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An Inbred Epilepsy-Prone Substrain of BALB/c Mice Shows Absence of the Corpus Callosum, an Abnormal Projection to the Basal Forebrain, and Bilateral Projections to the Thalamus

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BALB/c mice lack a corpus callosum in about 11% of the population. Two inbred substrains of BALB/c mice, epilepsy-prone (EP) and epilepsy-resistant (ER), have been examined to determine whether these substrains differ in regard to corpus callosum morphology. Further, this study addressed the issue of whether misrouted cortical axons form an aberrant pathway instead of the corpus callosum. Initial studies that examined fresh brain tissue of adult animals revealed normal corpora callosa in all ER mice but deficient or absent corpora callosa in all EP mice. Subsequently, DiI crystals were placed in the motor cortices of aldehyde-fixed brains of 2-week-old animals to investigate cortical projections in both inbred substrains of mice. Fluorescent microscopy revealed that all of the ER animals had normal corpora callosa, whereas all EP animals exhibited either reduced corpora callosa (partially callosal) or an absence (acallosal) of this structure. Both acallosal and partially callosal EP mice displayed an extensive, aberrant projection to the basal forebrain as well as bilateral projections to midline and intralaminar thalamic nuclei. The fibers projecting to the basal forebrain arose from the cortex, coursed toward the midline before turning ventrally along the midline, and appeared to terminate in the medial septal nucleus and the nucleus of the diagonal band. ER animals lacked this aberrant cortical projection to the basal forebrain. Electron microscopic results obtained from EP mice indicated that labeled axons in this aberrant pathway formed axosomatic, axodendritic, and axospinous synapses with the neurons in the medial septal/diagonal band complex. The function of the aberrant projection to the basal forebrain remains unknown but it may provide an abnormal excitatory input to a region that provides cholinergic and GABAergic input to the cerebral cortex and hippocampus. The additional projections to midline and contralateral intralaminar thalamic nuclei in EP mice may function to intensify the synchronization of bilateral discharges.

The corpus callosum interconnects the cerebral hemispheres of all known mammalian species. However, apparently due to a genetic defect, the corpus callosum has been found to be absent from the brains of some animals. For example, Wahlsten (1989) showed that about 11% of the BALB/c inbred strain of mice are acallosal. This phenotype in BALB/c mice displays incomplete penetrance (Wahlsten, 1989).

Previous work has shown that aged BALB/c mice are susceptible to audiogenic seizures (Gates and Chen, 1975). An intensive series of selective breeding and subsequent inbreeding has produced two substrains of BALB/c mice; one was bred for audiogenic seizure susceptibility, the other for seizure resistance (Dolina, 1992; Dolina et al., 1993). During the selective breeding, the susceptibility of epilepsy-prone (EP) mice increased with each generation, and the susceptibility of the epilepsy-resistant (ER) animals decreased. The subsequent inbreeding significantly increased the number and severity of seizures in EP mice. Currently, 92–96% of EP mice exhibit severe audiogenic seizures that include tonic convulsions and terminate in coma, whereas virtually no ER mice display seizures.

Since EP and ER mice are derived from the same BALB/c strain but differ in their seizure susceptibility, they appear to provide a useful model for studies of neural mechanisms of epilepsy. A considerable effort has been directed toward understanding the role of neurotransmitters in this animal model of epilepsy. Cortical serotonin deficiency and an increased ratio of glutamate:GABA in the hippocampus, cerebellum, neocortex, and brainstem were found in EP mice as compared to ER mice (Vriend et al., 1991; Dolina et al., 1993). Further, we examined the GABAergic neurons in the inferior colliculus and found greater numbers in the EP mice as compared to ER mice (Dolina et al., 1992). During the course of this latter study, it was noted that each of four adult EP animals lacked a corpus callosum, whereas each of four age-matched ER mice showed a normal corpus callosum. These pilot data prompted the present, more complete, study.

The present study was conducted to determine if the corpus callosum is absent in the EP substrain of BALB/c mice. Further, we wanted to determine whether cortical axons that normally form the corpus callosum might be misrouted to form an aberrant pathway. If some aberrant pathways were formed, it would be interesting to determine whether the axons

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of these projections form synapses. Carbocyanine dyes, such as DiI, have been shown to be extremely useful in mice for studies of the development of the normal corpus callosum (Ozaki and Wahlsten, 1992). To our knowledge, however, no study using these dyes has yet been conducted that addresses the subcortical projections of acallosal mice. DiI provides excellent visualization of axonal projections in the fixed brain, and can be photooxidized into an electron-dense reaction product to obtain data at the electron microscopic level (Lubke, 1993).

Materials and Methods

Animals

Subjects were adults or offspring of ER or EP substrains as described previously (Dolina et al., 1993).

Gross Inspection

Adult male and female mice from the 17th and 18th generations of the inbred ER and EP substrains (127 mice; 101 ER and 26 EP) were lightly anesthetized with ether and decapitated with a guillotine. The brains were removed immediately and examined for the presence or absence of a corpus callosum by making a mid-sagittal section. Anterior–posterior lengths of the corpora callosa were measured with a millimeter scale bar using a dissecting microscope to view the brains.

DiI Studies

Eighteenth and nineteenth generations of inbred substrains of BALB/c mice (33 EP and 23 ER) were used (Dolina et al., 1993). Animals of 13–14 postnatal days of age were anesthetized with sodium pentobarbital (50 mg/kg) and perfused with 0.9% saline followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. The fixed brains were removed and stored in 4% paraformaldehyde for 1–4 weeks. Small crystals (approximately 0.1–0.2 mm) of DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) were placed in the motor cortex just under the cortical surface (0.5 mm) using fine forceps and a dissecting microscope. The brains were kept in 1% paraformaldehyde at 37°C for 3–4 weeks to allow for DiI labeling of cortical axons. Vibratome sections of 60 μm were cut in the coronal plane and serial sections were stored in a 2% paraformaldehyde solution at 4°C. Sections were examined using an epifluorescence microscope with a rhodamine filter cube. Drawings of sections were made and the pattern of labeling was plotted to show the full rostral–caudal extent of DiI-labeled fibers in the forebrain. Care was taken to compare DiI labeling patterns in brains from EP and ER mice that were killed and processed in parallel, and had placements of DiI that were similar in size and location.

Photooxidation and Electron Microscopic Examination

Sections that displayed DiI labeling of fibers in the basal forebrain were selected for photooxidation and subsequent electron microscopic examination. These sections were treated at 20 min intervals, first with a rinsing solution of 0.05 M Tris, and then with a 0.1% diaminobenzidine (DAB) solution (10 mg of DAB in 10 ml of 0.05 M Tris at pH 7.6). The DAB-soaked tissue sections were illuminated with ultraviolet light from a Zeiss epifluorescence microscope with either a 20× or 40× objective, to convert the DiI fluorescence into a dark brown reaction product. This electron-dense reaction product allowed for the subsequent identification of labeled fibers using electron microscopy (Lubke, 1993). The photooxidized sections were processed for electron microscopy using a routine protocol including postfixation with osmium tetroxide, dehydration in alcohols, and embedding in Medcast. Thin sections from the medial septal/diagonal band complex were prepared and examined with a Philips CM10 electron microscope.

Results

Gross Inspection

Each of the 101 ER mice examined by gross inspection exhibited a normal corpus callosum, averaging approximately 4 mm in length (Wahlsten, 1989). In 20 of the 26 EP animals used for the gross inspection study, the corpus callosum was completely absent; the remaining six EP animals exhibited a partial corpus callosum. In these latter cases, the anterior–posterior length and dorsal–ventral thickness of the corpus callosum were less than that for ER mice.

DiI studies

Overview

Placement sites of DiI crystals in the cerebral cortex were identified by intense red fluorescence that included all of the cortical layers and a small portion of the underlying white matter. Areas that displayed intense fluorescence varied with the size of the initial DiI crystal and the length of diffusion time, but ranged in diameter from approximately 2 to 3 mm. Diffusion did not spread to the contralateral hemisphere or to the striatum.

Comparisons were made of the distribution of DiI labeling in EP and ER brains that were matched for size of DiI placement and for length of diffusion time. Results are presented in the photomicrographs found in Figures 1–3 and in the line drawings of representative sections shown in Figure 4.

DiI Labeling in Brains of ER Mice

Twenty-three ER mice were used for DiI labeling. Prominent DiI-labeled fibers were seen to leave the placement site and course medially to reach the corpus callosum (Fig. 1*A*). Labeled fibers were found in the anterior third of the corpus callosum, concentrated in its dorsal half. In ER mice, the sensorimotor cortices contralateral to the DiI placement site displayed many labeled axons in the subcortical white matter, many retrogradely labeled pyramidal neurons

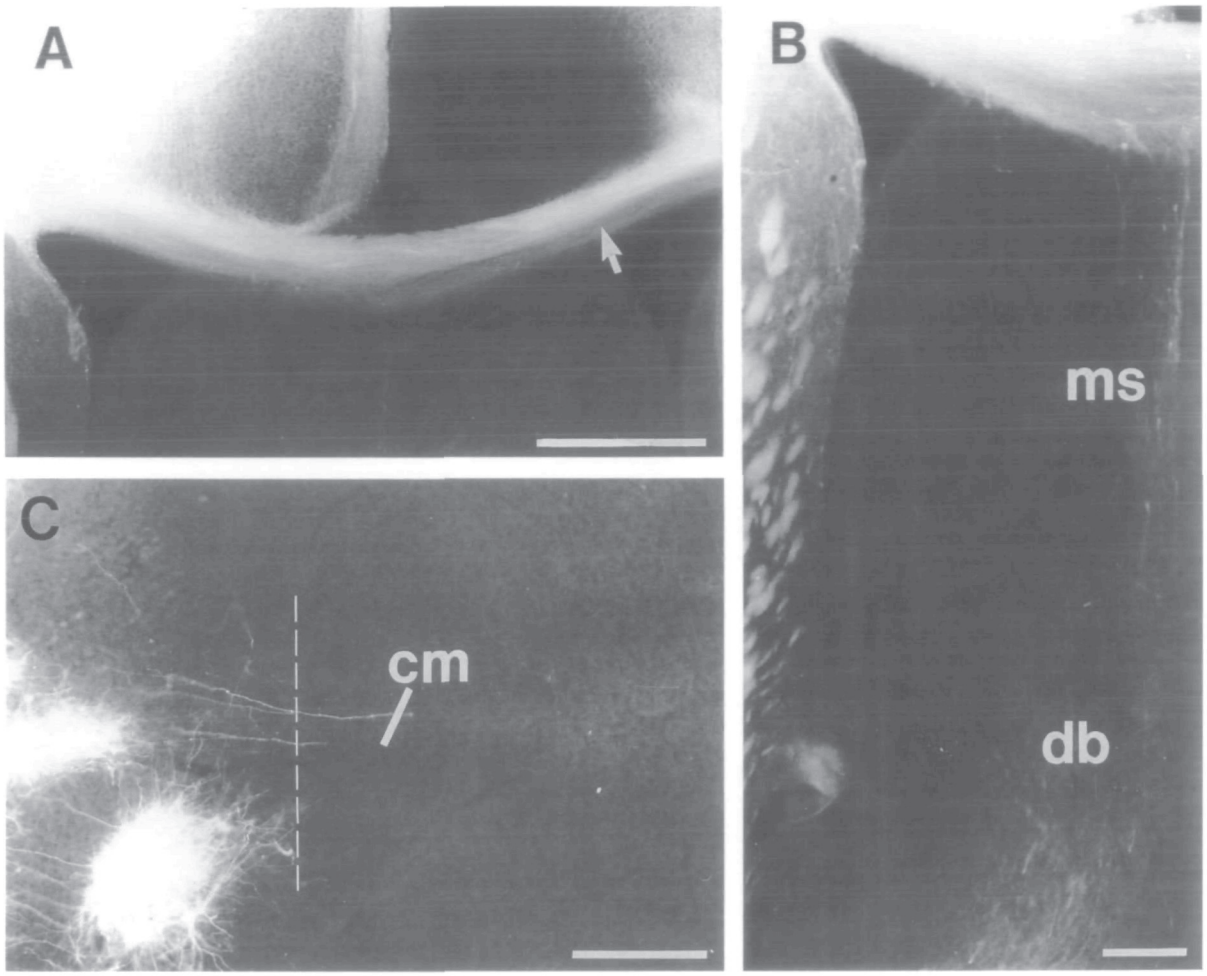

Figure 1. Fluorescent photomicrographs of DiI labeled coronal sections from an ER mouse. *A*, DiI labeling of fibers in the corpus callosum (*arrow*) and absence of labeled fibers in the medial septum. *B*, Medial septum (*ms*) and nucleus of the diagonal band (*db*) of an ER mouse; very few axons are seen. *C*, DiI labeling of corticofugal axons in the thalamic central medial (*cm*) nucleus, a few of which cross the midline (*vertical dashed line*). Scale bars: *A*, 500 μm; *B*, 250 μm; *C*, 200 μm.

scattered throughout the cortical layers, along with apparent axonal terminal fields (Fig. 4*A*, a–d).

DiI-labeled subcortical projections in ER mice included axons in the internal capsule, which terminated in the reticular, ventral posterolateral (VPL), and ventral posteromedial (VPM) nuclei of the thalamus (Fig. 4*A*, c, d). A few labeled axons were found in the central lateral, central medial, and reuniens nuclei of the thalamus (Fig. 1*C*). The striatum and the bed nucleus of the stria terminalis also were labeled (Fig. 4*A*, a–d). Retrogradely labeled neurons were found in the dorsal thalamus, including the VPL and VPM, as well as the posterior and central lateral nuclei. Retrogradely labeled neurons also were found in the basal forebrain, including the medial globus pallidus and the substantia innominata. A very few fibers and occasionally a retrogradely labeled neuron were seen in the nucleus of the diagonal band (Fig. 1*B*). These patterns of afferent and efferent projections are similar to those previously described for the rodent sensorimotor cortex (Wise and Jones, 1976; Stanfield, 1992). Descending projections to lower brainstem and spinal cord structures were not investigated in these studies.

DiI Labeling in Brains of EP Mice

Twenty-five of the 33 EP mice in the DiI labeling study lacked corpora callosa. The corpus callosum in each of the remaining eight EP mice was markedly reduced in size. In cases with partial corpora callosa, DiI-labeled fibers coursed medially from the placement site and reached the midline. Labeled axons could be seen crossing the midline via the corpus callosum (Fig. 2*A*). However, markedly fewer fibers crossed the midline compared to the corpus callosum in an ER animal (compare Figs. 1*A*, 2*A*). As labeled fibers reached the midline, many of them appeared to course ventrally to the medial septum and nucleus of the diagonal band (Figs. 2*B*; 4*C*, a, b). A few retrogradely labeled pyramidal neurons and apparent axonal terminal fields were seen in the contralateral cortex.

The 25 acallosal EP animals displayed no labeled fibers crossing the midline (Figs. 3*A*, 4*B*). Labeled axons were seen to exit the DiI placement site and course in a medial direction. As these labeled axons reached the midline, they turned ventrally and coursed along the medial wall of the hemisphere. These axons appeared to end in a terminal field in the medial septal nucleus and the nucleus of the diagonal band

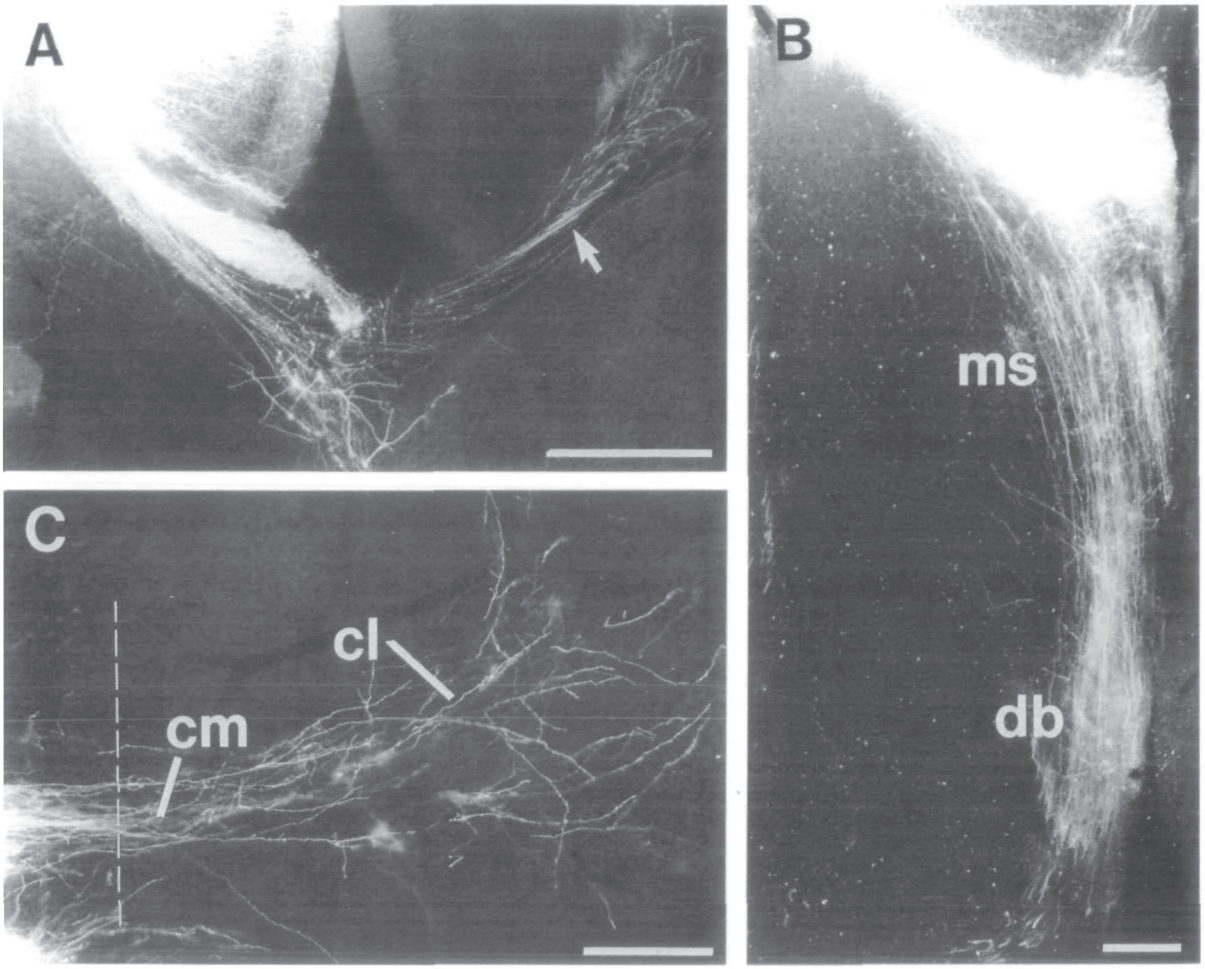

Figure 2. Fluorescent photomicrographs of DiI-labeled coronal sections from a partially callosal EP mouse. *A*, DiI labeling of fibers in the corpus callosum (*arrow*) shows a few axons crossing the midline and many labeled fibers coursing toward the medial septum. *B*, Medial septum (*ms*) and nucleus of the diagonal band (*db*) of an EP mouse; note the dense bundle of labeled axons. *C*, DiI labeling of corticofugal axons crossing the thalamic midline (*vertical dashed line*) to enter the central medial (*cm*) and central lateral (*cl*) nuclei of the contralateral hemisphere. Scale bars: *A*, 500 μm; *B*, 250 μm; *C*, 200 μm.

of the basal forebrain (Figs. 3*B*; 4*B*, a, b). The number of fibers coursing ventrally seemed to be inversely correlated with the number of fibers in the corpus callosum. For example, partially callosal EP mice had fewer labeled fibers coursing toward the basal forebrain compared with acallosal EP mice. For both partially callosal and acallosal EP mice, other subcortical labeling included axonal labeling in the internal capsule that terminated in VPL and VPM of the thalamus and the striatum. EP mice showed more extensive labeling of fibers in the thalamus, especially to the midline nuclei, than ER mice (compare Figs. 1*C*; 2*C*; 4*A–C*, sections d). A considerable number of axons coursed through the central medial nucleus to extend into the contralateral central lateral nucleus. The numbers of axons projecting contralaterally was always lower than that found in the ipsilateral nuclei of the thalamus. Similar to ER mice, labeled somata and dendrites were found in the dorsal thalamus, substantia innominata, and nucleus of the diagonal band (Figs. 2*B*, 3*B*). No retrogradely labeled neurons were seen in the anterior thalamic nuclei.

Electron Microscopic Analysis of Labeled Axons in the Medial Septal/Diagonal Band Complex

Acallosal and partially callosal EP mice were selected for study at the electron microscopic level. Electron microscopic examination of the medial septal/diagonal band complex revealed many axons containing electron-dense reaction product. Labeled axon terminals were analyzed in adjacent serial thin sections to confirm the presence of synaptic vesicles because the reaction product was very dense and obscured the vesicles in some sections. The adjacent sections were also helpful for the identification of synapses because the labeling often covered the pre- and postsynaptic membranes. Labeled axon terminals formed asymmetric synapses with cell bodies, dendrites and spines (Fig. 5*A–C*). At these locations, many nonlabeled axon terminals were observed forming asymmetric synapses (Fig. 5*A*). A few labeled somata and dendrites were found in the medial septal/diagonal band complex. The somata had numerous nuclear infoldings and an organelle-rich perikaryal cytoplasm (Fig. 6) similar to the features described for cholinergic and

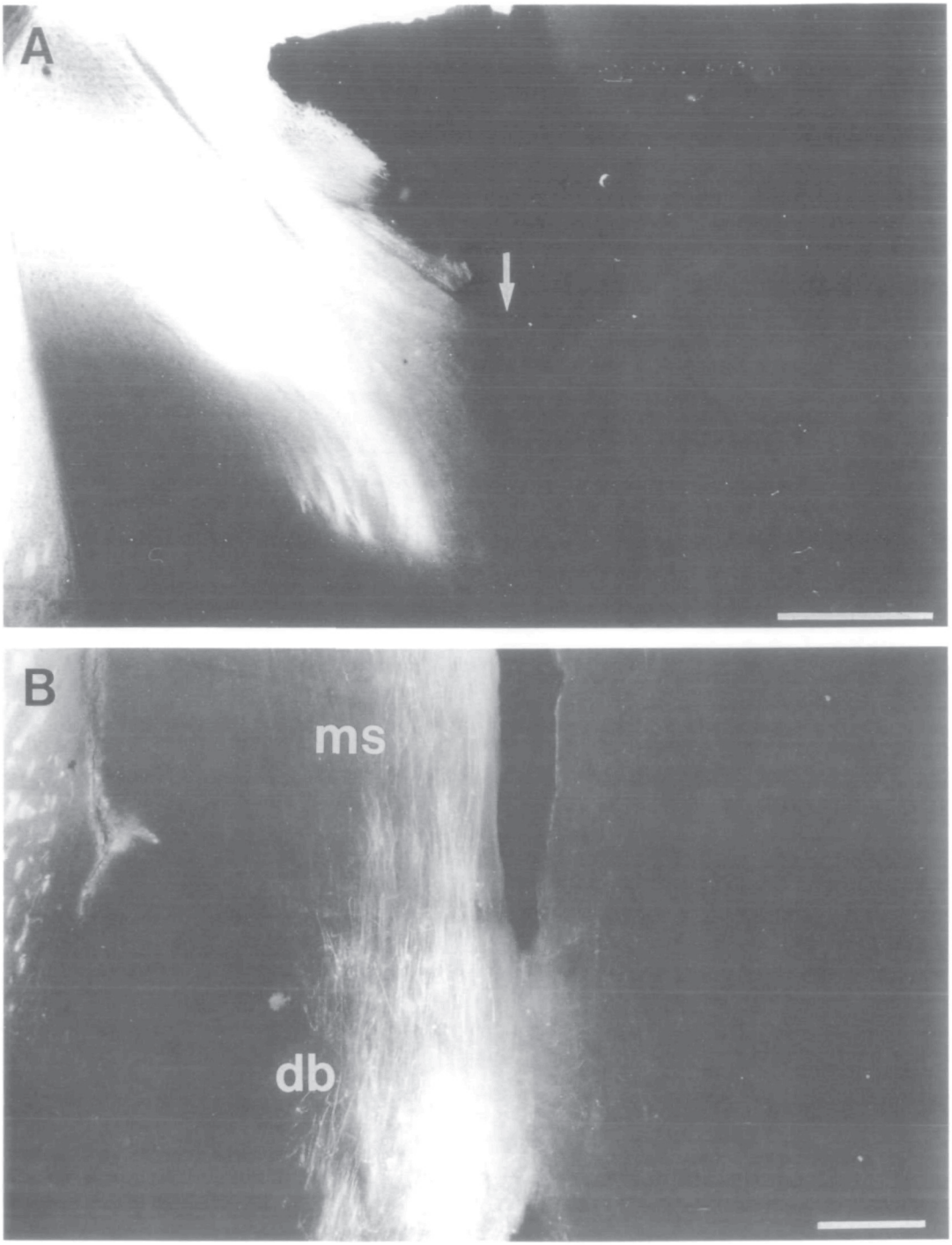

Figure 3. Fluorescent photomicrographs of DiI-labeled coronal sections from an acallosal EP mouse. *A*, DiI labeling of fibers shows axons coursing toward the midline (*arrow*), but they do not cross it. *B*, Medial septum (*ms*) and nucleus of the diagonal band (*db*) of this EP mouse, showing prominent labeling of axons. Scale bars: *A*, 350 μm; *B*, 250 μm.

GABAergic projection neurons in this region by Naumann et al. (1992). Labeled dendrites were aspinous and postsynaptic to many axon terminals, including some labeled ones.

Discussion

Three important findings were demonstrated in these studies. First, the inbred EP substrain of BALB/c mice showed a high percentage of acallosal brains whereas mice from the inbred ER substrain all displayed normal corpora callosa. Second, the somatosensory cortices of all EP mice had a prominent aberrant subcortical projection to the medial septum and nucleus of the diagonal band and bilateral projections to the midline and intralaminar nuclei of the thalamus. Third, electron microscopy of the axons in the aberrant pathway to the basal forebrain showed that they form asym-

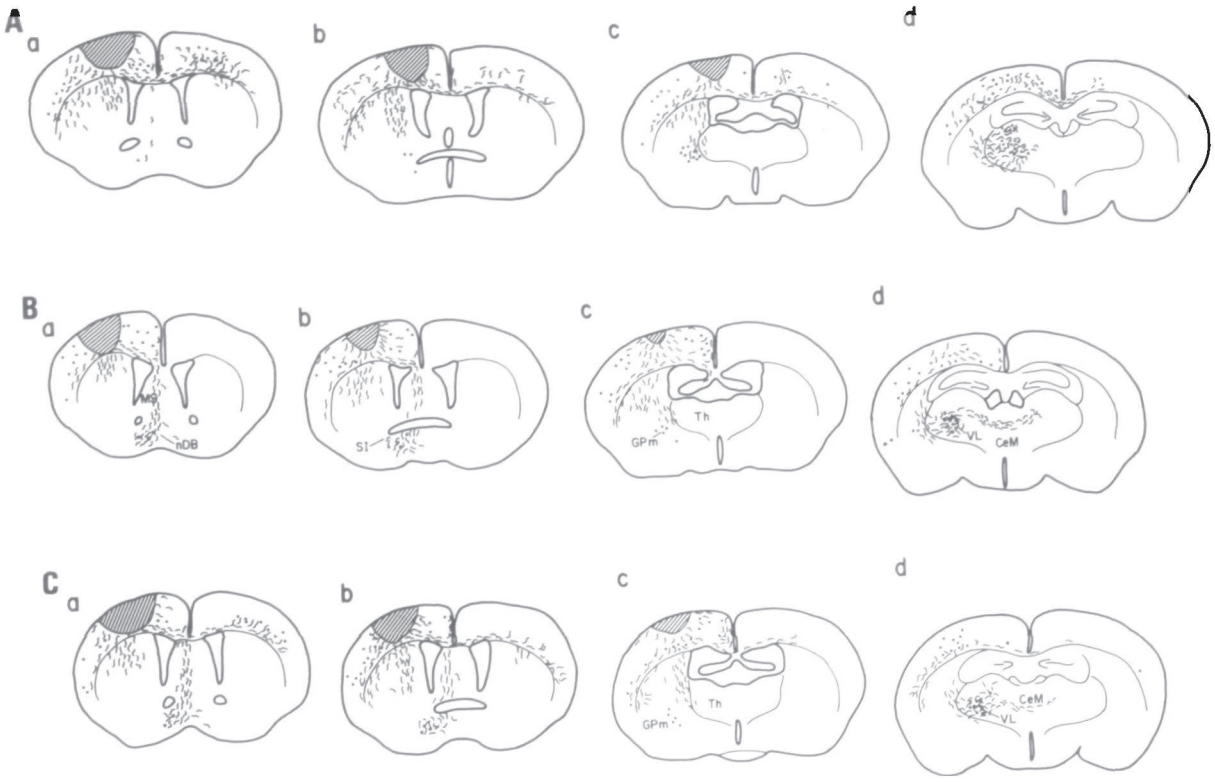

Figure 4. Line drawings of coronal brain sections showing the placement sites of DiI crystals (*hatched area*) and subsequent labeling of fibers (*lines*) and neuronal somata (*dots*) in the ER (*A*), acallosal EP (*B*), and partially callosal EP (*C*) mice. All of the placement sites included a similar size region of the cerebral cortex (compare *a–c* in *A–C*). Only the ER and partially callosal EP mice showed labeled fibers and somata in the contralateral cortex (*a–d* in *A* and *C*). Both partially callosal (*C*) and acallosal (*B*) EP mice displayed an aberrant projection to the medial septum (*MS*) and nucleus of the diagonal band (*nDB*) but had typical projections to the thalamus (*Th*), including the ventrolateral nucleus (*VL*). Additional thalamic labeling occurred in the central medial thalamic nucleus (*CeM*) of the acallosal and partially callosal EP mice. This latter projection was not observed in sections from ER mice. Retrogradely labeled cells were located in the substantia innominata (*SI*), thalamus (*Th*) and globus pallidus (*GPm*) of all mice.

metric synapses with somata, dendrites, and spines in the medial septal/diagonal band complex.

Percentage of Acallosal Mice in the Two Inbred Substrains of BALB/c Mice

Data from the gross brain inspection and the DiI labeling studies indicated that 76% of EP mice were acallosal (see data summarized in Table 1) and the remaining EP mice showed a markedly reduced corpus callosum. None of the EP mice had a normal corpus callosum. In contrast, all of the ER mice displayed a normal corpus callosum. In comparison with the 11% acallosal occurrence reported for the BALB/c strain (Wahlsten, 1989), the EP substrain of mice showed a much greater percentage of acallosal animals, whereas the ER substrain showed a much lower percentage. Thus, the inbreeding for seizure susceptibility separated the BALB/c strain into normal callosal and defective callosal substrains.

Table 1
Numbers of epilepsy-resistant (ER) and epilepsy-prone (EP) BALB/c mice studied for the presence, absence, or deficiency of a corpus callosum

	Corpus callosum		
	Present	Absent	Partial
Gross inspection (total of 127 animals)			
ER	101	0	0
EP	0	20	6
DiI microscopy (total of 56 animals)			
ER	23	0	0
EP	0	25	8

The Aberrant Subcortical Projection to the Medial Septum and Nucleus of the Diagonal Band

The absence of a normal corpus callosum in EP mice is associated with an aberrant cortical projection to the medial septal/diagonal band complex of the basal forebrain. Previous studies indicated that cortical projections in acallosal mice entered the dorsomedial portion of the septum (King, 1936; Ivy and Killackey, 1981b; Silver et al., 1982), but none of these studies described projections to the nucleus of the diagonal band or the presence of synapses formed by axons in this aberrant pathway. Both experimentally induced and genetically acallosal animals were used in these studies. Recent studies in rodents (e.g., Ivy and Killackey, 1981a; Olavarria and Van Sluyters, 1985) have indicated that considerably more cortical neurons send axons across the corpus callosum in infants than in adults. These studies further indicate that this initially exuberant projection is modified during early postnatal development, in that many of these cortical neurons retract their callosal axons. This process of retraction appears to be completed during the second

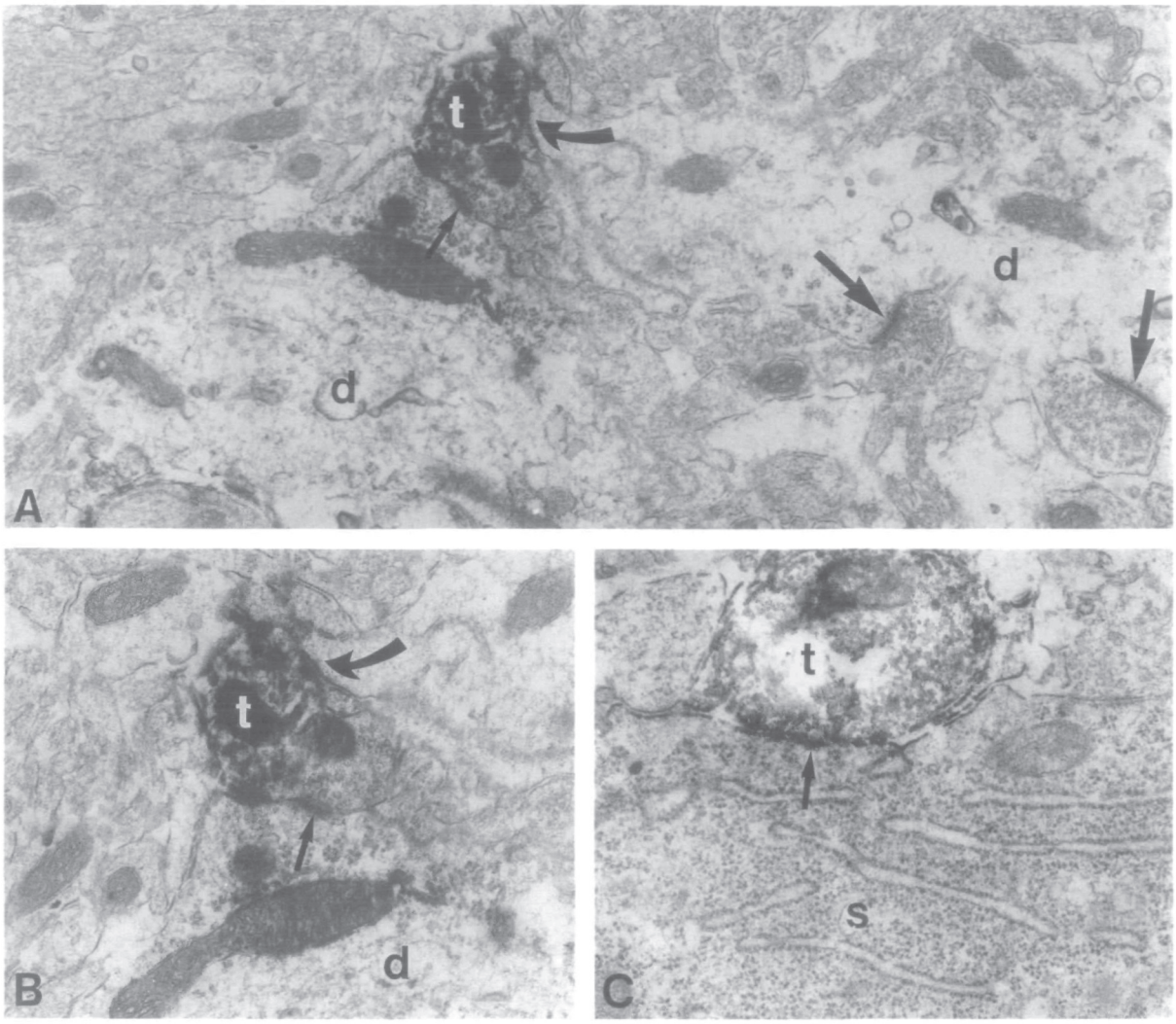

Figure 5. Electron micrographs from the vertical limb of the diagonal band of EP mice. *A* shows a labeled axon terminal (*t*) forming asymmetric synapses with adjacent dendrites (*d*). One synapse is with a dendritic shaft whereas the other appears to contact a spine that arises from the dendrite on the right. The synapses (*curved* and *small arrows*) are characterized by clustering of synaptic vesicles along the presynaptic membrane. Two unlabeled axon terminals form asymmetric axodendritic synapses (*large arrows*) with the same dendrites. *B* (*curved* and *small arrows*) shows the same two dendrites and the labeled axon terminal at a higher magnification. *C* shows an axosomatic synapse (*arrow*) formed by a labeled axon terminal (*t*) with a neuronal soma (*s*). Magnification: *A*, 22,000×; *B*, 28,000×; *C*, 29,000×.

postnatal week, and thus it seems unlikely that the prominent projection to the basal forebrain observed in the present studies would represent an exuberant, and transient, projection.

The cause of the aberrant projection is not understood. One possibility is that axons of cortical pyramidal cells that would form the corpus callosum are misguided into the basal forebrain. Perhaps the cells that in normal animals form the glial sling that guides cortical fibers across the midline are absent in EP animals (Silver et al., 1993). A glial sling that serves as a guide for growing callosal axons normally develops at the level of the septum early in fetal development. The observation that the aberrant projection to the basal forebrain in EP mice appears to arise in the region of the dorsal septum is supportive of the notion that absence of a glial sling results in misrouting of axons ventrally. Studies of fetal material to determine whether the glial sling develops in animals of the EP strain would be of great interest.

The presence of labeled fibers forming synapses in the medial septal/diagonal band complex of both acallosal and partially callosal EP animals suggests that this aberrant pathway may be functionally active and may demonstrate unique influences that have not yet been investigated in this model of genetic epilepsy. That is, the excitatory projection from the cerebral cortex that normally extends across the corpus callosum appears to be misguided to the basal forebrain, perhaps resulting in an aberrant cortical excitatory input to basal forebrain neurons. Experimental evidence indicates that populations of basal forebrain neurons, including those in the medial septum, diagonal band, and substantia innominata, provide cholinergic and also GABAergic projections to the neocortex and hippocampus (Freund and Antal, 1988; Gritti et al., 1993). The GABAergic projection selectively innervates GABAergic neurons in the Ammon's horn and dentate gyrus (Freund and Antal, 1988). It has been suggested that stimulation of GABAergic

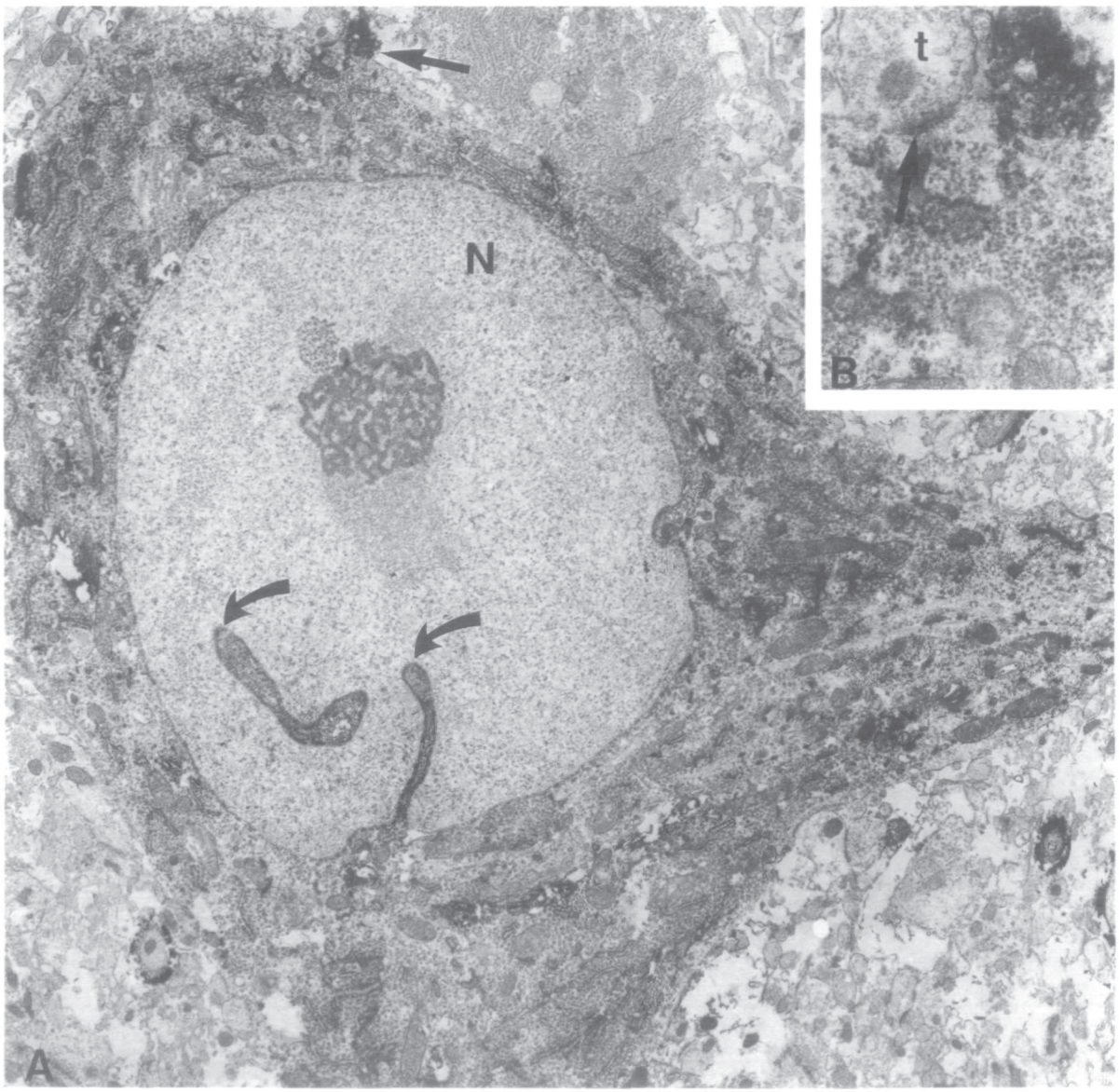

Figure 6. Electron micrographs of a retrogradely labeled neuron in the medial septal/diagonal band complex from an EP mouse. *A* shows that the photooxidized DiI reaction product is concentrated in the perikaryal cytoplasm but is absent from the nucleus (*N*). Note that the nucleus displays numerous infoldings (*curved arrows*). Two axon terminals (*straight arrow*) adjacent to this neuron are enlarged in *B*, which shows an unlabeled axon terminal (*t*) that forms a synapse (*arrow*) with this soma. A labeled terminal lies next to it but does not form an axosomatic synapse in this section. Magnification: *A*, 7000×; *B*, 27,000×.

neurons in the medial septum could disinhibit hippocampal neurons (Freund and Antal, 1988). Since the aberrant projection to the basal forebrain from the cerebral cortex in EP mice forms asymmetric synapses, a type that is thought to be excitatory (see pp 176–178 of Peters et al., 1991), some of the postsynaptic septal neurons may possibly drive this disinhibitory circuit and cause increased excitability in the hippocampi of EP mice.

Other Subcortical Projections in EP Mice

Although both ER and EP mice displayed similar ipsilateral projections to specific relay nuclei of the thalamus (VPL and VPM), the EP mice showed a bilateral projection to the midline and intralaminar nuclei. Crossed corticothalamic projections have been noted previously (Swanson and Cowan, 1977; Kaitz and Robertson, 1981), but they appear to originate in normal animals from medial or cingulate cortex. In the present experiments, placements of DiI involved somatosensory cortex and did not include the cingulate region, as was evidenced by the absence of retrograde labeling in the anterior thalamic nuclei of these cases. Therefore, this projection represents a novel projection to midline and intralaminar nuclei for these EP mice. Labeled axons in the contralateral thalamus of EP mice did not enter any of the specific relay nuclei.

Functional Relationship of Acallosal Brains to Epilepsy

Before discussing the role of the acallosal brain and the spread of epileptic activity in the forebrain of

animals, it is important to note that the inbred EP substrain of BALB/c mice was bred for its susceptibility to audiogenic seizures. In other rodents with audiogenic seizure sensitivity, such as the genetically epilepsy-prone rats (GEPRs), audiogenic seizures continue to occur despite the removal of the cerebral cortex (Kesner, 1966; Wada et al., 1970). Furthermore, the structures for initiation and propagation of audiogenic seizures in GEPRs appear to involve mainly brainstem structures, including the cochlear nuclei, lateral lemniscus, inferior colliculus, superior colliculus, and the reticularis pontine oralis nucleus of the reticular formation (Browning, 1986; Ribak et al., 1994). Thus, the role that the aberrant cortical projections to the basal forebrain play in the generation and spread of epileptic activity in EP mice remains unclear.

The fact that all inbred mice of the EP substrain are acallosal or partially callosal and all ER mice have corpora callosa is counterintuitive to our notion of the spread of epileptic activity between the cerebral hemispheres. In humans, sectioning of the corpus callosum was introduced 50 years ago as a palliative, surgical treatment for intractable epilepsy (Van Wagenen and Herren, 1940), and was subsequently confirmed by others (Wilson et al., 1977; Blume, 1984; Gates et al., 1984). However, other data suggest an inhibitory role of the corpus callosum, in that some patients experience more intense focal seizures after sectioning of the corpus callosum (Spencer et al., 1984).

The experimental animal data on the role of the corpus callosum in epilepsy are also inconclusive. Early work by Erickson (1940) and Sapirstein (1941) showed that the corpus callosum was a major pathway for secondary generalization of focal epileptiform discharges because callosotomy prevented the spread of excitation to the opposite cerebral hemisphere. Later studies using pentylentetrazole and penicillin as convulsants indicated that the corpus callosum served to synchronize interhemispheric widespread discharges in cats and monkeys (Marcus et al., 1968, 1969; Musgrave and Gloor, 1980). Recently, these results were confirmed in the lithium-pilocarpine model of epilepsy in rats where callosotomy prevented the development of electrographic seizures, status epilepticus, and death (Hirsch et al., 1992b). However, other experimental data indicate that the corpus callosum may serve an inhibitory function. For example, Eidelberg (1969) showed that activation of the corpus callosum could have an inhibitory influence on the contralateral cerebral cortex. Studies on experimental models of epilepsy using either kainic acid or amygdala kindling support this notion because sectioning of the corpus callosum had either no effect or a facilitatory effect on seizure development (Wada and Sato, 1975; McIntyre et al., 1986; Hirsch et al., 1992a). The results of these experimental studies indicate that callosotomy has no effect on the progression of temporal lobe seizures that involve the hippocampus whereas generalized seizures involving the neocortex are altered by this surgery.

Finally, another mechanism may exist in the EP substrain that could contribute to its seizures. All EP mice displayed a bilateral cortical projection to the midline and intralaminar nuclei of the thalamus. This projection to a region of the thalamus that has widespread cortical projections could lead to cortical synchrony. Experimental data by Jasper and Droogleever-Fortuyn (1947) support this notion because sectioning of the corpus callosum had no effect on bilateral cortical responses elicited by thalamic stimulation, whereas these responses were abolished by sectioning of the massa intermedia. Later, Penfield and Jasper (1954) proposed the centrencephalic hypothesis of epilepsy in which they suggested that the midline-diencephalic structures could synchronize the discharge of the two cerebral hemispheres. Andersen and Andersson (1968) have evidence that the thalamus is a pacemaker for the cortex and may generate spontaneous rhythms independently of the cerebral cortex. It is interesting to note that in a clinical study by Unterharnscheidt et al. (1968), 8 of 15 patients with callosal agenesis showed clear-cut bilateral synchrony. Therefore, the exuberant corticothalamic projections in EP mice may be one of the critical factors providing bilateral generalization of epileptic seizures.

In conclusion, the EP and ER substrains of BALB/c mice provide a unique model of genetic epilepsy. The presence of epileptic activity associated with acallosal and partially callosal brains provides an excellent opportunity to analyze physiological mechanisms that underlie epileptogenesis.

Notes

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