria terminalis, a rostral-medial portion of the nucleus accumbens, and the parataenial, midline, and reticular nuclei of the thalamus. Increased ¹⁴C-2-deoxyglucose uptake in each of these structures occurred ipsilaterally to the side of electrical stimulation, and the accumbens and parataenial nuclei were also labeled contralaterally.

Most of the same regions that exhibited increased ¹⁴C-2-deoxyglucose uptake also contained many Fos-like immunoreactive cells (compare Figs. 6, 7). The pattern of Fos-like immunoreactivity was largely bilateral, in contrast to the mainly unilateral pattern of ¹⁴C-2-deoxyglucose uptake. In general, robust

Figure 3. Bright-field photomicrographs that demonstrate Fos-like immunoreactivity associated with type III status epilepticus. *A*, Fos-like immunoreactive neurons in the amygdaloid complex and posterior piriform cortex. *B*, High-magnification view of stained cells in the basal amygdaloid nucleus. Typically, immunocytochemical staining is restricted to cellular nuclei, with the cytoplasm devoid of reaction product. *C*, Fos-like immunoreactivity in the ventral hippocampal formation. There is a dense concentration of stained nuclei in the dentate gyrus, and an abundance of stained cells throughout the ventral hippocampus and subiculum, the lateral entorhinal area, and the perirhinal cortex. The irregular spots of intense neuropil staining are also present in sections that were not exposed to the primary antibody, and are considered to be nonspecific. *D*, A corresponding section from an animal in which facial and forelimb clonus was evoked during the period of electrical stimulation, but status epilepticus failed to develop. Note the absence of specific cellular staining. For abbreviations, see Appendix. Scale bars: *A*, 400 μ m; *B*, 30 μ m; *C* and *D*, 900 μ m.

Fos-like staining was always observed in the anterior olfactory nucleus, the dorsal endopiriform nucleus, the nucleus accumbens, and the thalamic reticular nuclei. Cells in other regions, such as the midline thalamic nuclei, the lateral agranular insular cortex, the nucleus of the lateral olfactory tract, and the lateral entorhinal area were stained in some cases but not in others.

Within the medial prefrontal cortex, parts of the ventral hippocampal formation and the depths of the insular cortex, the patterns of activation associated with type II status match the distribution of efferent terminations from the basal nucleus of

the amygdala (Krettek and Price, 1977b; Fig. 8). This correspondence is most striking in the dorsal agranular insular cortex. Projections from the basal nucleus terminate in layers I, II, and V, and in dense bands that lie both superficial and deep to the claustrum, while the claustrum itself is largely avoided (Krettek and Price, 1977a; Fig. 8C). This distribution closely approximates the pattern of 14 C-2-deoxyglucose uptake in this region (Fig. 8A).

In contrast, portions of the ventral striatum that are known to receive substantial projections from the basal amygdaloid

Figure 4. Coronal autoradiographs that demonstrate the pattern of ^{14}C -2-deoxyglucose uptake associated with type I status. The only area to accumulate significant amounts of ^{14}C -2-deoxyglucose is the amygdalohippocampal area (*G*). Lesser amounts of ^{14}C -2-deoxyglucose are found in the bed nucleus of the stria terminalis (*D*), and the medial nucleus of the amygdala (*F*). For abbreviations, see Appendix.

Figure 5. Line drawings of coronal sections that depict the distribution of Fos-like immunoreactive cells associated with type I status in the same animal presented in Figure 4. Each dot represents a single stained neuron. The distribution of labeled cells in the amygdalohippocampal area (*G*) and the medial amygdaloid nucleus (*F*) closely matches the pattern of increased ^{14}C -2-deoxyglucose uptake. For abbreviations, see Appendix.

nucleus did not show significant increases in ^{14}C -2-deoxyglucose uptake during type II status. The basal nucleus gives rise to widespread fibers that are distributed to much of the ventral and dorsal striatum, except the anterior dorsolateral portion (Krettek and Price, 1978a,b; Kelley et al., 1982; Kita and Kitai, 1990; Fig. 8*C*). Most of the amygdalo-recipient areas failed to demonstrate more than subtle increases in ^{14}C -2-deoxyglucose uptake (Fig. 8*A*), despite evidence of intense activation of the basal nucleus itself (Figs. 6*F*, 7*F*). These same striatal regions did display robust ^{14}C -2-deoxyglucose uptake during type III status (see below; Fig. 8*B*).

Although type II status was most often induced by stimulation of the piriform cortex, it was striking that this largest part of the olfactory cortex did not show elevated ^{14}C -2-deoxyglucose uptake or Fos-like immunoreactivity. This again indicates that the site of stimulation was not necessarily involved in the anatomical substrate of status, and also that type II status is pri-

marily related to the amygdala and not the olfactory cortex. In addition, it is notable that most of the hippocampal formation, apart from the entorhinal cortex and the ventral subiculum, was not activated during type II status.

Type III status. The pattern of elevated ^{14}C -2-deoxyglucose uptake associated with type III status included all of the same regions activated in type II status, with the additional participation of other limbic and extralimbic structures (Fig. 9, Table 2). Specifically, these additional structures were the entire amygdaloid complex, ventral hippocampal formation, and ventral striatum, the olfactory bulb and olfactory cortex, all layers of the insular cortex, medial and lateral prefrontal cortical areas and related medial thalamic nuclei, and the substantia nigra pars reticularis. Animals that displayed only mild seizure behaviors (i.e., immobility with facial clonus) exhibited the same pattern of activation as those that developed more severe convulsive episodes (i.e., facial and forelimb clonus with rearing

Figure 6. Coronal autoradiographs that demonstrate the pattern of ^{14}C -2-deoxyglucose uptake associated with type II status. In addition to the amygdalohippocampal area, the basal nucleus of the amygdala (*F*), and related areas such as the agranular insula (*C*, *D*) and medial thalamus (*E*, *F*) were activated, but there is no seizure-related activity in the ventral dentate gyrus (*H*). For abbreviations, see Appendix.

Figure 7. The distribution of Fos-like immunoreactive neurons associated with type II status, in the same animal illustrated in Figure 6. The patterns of labeling with the two markers are very similar, although a few structures that had high levels of ^{14}C -2-deoxyglucose uptake did not contain Fos-labeled cells in this case. These structures, including the medial thalamus, nucleus of the lateral olfactory tract, ventral subiculum, and lateral entorhinal area, had substantial numbers of Fos-labeled cells in other cases of type II status. The overall distribution of Fos-like immunoreactivity is bilateral, while the pattern of increased ^{14}C -2-deoxyglucose is mostly unilateral. For abbreviations, see Appendix.

and falling). In most experiments, elevated ^{14}C -2-deoxyglucose uptake was largely restricted to the hemisphere that received the electrical stimulation, although a bilaterally symmetrical pattern was seen in a small minority of cases (about 5%).

Most of these regions with increased ^{14}C -2-deoxyglucose uptake also contained many Fos-like immunoreactive cells (Figs. 3A–C, 10), although the pattern of Fos-like immunoreactivity was predominantly bilateral. However, three structures, the parataenial and mediodorsal nuclei of the thalamus and the pars reticularis of the substantia nigra, never contained more than a few Fos-like immunoreactive cells, despite exhibiting robust ^{14}C -2-deoxyglucose uptake in all cases of type III status (compare Figs. 9, 10).

In the thalamus, ^{14}C -2-deoxyglucose uptake was elevated in the parataenial nuclei, the medial segment of the mediodorsal nucleus, the paraventricular, intermediodorsal, centromedial, and reuniens nuclei, and in the rostral-ventral portion of the reticular nucleus (Fig. 9E,F). A large number of Fos-like im-

munoactive cells were stained in the reticular nucleus, in the “shell” of intralaminar nuclei surrounding the parataenial nuclei, and in the intralaminar midline between the mediodorsal nuclei on both sides. In contrast, the parataenial nuclei and the medial segment of the mediodorsal nucleus were essentially free of Fos-like immunoreactivity (Fig. 10E,F).

Type IV status. Type IV status was induced in just three rats, but the pattern of ^{14}C -2-deoxyglucose uptake was identical in each case. This pattern involved the complete neural substrate associated within type III status, plus the entire dorsolateral neocortex, neostriatum, hippocampal formation, and most of the principal thalamic relay nuclei (Fig. 11). However, most regions of the anterior hypothalamus and brainstem, including the superior colliculus, showed no elevation of ^{14}C -2-deoxyglucose uptake. This pattern was bilaterally symmetrical in each case. No Fos immunocytochemistry data are available for this group.

Figure 8. Comparison of ^{14}C -2-deoxyglucose uptake during status epilepticus with the distribution of efferent fibers from the amygdala and piriform cortex. *A* and *B*, Patterns of ^{14}C -2-deoxyglucose uptake in the rostral forebrain during type II (*A*) and type III (*B*) status. In type II status there is activation of the dorsal endopiriform nucleus, the medial nucleus accumbens, and the deep layers of the agranular insular cortex, while in type III the entire amygdalo-recipient zone of the ventral striatum, piriform cortex, and insular areas all display high levels of ^{14}C -2-deoxyglucose uptake. *C* and *D*, The distribution of fibers from the basal amygdaloid nucleus (*C*) or piriform cortex (*D*), labeled by injections of the anterograde tracer ^3H -leucine. Note that the pattern of ^{14}C -2-deoxyglucose uptake in and around the rhinal sulcus during type II status matches the distribution of fibers from the basal amygdaloid nucleus, but there is no corresponding activation in the amygdaloceptive ventral striatum (nucleus accumbens and olfactory tubercle). In contrast, during type III status the pattern of ^{14}C -2-deoxyglucose uptake matches the entire, combined distribution of the amygdaloid and piriform cortical projections. *E* and *F*, Comparison of ^{14}C -2-deoxyglucose uptake in the ventral hippocampal formation during type II status (*E*) with the projections from the basal amygdaloid nucleus labeled by an injection of ^3H -leucine (*F*). The pattern of ^{14}C -2-deoxyglucose uptake matches the distribution of amygdaloid projections to the ventral subiculum, lateral entorhinal area, and perirhinal cortex. (The experiments with injections of the ^3H -leucine in the basal amygdaloid nucleus or piriform cortex were kindly provided by Dr. James P. Ray; see Ray and Price, 1992.) For abbreviations, see Appendix. Scale bars, 1 mm.

Figure 9. The pattern of ^{14}C -2-deoxyglucose uptake associated with type III status. In addition to the structures activated in type II status (compare Fig. 6) increased ^{14}C -2-deoxyglucose uptake is found in the entire amygdaloid complex, olfactory cortex, and prefrontal, insular, and perirhinal cortex, the ventral hippocampal formation, the ventral striatum and ventral pallidum, and the substantia nigra. For abbreviations, see Appendix.

Figure 10. The distribution of Fos-like immunoreactive neurons associated with type III status in the same animal presented in Figure 9. With the exception of the parataenial and mediodorsal thalamic nuclei, and the substantia nigra pars reticularis, each structure that displays high levels of ^{14}C -2-deoxyglucose uptake contains many Fos-like immunoreactive cells (compare Fig. 9). In addition to those seen with type II status, Fos-labeled cells are distributed throughout the ventral dentate gyrus, the piriform cortex, and the perirhinal and insular cortical areas in the rhinal sulcus. The distribution of Fos-like immunoreactive cells is largely bilateral, while the pattern of ^{14}C -2-deoxyglucose uptake is mostly unilateral. For abbreviations, see Appendix.

Discussion

These studies have characterized the neural substrates of both subconvulsive and convulsive forms of status epilepticus with ^{14}C -2-deoxyglucose autoradiography and Fos immunocytochemistry. The major finding is that persistent focal seizure activity induced by the electrical stimulation of the amygdala or the olfactory cortex leads to the development of one of four discrete levels of self-sustaining status epilepticus. Each of these is consistently associated with the activation of a distinct constellation of anatomical structures. A summary of the different types of status epilepticus and their correspondence to similar seizure states described by others is presented in Table 3.

The subconvulsive forms of status (i.e., types I and II) primarily involve subdivisions of the amygdaloid complex and some of their efferent projections. In the least severe level of

status, type I, the amygdalohippocampal area is the only structure in the forebrain to display significantly elevated ^{14}C -2-deoxyglucose uptake and Fos-like immunoreactivity (Fig. 12A). The amygdalohippocampal area is also involved in type II status, but this seizure state is mainly supported by a network of additional structures centered in the basal nucleus of the amygdala (Fig. 12B). The more convulsive forms of status (i.e., types III and IV) require the further recruitment of widespread regions throughout the ventral forebrain. In type III status, the patterns of seizure-related activity include the same neural elements as in type II, plus the entire amygdaloid complex, ventral hippocampal formation, olfactory cortex, and ventral striatum, the prefrontal and insular cortical areas, related thalamic and hypothalamic structures, and the substantia nigra pars reticularis (Fig. 12C). Type IV status, the most severe and widespread form of status, involves the bilateral activation of the same

Figure 11. The bilateral pattern of ^{14}C -2-deoxyglucose uptake associated with type IV status. The areas of increased ^{14}C -2-deoxyglucose uptake include all of the same structures that had accumulated large amounts of ^{14}C -2-deoxyglucose in type III status (compare Fig. 9), with the addition of the entire dorsolateral neocortex and striatum, the "principal" thalamic nuclei, and the dorsal hippocampus.

Table 3. Summary of four types of limbic status epilepticus

Status type	Behavioral manifestations	EEG patterns	Anatomical substrates	Correspondence to other models of status epilepticus
Type I	None	Variable epileptiform discharges (1/2–10 sec)	Amygdalohippocampal area	State of "seizure refractoriness" during rapid kindling ^a ; immobile status ^{b,c,f}
Type II	Discontinuous exploratory activities	Regular epileptiform discharges (1/1–2 sec)	Same as type I plus basal amygdaloid nucleus and some of its efferent projections, restricted parts of olfactory cortex	Exploratory or ambulatory status ^{b,c,d,f}
Type III	Same as type II, with recurrent episodes of facial and forelimb clonus	Same as type II, with discrete episodes of ictal discharges (>10 Hz)	Same as type II plus entire amygdaloid complex and olfactory cortex, ventral hippocampus, prefrontal cortex, ventral striatum, substantia nigra	Masticatory status ^{b,c,d,f} ; "score 4" EEG pattern ^{c,g}
Type IV	Continuous facial and forelimb clonus, with generalized clonus	Continuous ictal discharges	Same as type III plus entire neocortex, striatum, and "principal" thalamic relay nuclei	Clonic status ^{b,c} ; generalized status ^c

References: ^a Ackermann et al. (1989); ^b Handforth and Ackermann (1988a); ^c Handforth and Ackermann (1988b); ^d Handforth and Ackermann (1992); ^e Lothman et al. (1989); ^f McIntyre et al. (1991); ^g VanLandingham and Lothman (1991a).

structures, plus the entire dorsolateral neocortex, neostriatum, and most of the principal thalamic relay nuclei. These anatomical observations provide the basis for further experiments aimed at identifying the foci underlying type II and type III status, which are described in the following article (White and Price, 1993).

Correspondence of ¹⁴C-2-deoxyglucose autoradiography and Fos immunocytochemistry

Although it has been suggested previously that the induction of the *c-fos* gene could be exploited as a metabolic marker of neural activity in conjunction with ¹⁴C-2-deoxyglucose autoradiography (Morgan et al., 1987; Sagar et al., 1988; Dragunow and Faull, 1989), few studies to date have used these methods together in the same experimental preparation (e.g., Sharp et al., 1989). By comparing the patterns of ¹⁴C-2-deoxyglucose uptake and Fos-like immunoreactivity, it may be possible to identify brain regions in which synaptic inhibition counteracts or overwhelms excitatory synaptic activity. This is because elevated ¹⁴C-2-deoxyglucose uptake largely represents increased synaptic

activity, whether excitatory or inhibitory (Sharp, 1976; Mata et al., 1980; Di Rocco et al., 1989), while localization of Fos identifies postsynaptic neurons that were activated mainly by depolarization (Dragunow and Robertson, 1988; Le Gal La Salle, 1988; Sonnenberg et al., 1989).

In each case with conjoint ¹⁴C-2-deoxyglucose autoradiography and Fos immunocytochemistry, most regions that displayed increased ¹⁴C-2-deoxyglucose uptake also contained many Fos-like immunoreactive cells. Thus, the overall patterns of ¹⁴C-2-deoxyglucose uptake and Fos-like immunoreactivity from the same animals displayed a remarkable degree of correspondence. However, discordant patterns of labeling were found in the paratenial and mediodorsal thalamic nuclei and the substantia nigra pars reticularis. Each of these regions exhibited robust ¹⁴C-2-deoxyglucose uptake during type III status, without displaying Fos-like immunoreactivity. Another recent study that used *in situ* hybridization also reported a lack of *c-fos* expression within the substantia nigra following kindled seizures (Labiner et al., 1993). These findings are consistent with the presence of intense synaptic inhibition in each structure during type III status. The

Figure 12. Schematic representation of the anatomical substrate of limbic status epilepticus, types I–III. Seizure-related activity is represented by shading and by arrows between anatomically connected structures. A, Type I status is associated with the activation of the amygdalohippocampal area and its efferent projection to the posterior portion of bed nucleus of the stria terminalis. B, Type II status is supported by the basal nucleus of the amygdala and most of its efferent connections to the cortex and thalamus. Epileptiform activity invades the lateral entorhinal area and the ventral subiculum, but not the dentate gyrus or other parts of the hippocampal formation. Only restricted parts of the olfactory cortex and ventral striatum are involved. C, Type III status is associated with the activation of the entire anatomical substrate of type II status plus the additional recruitment of the ventral hippocampal formation, the entire amygdaloid complex, olfactory cortex, prefrontal, insular, and perirhinal cortex, ventral striatum, and the substantia nigra pars reticularis.

metabolic demands of inhibitory neurotransmission could account at least in part for the accumulation of ¹⁴C-2-deoxyglucose (Ackermann et al., 1984), whereas hyperpolarization of postsynaptic neurons, the shunting of depolarizing postsynaptic currents, or the sporadic occurrence of epileptiform potentials could explain the negative immunocytochemical results. A similar explanation of seizure-related ¹⁴C-2-deoxyglucose uptake within the substantia nigra has been proposed previously (e.g., Gale and Browning, 1986; Ackermann et al., 1989).

This interpretation is supported by evidence that major inhibitory projections into each structure are activated during type III status. Neurons in the thalamic reticular nucleus that use GABA as their neurotransmitter provide a powerful source of recurrent inhibition to the relay nuclei of the thalamus, including the parataenial and mediodorsal nuclei (Houser et al., 1980; Kelley and Stinus, 1984; Steriade and Deschênes, 1988; Chen and Su, 1990). In both type II and type III status, elevated ¹⁴C-2-deoxyglucose uptake and abundant Fos-like immunoreactive cells were found specifically in the rostral, ventral-medial part of the reticular nucleus, which is topographically related to the parataenial and mediodorsal nuclei (Jones, 1975; Kelley and Stinus, 1984; Chen and Su, 1990; Kuroda and Price, 1991). Likewise, the cells of origin of the GABAergic projection to the substantia nigra from the ventral striatum (Bunney and Aghajanian, 1976; Nagy et al., 1978; Preston et al., 1980) showed increased ¹⁴C-2-deoxyglucose uptake and significant Fos-like immunoreactivity in type III status.

On the other hand, it is difficult to exclude the possibility that neurons within these structures lack the cellular elements necessary for the induction of *c-fos* and related immediate-early genes during limbic seizure activity. In particular, excitatory bursting discharges have been observed within the substantia nigra pars reticularis during kindled limbic-motor seizures (Bonhaus et al., 1986, 1991). Thus, the high levels of ¹⁴C-2-deoxyglucose uptake and the absence of Fos-like immunoreactivity in the substantia nigra pars reticularis during type III status could either reflect the predominance of inhibitory neurotransmission or the failure of transient excitatory activity to induce the expression of *c-fos*.

In some regions of the brain, the opposite disparity was observed in which there was abundant Fos-like immunoreactivity but little or no specific increase in ¹⁴C-2-deoxyglucose uptake. This was found in most cases of type II or type III status, in which the patterns of ¹⁴C-2-deoxyglucose uptake were largely unilateral, while the distribution of Fos-like immunoreactivity was at least partly bilateral. The most likely explanation for this observation is that Fos immunocytochemistry may reflect transient increases in cellular activity better than ¹⁴C-2-deoxyglucose autoradiography. In this case, transitory propagation of epileptiform activity to the contralateral hemisphere might be sufficient to induce the expression of immediate-early genes, but too transient to result in the accumulation of significant amounts of ¹⁴C-2-deoxyglucose. Recently, McIntyre et al. (1991) reported a similar failure of ¹⁴C-2-deoxyglucose autoradiography to reflect EEG manifestations of status epilepticus in the hemisphere contralateral to the side of seizure induction. Thus, Fos immunocytochemistry may provide a more sensitive means of localizing the source of some epileptiform discharges.

Anatomical basis of limbic status epilepticus

The first two levels of status epilepticus defined here appear to be especially associated with two nuclei of the amygdala. It can

be suggested that these structures act as "epileptogenic foci," such that seizure activity in these nuclei drives or sustains epileptiform activity in other related structures. In type I status, the amygdalohippocampal area is the only region that displays evidence of persistent epileptiform activity, even though the electrical stimulation that induced seizure activity was applied at other sites and epileptiform activity propagated throughout widespread forebrain structures (Carnes and Price, 1987). Furthermore, the amygdalohippocampal area is always a participant in the more expansive levels of status epilepticus. Together, these observations indicate that the amygdalohippocampal area may have the lowest threshold of any structure in the limbic forebrain for the development and maintenance of status epilepticus activity. Moreover, because the site of electrical stimulation may not necessarily develop a focus of epileptiform activity, the induction of epileptogenic foci may be related more to the intrinsic properties and connections of neurons within discrete forebrain structures than to the level of applied stimulation or excitation. In a similar fashion, type II status is associated strongly with the basal amygdaloid nucleus, since this nucleus is always prominently activated and it has direct axonal connections to most of the other structures that participate in this level of status (see Fig. 12*B*). Because there were no instances in which the basal nucleus was engaged in epileptiform activity without the concurrent activation of the amygdalohippocampal area, the basal nucleus presumably has a higher threshold for the induction of an epileptogenic focus. Experiments reported in the following article test directly the hypothesis that the basal amygdaloid nucleus is the primary epileptogenic focus of type II and type III status (White and Price, 1993).

The pattern of EEG activity in type III status suggests that it may represent a more complex situation, which is not simply related to the presence of a localized epileptogenic focus. This type of status is characterized by the recurrent elaboration of well-defined episodes of fast "ictal" discharges, which emerge abruptly from a background of "interictal" activity like that seen in type II status. Such an explosive transition from the interictal to the ictal pattern suggests that type III status is associated with the development of additional instability within a neuronal circuit that promotes the rampant propagation of epileptiform activity throughout the ventral forebrain. The additional instability might be localized within the amygdala itself, but data from other studies suggest that the recruitment of the ventral hippocampal formation (including the entorhinal cortex) is critical for the widespread distribution of seizure activity throughout limbic circuits.

Combined electrophysiological and ¹⁴C-2-deoxyglucose autoradiographic studies have shown that stimulation of the entorhinal cortex leads to the emergence of limbic-motor seizures concurrently with the spread of epileptiform activity into the ventral hippocampus (Collins et al., 1983). More recently, Stringer and colleagues have described an intriguing paroxysm in the dentate gyrus, termed "maximal dentate activation," which may represent the full recruitment of the ventral hippocampal formation (Stringer and Lothman, 1989, 1990, 1992; Stringer et al., 1989, 1991). Indeed, these authors suggested that maximal dentate activation is a marker for the presence of "synchronized reverberatory epileptiform activity in hippocampal/parahippocampal circuits" (Stringer et al., 1991, p 341). They further speculated, as did Collins et al. (1983), that the dentate gyrus serves to gate the spread of epileptiform activity from the en-

torhinal cortex into the hippocampus (Stringer and Lothman, 1992).

The anatomical substrates of type II and type III status that were defined in this study support such a role for the dentate gyrus in "gating" the propagation of seizure activity. During type II status, the pattern of functional activation in the ventral temporal region involves the deep layers of the entorhinal cortex and the ventral subiculum, but not the dentate gyrus. In contrast, the anatomical substrate of type III status involves the entire entorhinal cortex, the dentate gyrus, and fields CA1–CA3 of the hippocampus. The recurrent development of discrete ictal episodes in type III status may be related to the spread of epileptiform activity from the amygdala into the entorhinal/subicular complex, which raises the level of activation to a degree capable of fully engaging the dentate gyrus and its projections through the hippocampus. If so, type III status may be distinguished from type II by an additional alteration in the excitability of hippocampal and parahippocampal circuits, such that the periodic invasion of epileptiform activity is more likely to occur. Propagation and amplification of epileptiform activity through the hippocampus and the entorhinal cortex would presumably lead to the sustained reverberation of activity among amygdaloid, olfactory, and ventral hippocampal structures (see White and Price, 1993).

Participation of amygdalostriatal projections in status epilepticus

The basal amygdaloid nucleus gives rise to fibers that terminate throughout most of nucleus accumbens and the olfactory tubercle (Krettek and Price, 1978a,b; Kelley et al., 1982; Fuller et al., 1987; Kita and Kitai, 1990). In spite of this, during type II status most of the ventral striatum failed to demonstrate elevated ^{14}C -2-deoxyglucose uptake and Fos-like immunoreactivity, which would indicate the presence of epileptiform activity. There are at least two possible explanations for this paradox. First, the subpopulation of amygdaloid neurons that gives rise to this striatal projection may not be active during type II status. This seems unlikely, simply because of the dense ^{14}C -2-deoxyglucose accumulation and the high proportion of Fos-like immunoreactive cells in the basal amygdaloid nucleus. Furthermore, McDonald (1991) recently demonstrated with double retrograde axonal tracers that a substantial portion of the amygdalostriatal fibers are axon collaterals of cells that project to regions of the medial and the lateral prefrontal cortex that displayed high levels of ^{14}C -2-deoxyglucose and many Fos-like immunoreactive cells during type II status.

An alternative explanation is that the activity of amygdalostriatal fibers is regulated within their terminal field, by the operation of some local neuromodulatory system. It has been suggested that dopamine has just such a modulatory role in the nucleus accumbens (Yim et al., 1991). Electrophysiological experiments have demonstrated that dopamine within the nucleus accumbens, applied either exogenously or by prior stimulation of dopaminergic projection neurons in the ventral tegmental area, attenuated the amplitude of EPSPs evoked by the stimulation of the basal amygdaloid nucleus (Yim and Mogenson, 1982, 1988). Parallel behavioral studies showed that the suppression of locomotion that followed activation of the basal nucleus was reversed by the infusion of dopamine into the nucleus accumbens (Yim and Mogenson, 1989). However, the present study did not find significant ^{14}C -2-deoxyglucose uptake

or Fos-like immunoreactive cells in the ventral tegmental area during type II status to demonstrate the specific activation of the "mesolimbic" dopaminergic system. Pharmacological and/or electrophysiological experiments are necessary to determine if dopamine or some other neuromodulator suppresses the activation of amygdalostriatal terminals in the ventral striatum during type II status.

Limbic-motor integration: the behavioral manifestations of status epilepticus

The two intermediate levels of status epilepticus (i.e., types II and III) are associated with a variety of subconvulsive or convulsive motor behaviors. However, these types of status primarily involve the "limbic system," and it is unlikely that the so-called "pyramidal" or "somatic" motor system is responsible for the behavioral manifestations of these seizure states. Motor cortical fields in the frontal and prefrontal regions did not display elevated ^{14}C -2-deoxyglucose uptake or significant Fos-like immunoreactivity in type II or type III status. Indeed, another study determined that these cortical fields are markedly *hypo*-metabolic in a seizure state that corresponds to type III status ("score 4" stage of VanLandingham and Lothman, 1991a). Instead, the behavioral manifestations of status epilepticus may be considered in terms of the indirect influences of limbic structures such as the amygdala on lower motor elements, mediated by descending projections to ventral and lateral regions of the brainstem (e.g., Price et al., 1987). Instead of directly eliciting explicit motor programs, these limbic projections appear either to trigger the expression of specific rhythmic motor patterns, or to alter the sensitivity of premotor and motor neurons to other descending or segmental inputs (see Holstege, 1991, for a recent comprehensive review).

The masticatory movements and excessive salivation that characterize mild facial clonus may be examples of specific limbic-motor interactions. It is likely that these activities are triggered by descending projections from the amygdala to the lateral tegmental field of the mesencephalon, pons, and medulla (Price et al., 1987). In particular, the central amygdaloid nucleus and the lateral part of the bed nucleus of the stria terminalis project to the supratrigeminal region, where premotor elements generate the motor program for the rhythmic contractions of mastication (Ohta and Moriyama, 1986; Takeuchi et al., 1988a,b; Holstege, 1991). Electrical stimulation of the amygdala induces a rhythm of alternating depolarizing and hyperpolarizing potentials in trigeminal motoneurons (Nakamura and Kubo, 1978). In addition, neurons in these same amygdaloid nuclei participate with the hypothalamus in the regulation of secretory parasympathetic outflow to the submandibular gland (Jansen et al., 1991). The propagation of epileptiform activity from the amygdala to the preganglionic neuronal pool within the medullary reticular formation could account for the heavy salivation associated with the development of facial clonus.

In addition to these effects, ictal activity within the limbic forebrain may lead to nonspecific change in excitability within premotor neuronal pools of the brainstem and spinal cord. Evidence for such modulatory influences comes in part from anatomical studies of the organization of descending projections from the amygdala and related parts of the hypothalamus to integrative centers in the periaqueductal gray and the lateral and ventral parts of the medullary tegmentum, some of which give rise to descending fibers that extend collaterals to all spinal levels

(Price et al., 1987; Holstege, 1991; Amaral et al., 1992). The highly diffuse organization of these projections from the brainstem suggests that they may exert a general effect on the activity of widespread motor and premotor neuronal elements, instead of evoking specific motor behaviors. Many of the descending projections from the periaqueductal gray use 5-HT as their neurotransmitter, which has been shown to modulate the excitability of spinal motoneurons and brainstem reticular formation neurons (McCall and Aghajanian, 1979; Elliott and Wallis, 1992; Stevens et al., 1992; Yomono et al., 1992). Such indirect limbic influences presumably increase the "gain" of premotor and motor neurons and thereby enhance the effects of direct excitatory inputs (McCall and Aghajanian, 1979; Cardona and Rudomin, 1983; Holstege, 1991; Kapp et al., 1992). The normal physiological role of these facilitatory interactions may be to exert a heightened state of arousal, in which the processing of sensory information and reflexive execution of conditioned responses are enhanced (e.g., fear-potentiated startle; LeDoux et al., 1988; Kapp et al., 1992; Davis, 1992).

Such enhancement of brainstem excitability may contribute to the exploratory locomotion of type II status and the generation of facial and forelimb clonus in type III status. In type II status, animals persistently meander about their cages in an automatic, side-to-side manner, except for momentary arrests of movement that coincide with the occurrence of each epileptiform discharge. The hypermobility of these animals may be the result of activation of the so-called "mesencephalic locomotor region" within the area of the pedunculopontine nucleus via projections from the central amygdaloid nucleus and the lateral part of the bed nucleus of the stria terminalis (Moon Edley and Graybiel, 1983; Holstege, 1991). Electrical stimulation of this brainstem area produces rhythmic stepping movements of the limbs through its connections with reticulospinal interneurons in the ventral medullary tegmentum (Shik and Orlovsky, 1976; Skinner and Garcia-Rill, 1984; Garcia-Rill and Skinner, 1987a,b). However, the coincidence of the single epileptiform discharges and the momentary arrests suggests that the induced rhythmic activity of the premotor elements within the mesencephalic locomotor region is overridden momentarily by other inputs that have a profound suppressive effect. Polysynaptic pathways from the amygdala and hippocampus through the nucleus accumbens and subpallidum have been reported to produce cessation of locomotion, and may be the source of these suppressive influences (e.g., Mogenson, 1987; Mogenson and Yang, 1991).

Similarly, the alternating clonic movements of the forelimbs in type III status may arise as descending inputs from the amygdala and hypothalamus increase the sensitivity of motor neurons within the cervical spinal cord to other inputs, possibly from muscle spindle afferents. The level of excitability may reach some threshold beyond which rhythmic oscillations in the firing patterns of motor elements are induced. In this view, forelimb clonus may be considered as a manifestation of a more general hyperreflexive condition within brainstem and spinal pattern generators. With this in mind, it is interesting to note that the "motionless-staring" posture assumed at the onset of ictal activity bears a striking resemblance to a decerebrate tonic-neck reflex (i.e., neck dorsiflexion with tonic flexion of one forelimb and tonic extension of the other). Further anatomical and physiological studies are necessary to determine the precise pathways and mechanisms by which forebrain seizure activity engages lower motor neuronal elements in the spinal cord.

Clinical correlations

Although there are obvious limitations in the application of experimental seizure models in the rat to human epilepsy, some speculation may be instructive. In particular, the clinical manifestations of type III status are similar to human seizure state known as complex partial status epilepticus, in which complex partial seizures occur repeatedly without complete mental clearing or a return to normal EEG activity between the ictal events (Gastaut and Tassinari, 1975; Treiman and Delgado-Escueta, 1983). Treiman and Delgado-Escueta (1988) have recognized two distinct behavioral and EEG phases that recur throughout most episodes of complex partial status epilepticus. One phase consists of a "continuous twilight state" with partial responsiveness and reactive automatisms. Associated with this phase are slow waves and low-voltage, fast activity in the electroencephalogram. These "interictal" patterns are interrupted by the other phase, which features discrete seizure events usually characterized by an arrest reaction and motionless staring, complete unresponsiveness, and orolimentary automatisms. The associated EEG pattern features medium-voltage, fast ictal discharges. The cycling of seizure activity between these two phases may correspond to the pattern of ictal activities observed in type III status, that is, the recurrent development of sustained seizures with facial and forelimb clonus from a background of single epileptiform discharges. If so, type III status in the rat could serve as a direct model of complex partial status epilepticus in humans.

Appendix

Abbreviations

Acc	nucleus accumbens
AHA	amygdalohippocampal area
AI _d	agranular insular cortex, dorsal division
AI _p	agranular insular cortex, posterior division
AON	anterior olfactory nucleus
B	basal nucleus of the amygdala
BNST	bed nucleus of the stria terminalis
CA1	hippocampal subfield CA1
CA3	hippocampal subfield CA3
C	central nucleus of the amygdala
Cl	claustrum
Co _p	posterior cortical nucleus of the amygdala
C/P	caudate/putamen
DG	dentate gyrus
DLO	dorsolateral orbital cortex
E	dorsal endopiriform nucleus
GP	globus pallidus
ic	internal capsule
IL	infralimbic cortex
L	lateral nucleus of the amygdala
LEA	ventrolateral entorhinal area
M	medial nucleus of the amygdala
MD	mediodorsal nucleus of the thalamus
NLOT	nucleus of the lateral olfactory tract
OT	olfactory tubercle
PAC	periamygdaloid cortex
PC _a	piriform cortex, anterior subdivision
PC _p	piriform cortex, posterior subdivision
PF	parafascicular nucleus of the thalamus
PL	prelimbic cortex
PR	perirhinal cortex
PT	parataenial nucleus of the thalamus
Rt	reticular nucleus of the thalamus
SI	substantia innominata
SN _r	substantia nigra pars reticularis
SUB	subiculum
TT _v	tenia tecta, ventral subdivision
VP	ventral-posterior nucleus of the thalamus

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