Chapter 1

Morphology and electroresponsive properties of thalamic neurons

To discuss gating processes in the thalamus during different normal and pathological conditions (see Chapters 6 and 7), we should first describe the types of neurons and neuronal networks as well as the modulation of intrinsic properties of thalamic neurons by synaptic activities in various behavioural states.

[1] Jones (1997) described different subdivisions of the human thalamus and compared them to nuclear systematizations in earlier morphological studies.

1.1 Nuclear systematization, morphology and immunoreactivity of thalamic cells

Thalamic nuclei can be systematized into sensorimotor (or relay), association, intralaminar, and reticular neuronal aggregates. The term relay indicates that those nuclei, among them visual lateral geniculate (LG), auditory medial geniculate (MG), and somatosensory ventroposterior (VP), transfer to cerebral cortex specific sensory signals arising in the ascending afferent pathway. This does not imply that such nuclei operate as mere relays, as if nothing would change between activities in afferent fibres and in thalamocortical axons. Indeed, the presence of local-circuit inhibitory neurons in various nuclei and the relations that thalamic relay neurons entertain with thalamic reticular (RE) inhibitory neurons, account for integrative processes in thalamic relay nuclei, mainly consisting of response selectivity higher than that recorded at prethalamic levels.

Before discussing the morphology, connections and properties of different neuronal classes in the thalamus, a brief account of the major thalamic nuclei is necessary. Figure 1.1 illustrates the nuclear groups in the cat, a species of choice for the study of many topics discussed in this monograph. The neuronal aggregates include some sensory (LG, VP), motor (ventroanterior, VA; ventrolateral, VL; ventromedial, VM), association (lateral posterior, LP; pulvinar, PUL; mediodorsal, MD), rostral intralaminar (centrolateral, CL; centralis medialis, CeM), and RE nuclei. The anterior nuclear group (anteromedial, AM; anteroventral, AV) is connected to the limbic system. The human thalamus (Jones, 1997)[1] comprises groups of nuclei that are similar to those

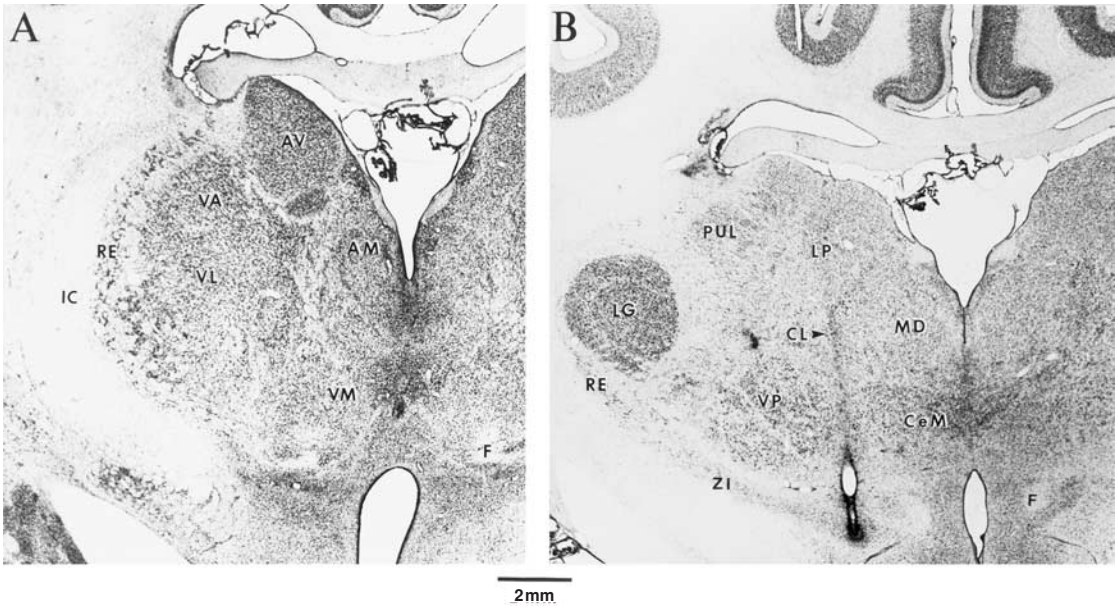

Fig. 1.1 Thalamic nuclei in the cat. Frontal (thionine-stained) sections at A 11 (A) and A 8 (B). Abbreviations: AM and AV, anteromedial and anteroventral nuclei; CeM, central medial nucleus; CL, central lateral nucleus (arrowhead points to a microelectrode track passing through the CL nucleus); F, fornix; IC, internal capsule; LG, lateral geniculate nucleus; LP, lateral posterior nucleus; MD, mediodorsal nucleus; PUL, pulvinar nucleus; RE, reticular nucleus; VA, VL, VM, and VP, ventroanterior, ventrolateral, ventromedial, and ventroposterior nuclei; ZI, zona incerta. Unpublished data by M. Steriade (1981).

previously described in felines and especially in Old World monkeys (Jones, 1985).[2] The sensory (LG, MG, two VP sectors), motor (VA, VL, VM), association (LP, MD), intralaminar (CL, CeM, and centrum medianum, CM), and RE, as well as some other thalamic nuclei are depicted in Figure 1.2.

The three major types of thalamic neurons are (a) those with cortical projections (called relay or thalamocortical, TC); (b) those whose axonal projection does not extend beyond the nucleus in which the soma is located (called local-circuit neurons or interneurons); and (c) those located in the RE nucleus. All TC neurons are glutamatergic and therefore excitatory, whereas both local-circuit and RE neurons use γ-aminobutyric acid (GABA) as neurotransmitter and are therefore inhibitory.

1.1.1 Thalamocortical neurons

Thalamocortical (TC) neurons are bushy (Figure 1.3A) and their variations are linked to soma size, large neurons projecting to middle and deep cortical layers, whereas small neurons project preferentially to superficial layers (Jones, 1985; Steriade *et al.*, 1997). After intracellular staining of one relay neuron, two closely apposed neurons may

[2] In a study focused on intralaminar nuclei of non-human primates, Jones and his colleagues (Hunt *et al.*, 1991) found that GABA-immunoreactive cells in thalamic intralaminar nuclei were only slightly fewer than in principal relay nuclei and assumed that previous reports of their absence or relatively low numbers probably resulted from failure to apply stereometric formulae that reveal the density of neurons per volume of tissue.

[3] In this *in vitro* study, dye-coupling of LG neurons was accompanied in a subset of cells (17%–19%) by spikelets, which survived application of antagonists of fast chemical synaptic transmission and were reversibly blocked by the gap junction blocker carbenoxolone. Spikelets are considered to be the electrophysiological correlate of electrotonic coupling via gap junctions (Perez-Velazquez & Carlen, 2000; see also Destexhe *et al.*, 1994a).

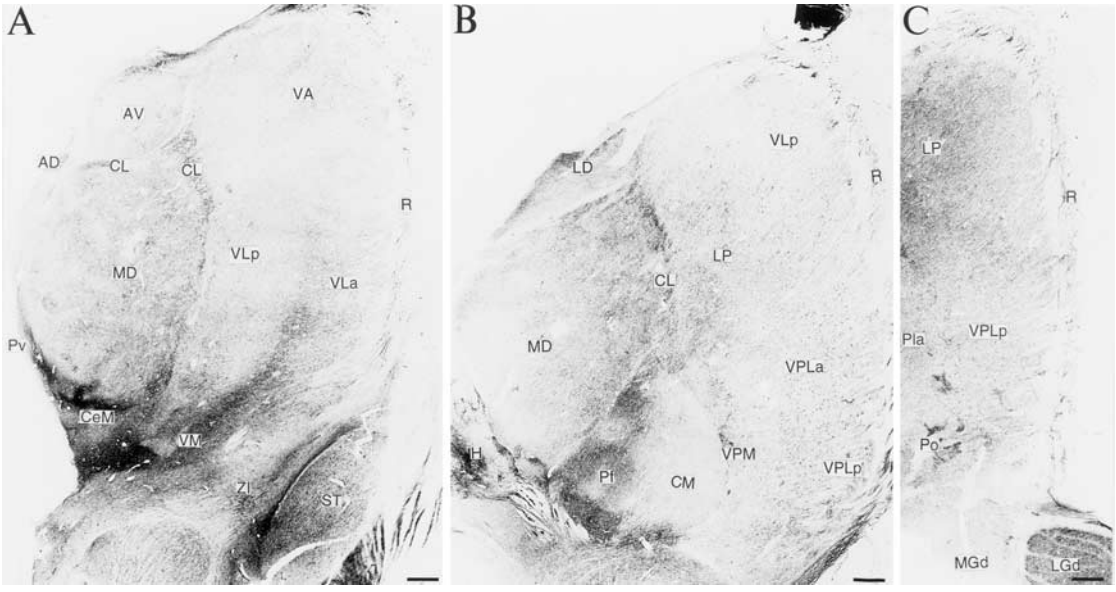

Fig. 1.2 Thalamic nuclei in humans. Frontal sections stained for acetyl cholinesterase. (A–C), three sections from anterior to posterior sites. Bar, 1 mm. Abbreviations (other than those explained in Figure 1.1): AD, anterodorsal nucleus; H, habenula; LD, laterodorsal nucleus; MGd, dorsal part of medial geniculate nucleus; Po and Pli, posterior and inferior pulvinar nuclei; Pv, paraventricular nucleus; R, reticular nucleus; ST, subthalamic nucleus; VLa and VLp, anterior and posterior parts of VL nucleus; VPLa, VPLp and VPM, anterior and posterior parts of lateral VP nucleus, medial VP nucleus. Modified from Jones (1997).

be found (Figure 1.3B); this likely reflects electrotonic coupling, as revealed in cat dorsal LG neurons (Hughes *et al.*, 2002a).[3]

In contrast to earlier hypotheses, which assumed that axonal collaterals of TC neurons contact local interneurons, there are no such intranuclear axonal collaterals in relay nuclei of the dorsal thalamus.[4] The only possible exception is the cat LG, in which axonal collaterals of TC neurons provide inputs to local intralaminar interneurons (Stanford *et al.*, 1983; Cox *et al.*, 2003). Thus, in general, TC neurons can communicate only through intermediary RE or neocortical neurons.

In some TC systems, the differences in soma size of relay neurons are paralleled by large variations in axonal conduction velocities of different types of cortically projecting neurons. For example, in the complex of motor-related nuclei, the antidromic response latencies of VM neurons projecting to cortical areas 4 and 6 are much longer (2.8–3 ms) than the antidromic response latencies of VA–VL neurons to stimulation of the same areas (1.8–2.3 ms) (Steriade, 1995a).[5] Despite variations in travel distances between the somatosensory thalamus and cortex, the latency from the mouse VP to a target cortical neuron is remarkably constant, generally *c.*2 ms (Salami *et al.*, 2003).[6] The highest conduction velocities (40–50 ms^{-1}) among all thalamic relay cells are displayed by a special neuronal class recorded from the large-cell

[4] The absence of intranuclear axonal collaterals in VL, VP and other relay thalamic nuclei, as demonstrated by intracellular staining (Steriade & Deschênes, 1984) and Golgi staining (see Jones, 1985),[2] stands in contrast with earlier hypotheses postulating that such presumed recurrent axonal collaterals would play a role in setting up postinhibitory rebound excitations and rhythmicity of discharges during spindles, by acting on local inhibitory interneurons (Andersen & Andersson, 1968). It is now recognized that sleep spindles are generated by thalamic reticular (RE) neurons and interactions with TC neurons (see Steriade *et al.*, 1985, 1987a).

[5] These differences between the conduction velocities of VM and VL–VA neurons projecting to the motor cortex match the longer latencies of VM responses evoked by stimulation of cerebellar fastigial nucleus (*c.*3 ms), compared with the VA–VL responses evoked by stimulation of cerebellar interpositus or dentate nuclei (1.7 and 2.4 ms, respectively).

[6] The authors calculated the conduction velocity from excitatory postsynaptic currents (EPSCs) in layer IV neurons by stimulating the VP complex and the white matter (WM)

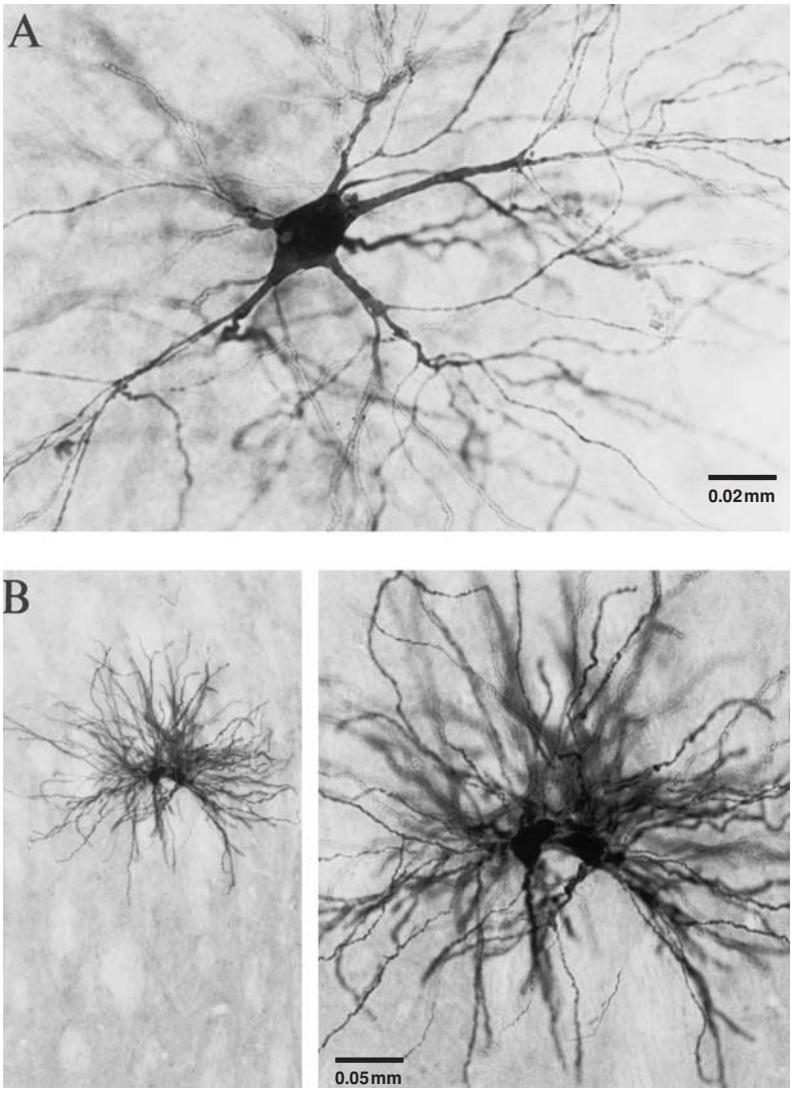

Fig. 1.3 Morphological features of thalamocortical (TC) neurons. (A) Intracellular staining (neurobiotin) of a relay neuron from the ventrolateral (VL) nucleus of the cat. The neuron had a soma 20 μm in diameter with radiating tufted dendrites, characteristic of relay neurons. (B) Two neurons were found close to each other, after one neuron was intracellularly stained (see text). (A) Modified from Contreras & Steriade (1995); (B) unpublished data by D. Contreras & M. Steriade.

[6] (*cont*). in mouse thalamocortical slices. The conduction velocity was found to be 10-fold faster between the thalamus and the WM than from WM to layer IV neurons (despite the fact that the VP–WM path is much longer and more variable in length), this difference being accounted for by the heavily myelinated VP–WM path, compared with the much weaker myelination in the WM–cortex path. Thus, most of the conduction time is spent travelling the intracortical pathway. Other systems, such as the olivocerebellar (Sugihara *et al.*, 1993) and amygdaloperirhinal (Pelletier & Paré, 2002) pathways, similarly show that input timing needs to be within a certain window. These results support the idea that conduction velocities of axons in given systems are adjusted to compensate for variations between the input source and target neurons.

part of the rostral intralaminar nucleus CL, antidromically activated from cortex at latencies of 0.4–0.5 ms (Steriade & Glenn, 1982).

Studies on neuronal immunoreactivity of non-human primates (Hashikawa *et al.*, 1991) showed that parvalbumin-containing neurons in the MG complex prevail in the ventral MG nucleus and project to layer IV of the primary auditory cortex, whereas calbindin-containing neurons prevail in the magnocellular part of the MG complex and project to layer I. In the human thalamus, calretinin immunoreactivity is weak in the geniculate nuclei but strong in the midline and anterior intralaminar nuclei (Fortin *et al.*, 1998).

1.1.2 Local-circuit neurons

Local-circuit GABAergic thalamic interneurons are not always considered in didactic schemes of thalamic circuits because, although they constitute 20%–30% of neurons in all thalamic nuclei of cats and

primates, including intralaminar nuclei (Jones, 1985), as well as in the dorsal LG nucleus of rats, they are virtually absent from other thalamic nuclei of rodents (Steriade *et al.*, 1997). Most slice studies are conducted on rodents, even though local thalamic interneurons may play an important role in the investigated function.

Local interneurons are characterized by their small soma and complex dendrites with *en passage* and terminal swellings. The output of local interneurons arises from axon terminals that form inhibitory synapses onto somata and dendrites of relay neurons but also, importantly, from the dendritic appendages of interneurons that are equipped with presynaptic vesicles, known as F2 terminals (Jones, 1985; Guillery, 1969; Montero, 1986). The latter contact the dendrites of TC neurons and form symmetrical (inhibitory) profiles within the triadic circuitry of synaptic aggregations called glomeruli (see Figure 16 in Steriade, 2001a).

The role of presynaptic dendrites of local interneurons in generating a peculiar type of inhibitory postsynaptic potential (IPSP) is discussed in Section 1.2. Some interneurons may not possess an obvious axon in dorsal thalamic nuclei other than the LG.

1.1.3 Reticular neurons

Reticular (RE) neurons have long dendrites (Figure 1.4), whose secondary and tertiary branches possess vesicle-containing appendages that form synapses on the dendrites of neurons in the same nucleus (Deschênes *et al.*, 1985; Yen *et al.*, 1985). Axoaxonic synapses have also been described in the rat RE nucleus (Pinault *et al.*, 1997). More recently, it was shown in slices maintained *in vitro* that RE neurons of rats and mice are electrically coupled and that electrical synapses require C×36 (Landisman *et al.*, 2002), the predominant type of connexin in gap junction channels (Condorelli *et al.*, 2000; Rash *et al.*, 2000; Venance *et al.*, 2000). The electrophysiological correlates of central neurons coupled by gap junctions are spikelets (Hughes *et al.*, 2002). Experiments *in vivo* on cat RE neurons showed the presence of spikelets during and outside RE cells' oscillatory activity (Fuentealba *et al.*, 2002, 2004a) (Figure 1.5). The spikelets in RE neurons are significantly different from excitatory postsynaptic potentials (EPSPs) and fast prepotentials (FPPs) (Figure 1.6), which are triggered by synaptic mechanisms. The fact that spikelets and EPSPs are different events in RE neurons results from two major features (Fuentealba *et al.*, 2002, 2004a). First, the rising phase in spikelets peaked at *c.*0.5 ms and the decaying phase at *c.*2 ms, whereas the same phases peaked at *c.*1 ms and *c.*4 ms in EPSPs (Figure 1.6A). Second, spikelets were unable to elicit full action potentials, even during states of membrane depolarization close to firing threshold, whereas EPSPs led to cell firing at the same level of depolarization (Figure 1.6B). It is then possible that fast events displaying very short durations, such as spikelets, do not generate full action potentials even if they reach the threshold for spike generation. Spikelets could also be distinguished from fast prepotentials (FPPs), which are usually considered as dendritic spikes triggered by synaptic volleys (Figure 1.6). FPPs are characterized by

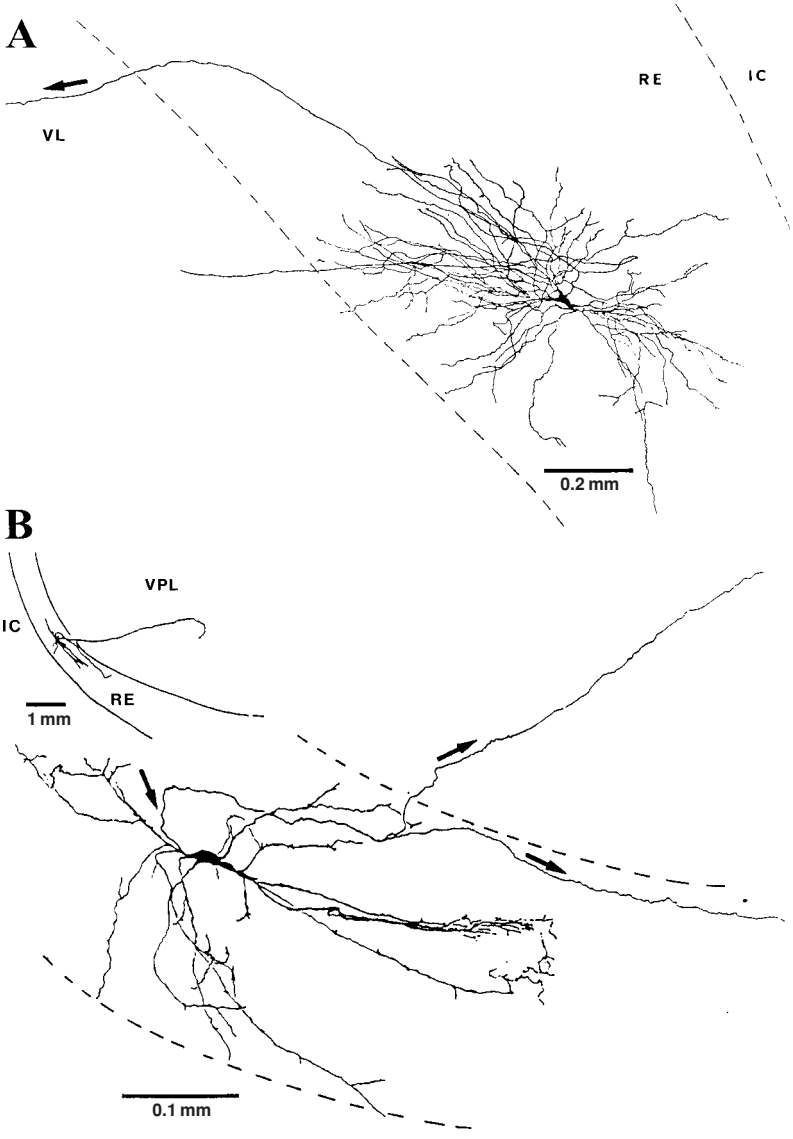

Fig. 1.4 Morphology of intracellularly stained cat thalamic reticular (RE) neurons. (A) RE neuron located in the rostral pole of the nucleus, reconstructed from horizontal sections after intracellular staining. Note the long extent of dendrites and main axonal branch running caudally towards the thalamic ventrolateral (VL) nucleus. (B) Intracellularly injected neuron in the somatosensory sector of the RE nucleus showing, at two different magnifications, the dendritic field extending across the full thickness of the RE nucleus and the axon running to the ventroposterolateral (VPL) nucleus. Modified from (A) Steriade and Deschênes (1984) and (B) Yen *et al.* (1985).

a rapid falling phase and an initiation at *c.*5–6 mV below the usual firing level. These synaptic events are efficiently triggered by corticothalamic volleys. The amplitudes of FPPs are much greater than those of spikelets, and their time-course are also different (see scaled spikelet and FPP in Figure 1.6A). Finally, in contrast to spikelets, FPPs are mainly present during periods of membrane depolarization and are virtually absent at membrane potentials more negative than –70 mV. These results *in vivo* (Fuentealba *et al.*, 2002, 2004a) led to the conclusion that spikelets represent one of the factors that account for the generation of synchronized rhythms, such as spindles, in the isolated thalamic RE nucleus. Experiments *in vitro* led to a similar conclusion (Long *et al.*, 2004).

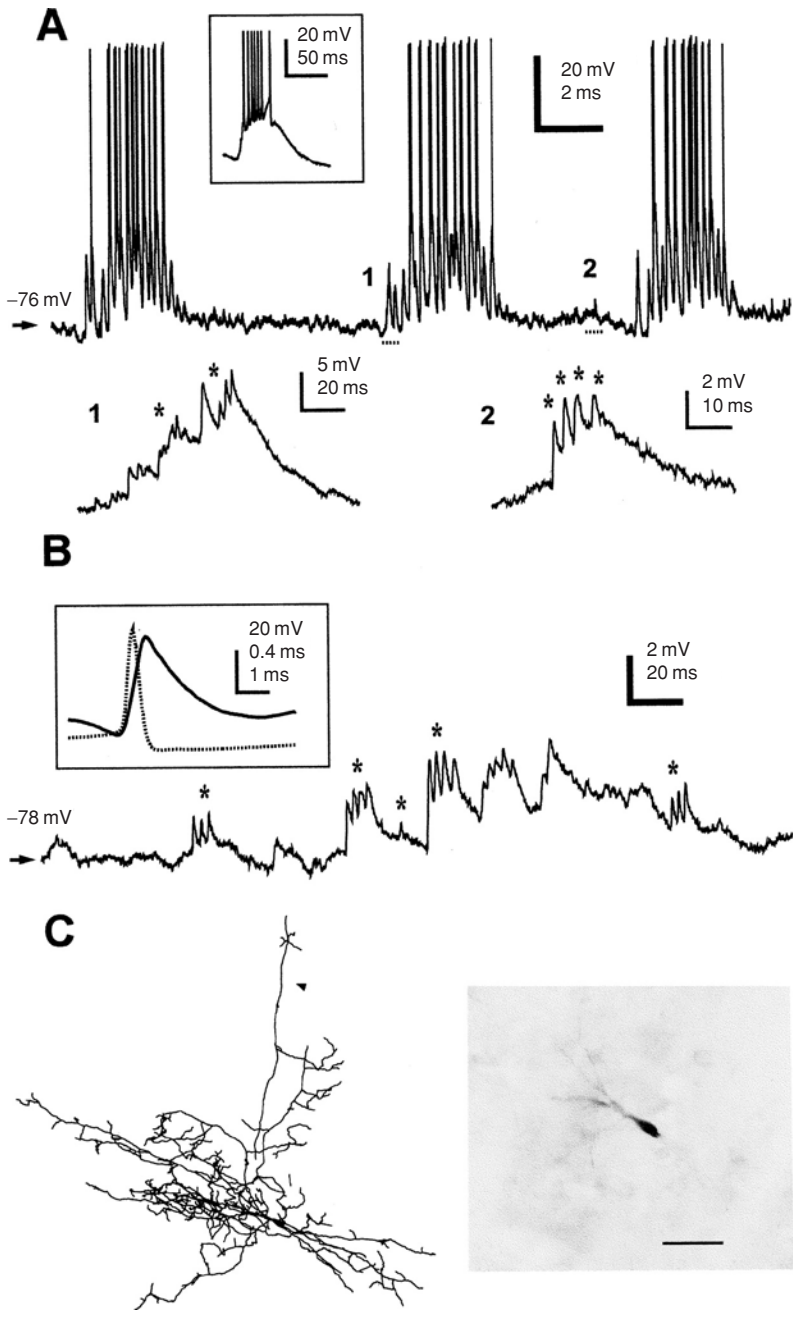

Fig. 1.5 Spikelets during spontaneous activity of cat RE neurons. Barbiturate anaesthesia. (A) Spike-bursts over the depolarizing envelope of spindles. Typical low-threshold spike-burst of RE cell expanded in inset. Epochs marked 1 (at the onset of a spindle sequence) and 2 (during interspindle lull) are expanded below and show spikelets (asterisks), i.e. fast-rising and low-amplitude events occurring in isolation or in clusters. (B) Another RE neuron displaying spikelets (asterisks) occurring in isolation or in clusters. Inset shows the average ($n = 500$) of spikelets (solid line; calibration bar 0.4 mV), scaled with the average ($n = 500$) of full action potentials (dotted line; calibration bar 20 mV). (C) Intracellularly stained (neurobiotin) RE neuron located in the rostrolateral sector of the nucleus. Photograph (right) and reconstruction (left). Arrowhead indicates the axon to the dorsal thalamus. Calibration bar within the photograph, 0.1 mm for RE neuron in the photograph and 0.15 mm for the reconstructed neuron. Modified from Fuentealba *et al.* (2004a).

Thus, contrary to TC neurons, RE neurons form an interconnected network, particularly well suited for the generation of oscillatory activity which can occur even in the isolated RE nucleus (see Chapter 6). The most effective mechanism for generating oscillations within the RE nucleus is probably provided by $GABA_A$-receptor-mediated synapses among RE neurons. Indeed, computational studies showed that RE neurons densely interconnected with

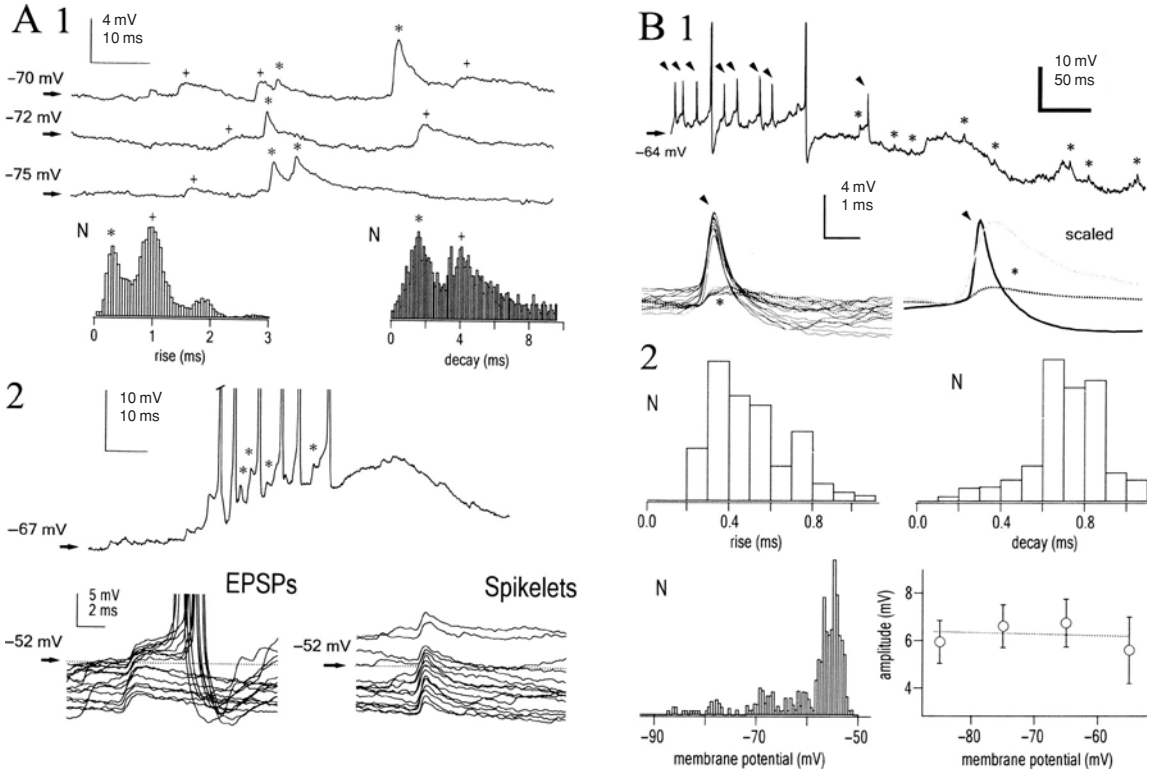

Fig. 1.6 Spikelets and EPSPs as well as FPPs are different types of depolarizing event in cat RE neurons. (A1) Barbiturate anaesthesia. The top three traces, from the same RE neuron, show two types of depolarization: spikelets (*) and EPSPs (+). Below, two histograms show the distribution of the rising and decaying phases (left and right, respectively) in the two types of event. (A2) Ketamine–xylazine anaesthesia in another RE neuron. Spikelets (*) are present during the firing of RE cell (spikes truncated). Note different rising phases in spikelets and some EPSPs that give rise to action potentials. Below, superimposed traces from the same neuron showing EPSPs and spikelets. Modified from Fuentealba *et al.* (2004b). (B) Another RE neuron recorded under ketamine–xylazine anaesthesia. (B1) Top trace displays FPPs (arrowheads) and spikelets (*). Action potentials truncated. Below, superimposition of single events (left) and averages ($n = 100$) showing both FPPs and spikelets (right); the grey trace shows the averaged spikelet scaled ($\times 5$) for comparison. (B2) Upper histograms show the rising and decaying phases (left and right, respectively) of FPPs. Bottom left histogram shows the voltage sensitivity of FPPs. Bottom right histogram shows voltage independency of the amplitude of FPPs. Each point is the average of ten points taken from intervals of 10 mV. Modified from Fuentealba *et al.* (2004a).

$GABA_A$ synapses produced synchronous oscillations within the frequency range of spindles (Destexhe *et al.*, 1994a), as demonstrated by experiments on the isolated RE nucleus (Steriade *et al.*, 1987a). In contrast, networks of model RE neurons fully interconnected through $GABA_B$ synapses failed to synchronize within this frequency range (Fuentealba *et al.*, 2002, 2004a). Experiments *in vitro* similarly showed

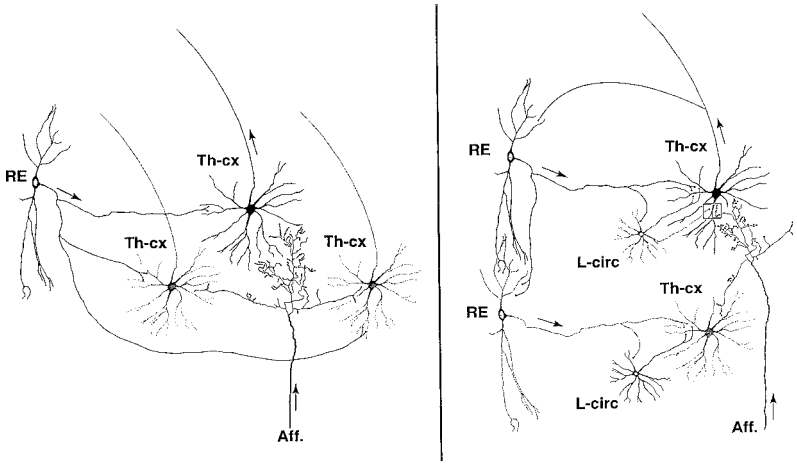

Fig. 1.7 Tentative schemes of operations in synaptic networks involving TC (Th-cx), local-circuit (L-circ), and RE thalamic cells. Left panel does not represent L-circ cells, as is the case of most relay nuclei in rodents. Right panel also depicts L-circ cells, as is the case in felines and primates. *Left panel:* activity in the afferent (Aff) prethalamic axon prevalently excites Th-cx in the centre. The inhibition exerted by the RE neuron is distributed equally to the three Th-cx neurons, but it mainly affects the two cells at the periphery because of the reduced amount of afferent excitation to these cells. *Right panel:* interactions between RE, L-circ and Th-cx neurons. In top Th-cx cell, which receives prevalent excitation from the Aff axon, directly connected RE neurons contribute to further enhancement of this relevant activity by inhibiting the pool of L-circ inhibitory elements (see Steriade *et al.*, 1984a). Simultaneously, the activity in adjacent RE sectors (bottom RE neuron) is suppressed by axonal collateralization and dendrodendritic inhibition among RE neurons (Deschênes *et al.*, 1985; Yen *et al.*, 1985; Pinault *et al.*, 1997). The consequence would be the released activity of target L-circ neurons and inhibition of weakly excited Th-cx neurons in thalamic areas adjacent to the active focus. This operation postulated a mechanism for inhibitory sculpturing in the thalamus. Modified from Steriade (1991), based on experiments by Steriade *et al.*, 1986; scheme kindly redrawn by E. G. Jones.

that rodents express very little $GABA_B$ responses in RE neurons (Ulrich & Huguenard, 1996).[7] Studies using expression of $GABA_B$ receptor gene transcripts in primate thalamus also concluded that there is a low level of $GABA_B$ receptors in the RE nucleus, in contrast with the high density of $GABA_B$ receptors in the dorsal thalamus (Muñoz *et al.*, 1998).

1.2 Intrathalamic and thalamocortical neuronal networks

We will first discuss the intrathalamic relations between TC and GABAergic local-circuit and RE neurons. Next, we will deal with reciprocal connections between TC and neocortical neurons.

[7] In another experimental and modelling study, the same team (Zhang *et al.*, 1997) also placed emphasis on $GABA_A$ receptors in the RE neurons and concluded that their prolonged inhibitory postsynaptic currents (IPSCs) are consistent with studies of spindle synchrony in an interconnected network of RE inhibitory neurons, as previously demonstrated experimentally (Long *et al.*, 2004) and in computational studies (Fuentealba *et al.*, 2002, 2004a). Other experiments, in ferret slices, have shown that $GABA_B$ responses to glutamate applied in the perigeniculate sector of the RE nucleus are also present, since the rebound spike-bursts were blocked by an antagonist of $GABA_B$ receptors (Sanchez-Vives *et al.*, 1997).

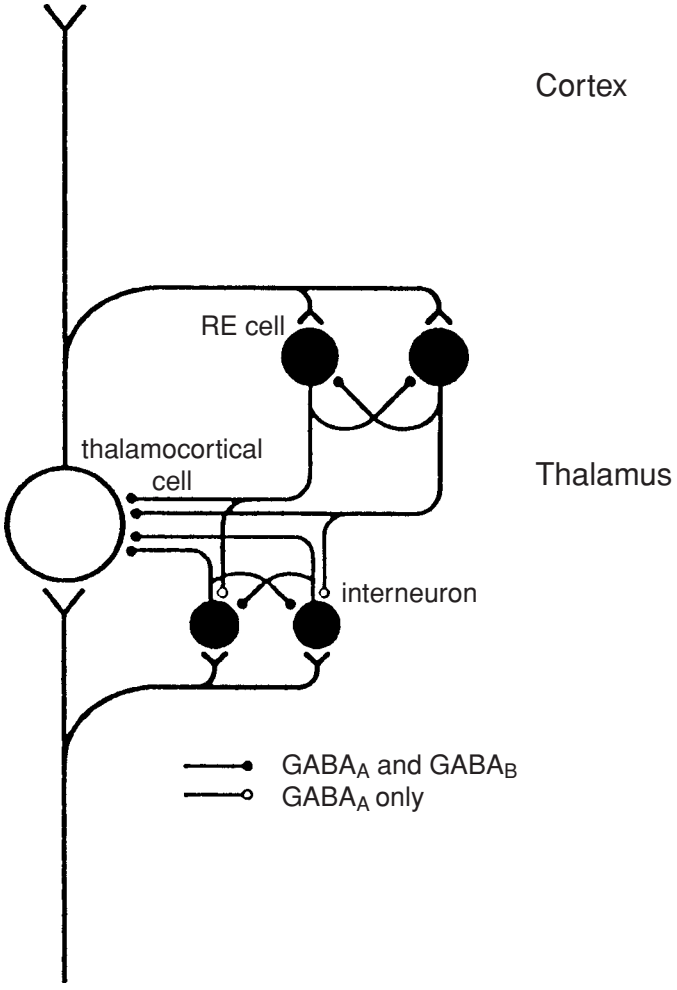

Fig. 1.8 Scheme of intrathalamic circuits, based on intracellular recordings *in vitro* in thalamic slices from rat LG TC cells and local interneurons. Note RE–interneuron inhibition, similar to that postulated in Figure 1.7 and experimental data on other thalamic nuclei *in vivo* (Steriade *et al.*, 1985). Modified from Zhu & Lo (1999).

1.2.1 Intrathalamic neuronal networks

Basically, (a) ascending fibres from specific systems contact both TC and local-circuit neurons (Jones, 1985; Steriade *et al.*, 1997);[8] (b) the axons of the local interneurons contact TC neurons and their presynaptic dendrites contact other interneurons (Jones, 1985; Steriade *et al.*, 1997)[9] (Figure 1.7, right panel); (c) RE neurons project to virtually all TC neurons, with the exception of anterior nuclei of cats (Steriade *et al.*, 1984), as well as to local interneurons[10] (Figure 1.7, right panel; Figure 1.8).

One of the two outputs of local-circuit inhibitory interneurons, which arises from their dendritic appendages generates a peculiar type of IPSP in TC neurons (Paré *et al.*, 1991).[11] This IPSP is the earliest in the sequence of IPSPs, thus preceding the $GABA_{A\text{-}B}$ sequence (Hirsch & Burnod, 1987; Crunelli *et al.*, 1988). To circumvent the possible intervention of inhibitory processes arising in thalamic RE nucleus, the complete sequence of IPSPs in TC neurons was induced by inhibitory local-circuit cells in the anterior nuclei that, in felines, are devoid of

[8] Govindaiah and Cox (2004) used parasagittal slices from rat LG nucleus to preserve the optic tract (OT) input, recorded intracellularly from relay cells and local interneurons, and revealed that OT tetanic stimulation activated metabotropic glutamate receptors (mGluRs) located on presumed presynaptic GABA-containing dendrites of interneurons, which led to increased inhibition in target thalamocortical neurons. Using a series of pharmacological manipulations in the bath, the authors concluded that the increased IPSPs in TC cells were not due to suprathreshold depolarization of the interneurons at the somatic level, but to OT-induced activation of mGluRs that are presumably localized on presynaptic dendrites of LG interneurons. Thus, they hypothesized that synaptic activation of mGluRs on presynaptic dendrites of LG interneurons increases the release of GABA from these dendrites, without influencing the axonal output, and may modulate synaptic transmission at retino-LG synapses, thus representing a focal form of information integration (see also Steriade, 2004a).

[9] See Figure 16 in Steriade (2001a).

[10] About 8%–10% of RE neurons project to local inhibitory interneurons (Liu *et al.*, 1995). Whole-cell recordings in slices of rat LG nucleus showed that stimulation of the thalamic RE