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Evolution of GABAergic circuitry in the mammalian medial geniculate body

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ABSTRACT **Many features in the mammalian sensory thalamus, such as the types of neurons, their connections, or their neurotransmitters, are conserved in evolution. We found a wide range in the proportion of γ-aminobutyric acidergic (GABAergic) neurons in the medial geniculate body, from <1% (bat and rat) to 25% or more (cat and monkey). In the bat, some medial geniculate body subdivisions have no GABAergic cells. Species-specific variation also occurs in the somesthetic ventrobasal complex. In contrast, the lateral geniculate body of the visual system has about the same proportion of GABAergic cells in many species. In the central auditory pathway, only the medial geniculate body shows this arrangement; the relative number of GABAergic cells in the inferior colliculus and auditory cortex is similar in each species. The range in the proportion of GABAergic neurons suggests that there are comparative differences in the neural circuitry for thalamic inhibition. We conclude that the number of GABAergic neurons in thalamic sensory nuclei may have evolved independently or divergently in phylogeny. Perhaps these adaptations reflect neurobehavioral requirements for more complex, less stereotyped processing, as in speech-like communication.**

In mammalian phylogeny, the types of neurons and their basic sensory and motor circuits are highly conserved. In the spinal cord, thalamus, and cerebral cortex, for example, the main kinds of cells and their ordinal position in the synaptic sequence are similar in all vertebrates, and there is a corresponding continuity in neuronal structural and physiological organization (1, 2). These parallels are evident despite species differences in the relative size of nuclei, the types and concentration of peripheral receptors, the size of the spinal and other tracts, or in the number and complexity of thalamic nuclei and cortical areas (3). This continuity suggests that comparative differences in the mammalian central nervous system are largely a matter of nuclear size and areal elaboration, while the principal features of the underlying neuronal circuitry are assumed to be largely conserved. We present evidence here that the intrinsic architecture of the medial geniculate body (MGB) has a species-specific arrangement. This finding implies that the physiological substrates for intrinsic auditory thalamic processing may take several phylogenetic forms.

The MGB is prominent in mammals and it is essential for hearing (4). Local circuit neurons represent about one-quarter of the cells in the cat MGB and are thought to play a role in controlling the flow of information from the thalamus to the cortex (5). The question addressed here is whether intrinsic neurons are present in similar numbers in different mammals. If they are not, then local circuits to which they contribute may have more than one basic design, and intrathalamic processing sequences could be species-specific.

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The ventral division of the MGB is the focus of this study because it plays an important role in normal hearing and is the conduit for ascending input to the primary auditory cortex. It contains projection (type I) neurons with bushy dendrites and smaller local (type II) stellate cells (6). The type I and II cells participate in circuits for local tonotopic and binaural processing (5, 7). Type I cells send their axons to primary auditory cortex (8), which projects to other cortical (9) and subcortical (10) areas. Type I cells are large, have well-myelinated axons (11), and may be glutamatergic (12), whereas type II neurons are small, have unmyelinated axons and dendrites that are presynaptic to type I cells (13), and use γ-aminobutyric acid (GABA) as their transmitter (14). The ratio of type I:II cells may provide a framework for thinking about the comparative operations of thalamic nuclei. Differences between species could reveal whether the proportion of type II cells forms a continuum or represents discrete patterns of intrinsic inhibition. These competing hypotheses have different implications for MGB function and for the evolution of auditory thalamic processing.

METHODS

Adult mustache bats, *Pteronotus p. parnellii* ($n = 10$), Sprague–Dawley rats ($n = 10$), cats ($n = 12$), and macaque monkeys ($n = 4$) were anesthetized deeply and perfused transcardially. For glutamic acid decarboxylase (GAD), a normal saline wash preceded fixation with 0.5% zinc salicylate in 10% unbuffered formalin (15; see refs. 16, 17). Frozen sections, 25-μm-thick, were treated with blocking serum [10% normal rabbit serum (NRS)] for 1 hr and incubated overnight at 4°C in sheep-anti-GAD [GAD-1440 (18)] diluted 1:2000 in 0.5 M Tris with 2% NRS. For GABA, the perfusate was 0.1 M phosphate buffer, 4% paraformaldehyde, and 0.25% glutaraldehyde. Vibratome sections, 50-μm-thick, were placed in 10% normal goat blocking serum (NGS) for 1 hr and incubated overnight in rabbit-anti-GABA (INCstar; Stillwater, MN) diluted 1:5000 or in rabbit-anti-GABA (R.J. Wenthold, National Institutes of Health, Bethesda, MD) diluted 1:2000 in 0.01 M phosphate-buffered saline with 2% NGS. The immunoperoxidase procedure was avidin–biotin (Vector Laboratories; Burlingame, CA) with diaminobenzidine as the chromagen. For postembedding immunocytochemistry the fixative was 2% paraformaldehyde and 3% glutaraldehyde. Vibratome sections, 200-μm-thick, were osmicated and flat embedded in epoxy resin. Semithin sections were etched on the slide, incubated in the above antisera, and then treated with streptavidin–biotin–peroxidase (Kirkegaard & Perry; Gaithersburg, MD) and cobalt-nickel diaminobenzidine. Neither omission nor absorption controls (GABA) nor preimmune serum controls (GAD) resulted in any specific immunostaining (19).

Abbreviations: GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; MGB, medial geniculate body.

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RESULTS

Immunoreactive Neurons. Each of the four species had many GABAergic cells in the brain stem (Fig. 1). However, there was a 50-fold difference in the proportion of such neurons in the ventral division of the MGB. The values ranged from <1% in the bat (Fig. 1*A*) and rat (Fig. 1*B*), to ≈25% in the cat (Fig. 1*C*) and monkey (Fig. 1*D*). In sharp contrast, the inferior colliculus (Fig. 2 *E–H*), whose neurons are presynaptic to those of the MGB (20, 21), and the auditory cortex (Fig. 2 *I–L*), whose cells are the target of MGB projections (22–24), had many GABAergic neurons in each species. The proportion of such cells in these regions was estimated to be 20–25%. All illustrations are from GABA immunostained material; the results in GAD preparations (data not shown) were indistinguishable from the former.

The size and shape of GABAergic neuronal perikarya in the auditory thalamus was consistent with the profiles of type II cells as described in morphological studies (11, 13, 23). These neurons had a flask-shaped soma and sparse, thin primary processes. In the cat and monkey, they were more diverse, and most had a soma <15 μm in diameter.

The distribution of GABAergic cells was not uniform within the MGB subdivisions in all species. The few immunopositive neurons in the bat were concentrated in the dorsal division, and in the rat the ventral division had the largest number of GABAergic cells. There were no GABAergic cells in the bat medial division, and very few in the ventral division. In the cat and monkey, they were distributed more evenly in the ventral, dorsal, and medial divisions.

Immunoreactive Axon Terminals (Puncta). A second facet of GABA processing in the auditory thalamus was the many and varied axon terminals (puncta), which arise from intrinsic (Golgi type II) or extrinsic (projection) sources. In each MGB subdivision, including nuclei with no or few immunoreactive neurons, there were many granular, oval immunostained profiles ≈0.5 μm in diameter near immunonegative neurons and in the neuropil in every species (Fig. 2). Puncta were especially numerous and heterogeneous in the cat and monkey; large, globular endings >4 μm in diameter were rare in the bat and rat. Puncta in each subdivision had a characteristic density and architecture. They were most numerous and medium-sized in the ventral division and sparser and smaller in the dorsal division; in the medial division, they were abundant and tended to be larger and coarser. The findings reported above were

FIG. 1. Neurons immunostained for GABA. (*A* and *B*) The bat and rat MGB had few GABAergic cells (black dots) and far more elsewhere. (*C* and *D*) Both cat and monkey MGB had many GABAergic neurons. The results in GAD material were identical. AC, primary auditory cortex; APt, anterior pretectum; Aq, aqueduct; CG, central gray; CP, cerebral peduncle; D, dorsal or dorsal nucleus of MGB; DD, deep dorsal nucleus of dorsal division of MGB; DS, dorsal superficial nucleus; EW, Edinger–Westphal nucleus; Fx, fornix; IC, inferior colliculus; IThP, inferior thalamic peduncle; L, lateral; LGN, lateral geniculate nucleus, LGNd, LGN, dorsal part; LGNv, lateral geniculate nucleus, ventral part; LP, lateral posterior nucleus; M, medial or medial division of MGB; ML, medial lemniscus; MRF, mesencephalic reticular formation; MZ, marginal zone of MGB; MGB, medial geniculate body; OR, optic radiation; OT, optic tract; Ov, ventral division, ovoid subnucleus of MGB; PC, posterior commissure; Pt, pretectum; PulI, pulvinar nucleus, inferior part; PulL, pulvinar nucleus, lateral part; SC, superior colliculus; Sg, suprageniculate nucleus of MGB; SN, substantia nigra; Spf, subparafascicular nucleus; SpN, suprapeduncular nucleus; StN, subthalamic nucleus; V, ventral or ventral division of MGB; Vl, ventral nucleus, lateral part of MGB; Vm, ventral nucleus, medial part of MGB; Vpl, ventral posterior nucleus, lateral part (ventrobasal complex); Vpm, ventral posterior nucleus, medial part (ventrobasal complex); wm, white matter; I, layer I; IIIr, oculomotor nerve root. Vibratomed sections are 50-μm-thick (Planapochromat, numerical aperture, 0.13, ×19).

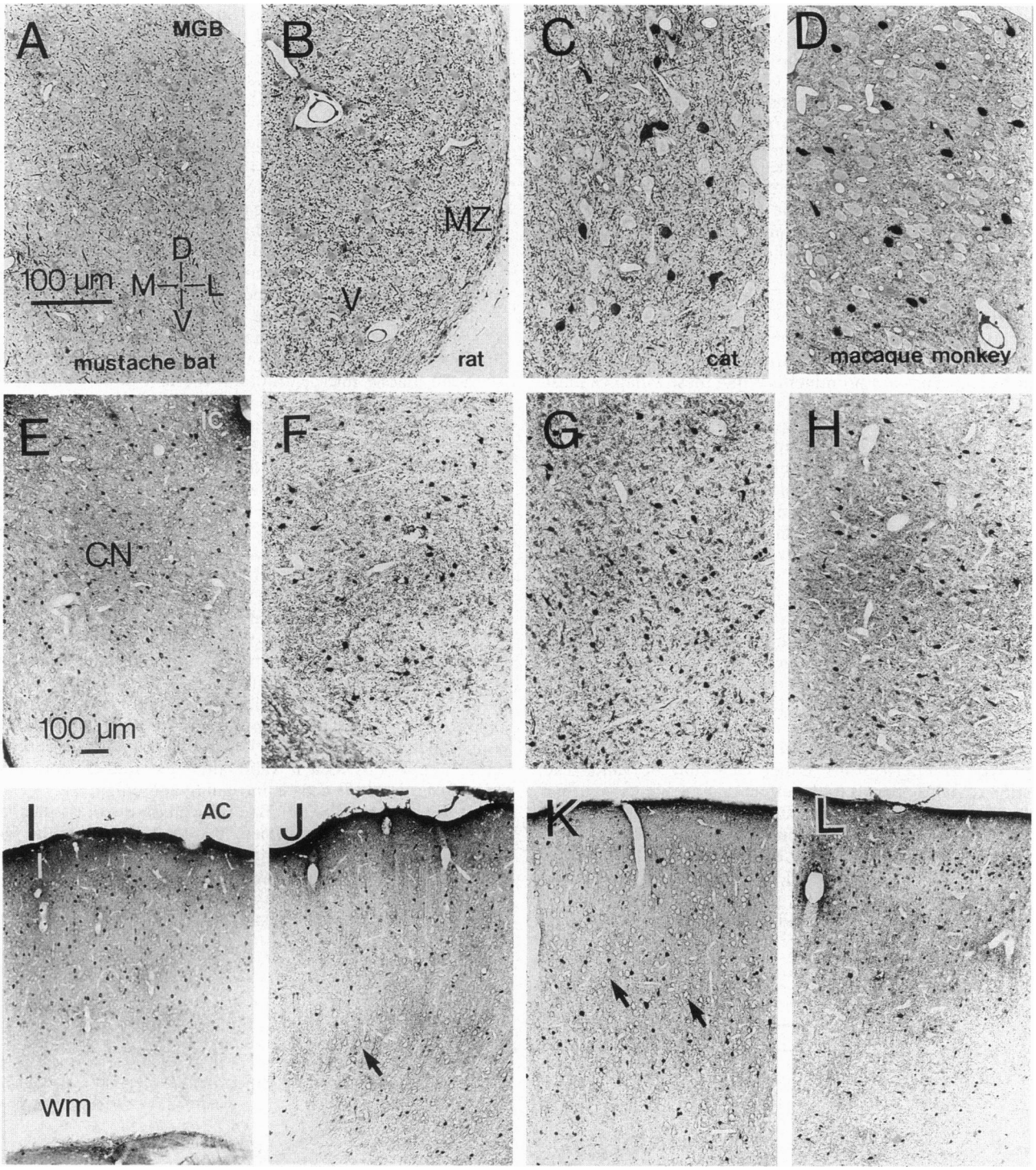

FIG. 2. Neurons and puncta immunostained for GABA. (*A–D*) In the ventral division of the MGB, the bat (*A*) and rat (*B*) had abundant GABAergic puncta but few GABAergic neurons; the cat (*C*) and monkey (*D*) had more neurons and a wide range of puncta. The central nucleus of the inferior colliculus had many GABAergic cells in each species. (*I–L*) The primary auditory cortex also contained numerous GABAergic neurons. These patterns were confirmed in GAD material (data not shown). Arrows in *J* and *K* indicate puncta-ringed, immunonegative neurons. (*A–D*) Semithin sections are 1.5-μm-thick (Planapochromat, numerical aperture, 0.7, ×250). (*E–L*) Vibratomed sections are 50-μm-thick (Planapochromat, numerical aperture, 0.4, ×125). Scale in *A* applies to panels *A–D*; scale in *E* applies to panels *E–L*.

corroborated in the GAD preparations. In the GABA material, the thick sections and the plastic embedded tissue had identical patterns of immunoreactivity.

DISCUSSION

The number of GABAergic neurons in the MGB shows striking phylogenetic diversity. The proportion of GABAergic neurons is species-specific and ranges from <1% to >25%. This result aligns the MGB with some thalamic sensory nuclei (ventrobasal complex; Vb: Vpl, Vpm) and distinguishes it from at least one other (lateral geniculate body; LGN), whose GABAergic composition is apparently more conserved. This finding raises the question of the physiological processes to which inhibitory cells may contribute in the auditory thalamus. If the proportion of GABAergic neurons is a valid index of some function, then the nature of that function (and its

presumptive absence or modification in some rodents and bats) remains to be explained. Our results were identical in thick frozen and in semithin plastic embedded material immunostained with antibodies to GABA and in thick frozen sections immunoreacted with antisera to GAD.

A profile of auditory thalamocortical circuitry based upon structural (13), connectional (5), and immunocytochemical (present results and ref. 25) work in the cat ventral division (Fig. 3) is now available. In this scheme, GABAergic Golgi type II cells provide axodendritic (Fig. 3*a*) and dendrodendritic (Fig. 3*b*) input to the intermediate and distal segments of the type I principal cell dendrites. Such endings should be sparse in the mustache bat, as they are in the rat (26). Inhibitory and excitatory input from extrinsic sources converges on the type I cell; not shown are aminergic brain stem projections (31) whose role is probably nonauditory. Both the axodendritic and the dendrodendritic inputs of type II cells to projection neurons are prominent. While these synapses must affect the output of the relay cell, their precise role in thalamic processing is uncertain (5). In species with few GABAergic neurons, the frequency of dendrodendritic synaptic arrangements (glomeruli or nests), to which such intrinsic cells often contribute, is reduced correspondingly (32). The paucity of Golgi type II cells in the opossum (33) and mustache bat (17, 34) has prompted similar speculations with respect to MGB intrinsic architecture. This result supports the idea that the thalamic auditory and somatic sensory nuclei are sites of evolutionary flux with regard to inhibitory interneurons.

Perhaps the comparative rarity of Golgi type II cells identifies a class of thalamic nuclei whose synaptic arrangement is distinct and disjunct from that in species with many GABAergic cells. An alternative interpretation is that this is a difference in degree and not in kind, and that the distribution of intrinsic cells is continuous, as are the function(s) they represent. Without more data on the physiological actions of Golgi type II interneurons, either view may be valid. The question remains open as to the effect that a few such neurons might have, and whether it is similar in species with different proportions of local circuit neurons. The cardinal physiological features of the mustache bat's MGB (22) closely resemble those in the cat (35). Both species have an orderly tonotopic map of the frequency spectrum, probable spatial segregation of thalamic aural subregions, and analogous arrangements of brain stem and cortical input. Species differences include the somewhat sharper tuning of the bat neurons and the preferential response of some cells to combinations of different tones. It seems unlikely that the substrate for narrow tuning or sound-evoked inhibition is a large population of intrinsic GABAergic neurons, because such physiological responses occur both in the mustache bat (36, 37) and the squirrel monkey (38) and because these attributes are represented robustly at prethalamic levels (35, 39, 40). If combination sensitivity in the mustache bat auditory thalamus depends on GABAergic influences, then these must arise chiefly from extrathalamic origins. A candidate source for such an influence is the GABAergic neurons in the inferior colliculus, which in the cat provide robust projections directly to the MGB (27). While this does not exclude intrinsic GABAergic cells from such roles, it argues that they are not always essential (and probably not equivalent functionally) at each synaptic station.

Even among microchiroptera, there is no singular or stereotyped arrangement of GABAergic elements in the MGB. The horseshoe bat (*Rhinolophus ferrumequinum*) has many more immunostained cells than the mustache bat (41), and the pallid bat (*Antrozous pallidus*) has even more than in the horseshoe bat, although fewer than the cat or the monkey (unpublished observations). This fact implies that a nucleus otherwise regarded as phylogenetically conserved may have different circuitry and, by extension, both common interspecific and species-specific arrangements. Moreover, this position is consistent with the view that some hindbrain and midbrain auditory nuclei are stereotyped in structure and function and may be more conserved in evolution than forebrain structures (40). These brain stem auditory nuclei seem to share phylogenetic continuity in neuronal structure and synaptic architecture, and to have much in common physiologically; this argues that they may be more stereotyped in form and function than auditory thalamic (but not cortical) centers (42, 43). If this idea is valid, then the role of even a few GABAergic MGB cells is intriguing and enigmatic.

If the proportion of GABAergic cells in the mustache bat is a simple function of brain size, then their number in the MGB should resemble that in pre- and postthalamic auditory centers. Because these neurons are plentiful in both the inferior colliculus and the auditory cortex and comparable to the number in the cat and monkey, we conclude that there must be

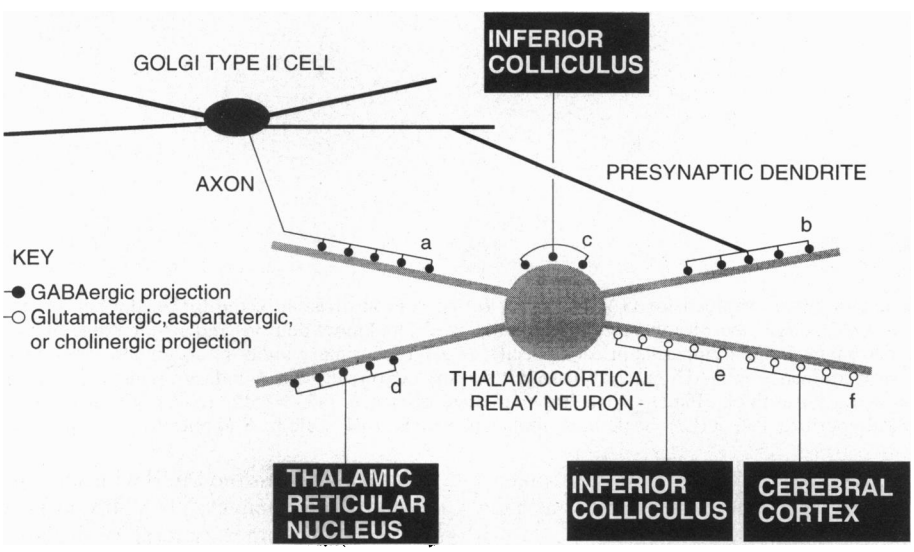

FIG. 3. Schematic synthesis of neural circuitry in the ventral division of the MGB. The ultrastructural relations are based mainly on work in the cat, and only the primary features are shown. Black dots represent presumptive axon terminals. Dendrodendritic inputs could modulate principal cell spike timing (5). In the bat and rat, interneuronal input from axodendritic (*a*) and dendrodendritic (*b*) synapses should be reduced or absent (26). Other GABAergic projections, from the inferior colliculus (*c*) (27, 28) and thalamic reticular nucleus (*d*) (23), are present; the latter may affect attention (29). Excitatory inputs arise from the inferior colliculus (*e*) (30) and auditory cortex (*f*) (5).

a fundamental physiological difference in the MGB. If these cells subserve computational complexity relating to the functional demands imposed by echolocation, then it is counterintuitive that the bat's highly developed auditory system should have so many such cells elsewhere (19) and so few in the MGB; it is equally puzzling that the rat, whose auditory system does not seem to require the rigorous temporal constraints inherent in echolocation or in the acquisition of prey in acoustically challenging environments, has a similar inhibitory architecture. The proposition that tiny mammals with proportionally smaller brains and reduced neuropil invariably have fewer interneurons (44) is not supported.

Without more data on the physiology of interneurons, functional inferences must be made cautiously. We speculate that the number of these cells reflects the complexity and richness of species-specific auditory communication. Insofar as such signals are speech-like—and, by implication, less stereotyped, more labile, and responsive to flexible syntactic and semantic demands—their interneuronal substrates may likewise be more numerous or complex. This prediction can be examined in species with different communication repertoires. Another view is that these neurons could play a part in the thalamic mediation of behavioral plasticity. The medial division, which is implicated in such a role (45), has no GABAergic cells in the bat, and few in the rat; in the cat and the monkey, these neurons are far more abundant.

No single picture accurately captures the range of thalamic GABAergic organization (4, 17, 19). About 20% of LGN neurons are GABAergic in rats (46), cats (47), and monkeys (48). In contrast, the ventrobasal complex (Vb), like the MGB, has no GABAergic cells in lizards (49), rats (50), and the mustache bat (Fig. 1*A*: ventral posterior lateral and medial nuclei), a few in rabbits (51), and many more in cats (52) and monkeys (53). The evolution of intrinsic inhibitory circuitry in certain mammalian dorsal thalamic nuclei thus embodies independent and divergent adaptations.

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