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A Monoclonal Antibody to Limbic System Neurons

Abstract. *A monoclonal antibody produced against hippocampal cell membranes labeled the surface of neurons in the rat limbic system. With a few exceptions, all nonlimbic components were unstained. This specific distribution of immunopositive neurons provides strong evidence of molecular specificity among functionally related neurons in the mammalian brain and supports the concept of a limbic system.*

The limbic system, a group of interrelated brain areas, was first described by Broca (*1*) more than 100 years ago. Fifty years later, Papez (*2*) hypothesized that the limbic system is the neuroanatomical substrate of emotion. The organization of the limbic system and other functional neural systems is continually being redefined in anatomic and physiological terms. One reason for this may be the disagreement over how the parts of a system become interrelated and, indeed, what constitutes a functional system in the brain.

If limbic structures do become integrated into a functional system then they might also be expected to display a unique relation at the molecular level, ultimately adding a new dimension to structure-function relations in the central nervous system (CNS). This now can be examined with new molecular approaches. For example, monoclonal antibodies generated thus far have revealed the molecular heterogeneity of brain cell types (*3*), chemical gradients in the developing CNS (*4*), and adhesion factors that may be involved in axon growth (*5*). We are developing monoclonal antibodies that are specific for cell classes or systems in both the differentiating and mature CNS, and have recently produced a monoclonal antibody that demonstrates molecular specificity at the systems level. The antibody, described in this report, exposes an antigenic determinant found almost exclusively in cortical and subcortical regions composing the limbic system.

Monoclonal antibodies were produced by conventional procedures (*6*). BALB/c mice were immunized three times with a crude membrane preparation (0.5 mg per injection) obtained from adult rat hippocampus. After fusion of the mouse spleen cells with the myeloma cell line NS-1, cells were distributed into 96-well plates containing HAT (hypoxanthine, aminopterin, and thymidine) selection medium. Screening for antibodies was performed 10 to 14 days after fusion by indirect immunofluorescence on paraformaldehyde-fixed cryostat sections through the hippocampus. Hybridomas that elicited positive staining of any cellular elements were expanded and cloned by the limiting dilution method (*6*). Eighteen of the original 48 positive clones remained stable and continued to produce antibodies. Only one line, clone 2G9, produced an antibody that stained in a regionally specific fashion. The others stained more ubiquitously, although a few were either neuron- or glia-specific. Clone 2G9 produced an antibody of the immunoglobulin G (IgG) 2a subclass (*7*) that labeled all neurons in the hippocampus.

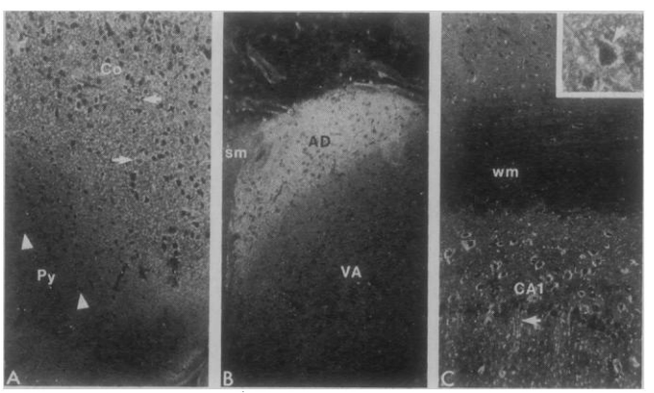

Fig. 1. Photomicrographs showing the specificity of 2G9 monoclonal antibody staining in limbic system regions of the rat brain. (A) Coronal section through the basal forebrain (×125). Note the clear border between the dense immunofluorescence in the corticoamygdaloid nucleus (*Co*) and the unstained pyriform cortex (*Py*). Punctate immunofluorescence surrounds the silhouettes of amygdaloid neurons (arrows). Neurons in the pyriform region are not specifically stained (arrowheads). (B) Lower power micrograph of a coronal section through the rostral thalamus (×80). There is heavy immunofluorescent staining of the anterodorsal thalamic nucleus (*AD*). This contrasts with the dark, unstained region corresponding to the motor thalamus, the ventroanterior nucleus (*VA*). Note that only the neurons in the AD are surrounded by the immunoreactivity (*sm*, stria medullaris). (C) Stained neurons in the CA1 region of the hippocampus (×125). Overlying white matter (*wm*) and motor cortex are unstained. Immunofluorescence is particularly evident on the surface of large neurons and their apical dendrites (arrows). A hippocampal cell viewed at higher magnification (inset) reveals the surface distribution of stain on all 2G9-positive neurons (×320).

Table 1. Regional distribution of monoclonal antibody 2G9 staining in rat brain.

Region	2G9 staining*	Evidence of limbic system relation (reference)
Cerebral cortex		
Medial prefrontal (area 32)	+4	(*10*)
Sulcal prefrontal (area 8)	+3	(*10*)
Insular (area 14)	+3	(*1, 2, 10*)
Anterior cingulate (area 24)	+3	(*10*)
Posterior cingulate (area 23)	+2	(*1, 2, 10*)
Subiculum (areas 27 and 49)	+4	(*1, 2, 10*)
Entorhinal (area 28)	+4	(*1, 2, 10*)
Hippocampus	+4	(*1, 2, 10*)
Pyriform (area 51a and b)	0	(*1, 2, 10*)
Sensorimotor	0	
Auditory	0	
Visual	0	
Basal telencephalon		
Septum	+4	(*1, 2, 10*)
Olfactory tubercle	+3	(*1, 2, 10*)
Olfactory bulb	0	
Nucleus accumbens	+4	(*10*)
Bed nucleus stria terminalis	+4	(*10*)
Caudate putamen	+2	(*10*)
Amygdala	+4	(*1, 2, 10*)
Diencephalon		
Anterior hypothalamic nucleus	+4	(*10*)
Preoptic area	+4	(*10*)
Paraventricular nucleus	0	(*10*)
Periventricular nucleus	+1	(*10*)
Arcuate nucleus	0	(*10*)
Dorsomedial nucleus	+3	(*10*)
Ventromedial nucleus	+4	(*10*)
Lateral hypothalamic area	+4	(*10*)
Supraoptic nucleus	+3	(*10*)
Mammillary bodies	+3	(*1, 2, 10*)
Habenula	+2	(*10*)
Anterior thalamic nuclei	+4	(*1, 2, 10*)
Medial dorsal nucleus	+4	(*1, 2, 10*)
Paraventricular thalamic nuclei	+4	(*10*)
Lateral dorsal nucleus	+2	(*10*)
Ventroanterior, ventrolateral nuclei	0	
Ventrobasal complex	0	
Lateral geniculate nucleus	0	
Medial geniculate nucleus	0	
Brainstem		
Interpeduncular nucleus	+3	(*10*)
Periaqueductal gray	+4	(*10*)
Superior colliculus (superficial layers only)	+4	
Inferior colliculus	0	
Ventral tegmental area	+3	(*10*)
Substantia nigra	+3	(*10*)
Mesencephalic raphe nuclei	+2	(*10*)
Locus coeruleus	+2	(*10*)
Parabrachial nucleus	+4	(*10*)
Dorsal tegmental nucleus	+3	(*10*)
Ventral tegemental nucleus	+3	(*10*)
Cranial nerve nuclei III, IV, V, VI, VII, XI, XII	0	
Red nucleus	0	
Cochlear nuclei	0	
Vestibular nuclei	0	
Nucleus tractus solitarius	+4	(*10*)
Nucleus of dorsal motor vagus	+4	(*10*)
Reticular formation	+2	(*10*)
Pontine and medullary raphe nuclei	0	
Cerebellum		
Deep cerebellar nuclei	0	
Purkinje cells	0	
Granule cells	0	
Molecular layer	+1	
Spinal cord		
Intermediolateral cell column	+2	(*10*)
Lamina II	+3	
All other laminae	0	

*Density of staining was scored subjectively as +4 (high), +3 (moderate), +2 (low), +1 (barely detectable), or 0 (not detectable).

The distribution of 2G9 antibody staining throughout the CNS was assessed by immunofluorescence and immunoperoxidase methods. Twenty-four female Sprague-Dawley albino rats were fixed by vascular perfusion with one of four fixatives (*8*). Cryostat sections (10 μm thick) were collected every 30 μm through the entire neuraxis. Immunostaining was performed in the absence of detergent (*9*) with a 1:500 dilution of ammonium sulfate precipates from culture supernatant or a 1:2000 dilution of ascites fluid. Fluorescein-conjugated rabbit antibody to mouse IgG (Dako, Accurate Scientific) was used to visualize antigen. Immunoperoxidase staining of alternate sections revealed an identical pattern of staining. Supernatants from other IgG-producing hybridomas served as controls and never elicited positive staining.

Complete regional mapping of staining with the 2G9 monoclonal antibodies showed a striking correlation between the presence of immunoreactivity and conventionally defined limbic regions or areas receiving direct projections therefrom (Table 1) (*10*). The specificity of the staining was particularly evident when we examined classically defined limbic and adjacent nonlimbic regions (Fig. 1). For example, neuronal staining in the frontal cortex precisely defined areas 32 and 8, which are innervated by limbic thalamus (*11*). A similarly distinctive border was present between the 2G9-positive limbic cortico-amygdaloid nucleus and the adjacent unstained olfactory region, pyriform cortex area 51b (Fig. 1A). In the thalamus, we could discriminate the borders between immunopositive anterior and mediodorsal nuclei from adjacent unstained motor and sensory thalamic nuclei (Fig. 1B). In view of the massive and reciprocal projections between the limbic system and the hypothalamus (*10*), we expected that most hypothalamic nuclei would be 2G9-immunopositive; this was indeed the case (Table 1). Specific staining was also evident in areas known to receive limbic innervation but usually not classified as limbic structures. For example, we saw immunoreactivity in only dorsomedial and ventral regions of the caudate putamen, which receive input from the amygdala (*10*); the lateral striatal areas that contain a sensorimotor projection were not immunopositive. In the brainstem the only nuclei stained were those that receive descending limbic input, such as the tegmental nuclei and nucleus tractus solitarius (Table 1).

There were few exceptions to the limbic identity of the immunostaining. Moderate staining was present in the superfi-

cial layers of the superior colliculus and lamina II of the spinal cord and extremely light immunoreactivity was present in the cerebellar molecular layer. Staining of these regions may represent localization of a different antigen with the same antigenic determinant as we found in the limbic system, or may indicate a true functional relation between these and the other classically defined limbic structures. For example, the stain in lamina II is present on cells innervated by afferents concerned with sensations that have a strong emotional component, such as pain and sex.

The density of 2G9 staining varied among areas (Table 1). At the light microscopic level, the immunofluorescence appeared to reside on the surface of neurons and their dendritic processes (Fig. 1C). Glial cells were not immunopositive. Preliminary electron microscopic immunocytochemical data on prefrontal cortex and amygdala confirm the presence of stain on the soma and dendrites of the neurons; thus far, we have not seen immunoreactivity on axons in these regions.

We have identified, therefore, a molecule whose distribution in the vertebrate brain is correlated with the conventional anatomic organization of the limbic system. In addition to its possible functional importance, the antigen may serve as a marker during the assembly of limbic pathways. Only in invertebrates have monoclonal antibodies previously been generated that detect neurons composing a functional system (*12*). Thus, the limbic system antigen is part of an increasingly complex cell-surface molecular organization in the vertebrate CNS. In previous studies three groups of surface molecules have been described: (i) cell class–specific molecules such as those on neuronal or glial cells (*3*); (ii) cell type–specific molecules, which may be present on one type of neuron in many different brain regions (*1*); and (iii) molecules that are nonspecific with respect to cell type, but whose distribution in the CNS may be related to a specific developmental event (*4, 5*). We now propose a fourth class, molecules distributed in a system-specific manner on neurons that are interconnected.

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Activation of Pontine Cholinergic Sites Implicated in Unconsciousness Following Cerebral Concussion in the Cat

Abstract. *Low levels of cerebral concussion in the cat produce reversible behavioral suppression presumably associated with unconsciousness. This injury is also associated with increased rates of glucose utilization in regions within the dorsomedial pontine tegmentum. Microinjection of carbachol into these regions produced behavioral suppression resembling that following concussion. These data, together with previously published observations on cholinergic responses to brain injury, suggest that concussive unconsciousness may be attributable in part to activation of cholinergic pontine sites.*

Cerebral concussion is usually defined as a reversible syndrome occurring without detectable pathology and principally characterized by immediate loss of consciousness. The neurophysiological basis of the concussive syndrome is not known. Most investigators have related the consequences of cerebral concussion

Table 1. Local cerebral glucose utilization (LGU) in midbrain regions of uninjured control cats ($N = 4$) and cats subjected to experimental cerebral concussion ($N = 4$). Values of LGU are means (± standard errors) of readings from four to eight sections studied in each cat. All differences between control and injured animals were statistically significant (*t*-tests for independent samples, $\alpha = 0.05$). Regions correspond to those from P1.0 to P2.0 (Fig. 1B).

Area	Glucose utilization (μmole per 100 g per minute)		Change (%)
	Control	Concussion	
Gray matter			
Inferior colliculi	73.9 ± 2.1	56.8 ± 2.0	−23
Ventral central gray	48.6 ± 3.4	33.9 ± 1.7	−30
Central superior nucleus	48.9 ± 4.0	36.7 ± 1.2	−25
Locus coeruleus	38.2 ± 2.6	27.6 ± 1.2	−28
Tegmental reticular nucleus	36.9 ± 2.9	32.2 ± 1.2	−13
Dorsomedial pontine tegmentum	47.6 ± 2.1	57.3 ± 1.7	+20
Nucleus of lateral lemniscus	54.8 ± 3.7	36.9 ± 1.4	−32
White matter			
Pyramidal tract	28.0 ± 2.0	22.3 ± 1.6	−20
Brachium conjunctivum	31.6 ± 3.2	23.5 ± 1.2	−25
Medial longitudinal bundle	32.7 ± 2.3	25.0 ± 1.9	−24
All regions	44.2 ± 3.7	35.2 ± 3.2	−21