mammals are promiscuous or polygamous. Facultative monogamy is, however, a possibility[13]. It has not been reported in populations of house mice, but this widely dispersed species is well known to be ecologically exceptionally versatile[14,15]. The house mouse is not only capable of thriving in a great variety of environments: studies of free-living populations suggest also that, in different cold climates, there are alternative genotypes and phenotypes, each suited to severe conditions[16].

Our experiments on wild mice in captivity have enabled us to compare eskimo mice with controls in the same environment. The superior growth rate, fertility and other features of the cold-adapted mice have been observed in stocks derived from Scottish as well as Australian populations[2,3]. Such comparisons, together with cross-fostering[17], have shown that cold-adaptation depends on a combination of direct ontogenetic responses, genetical changes and also alterations in maternal performance which favour the survival and growth of the young. Similar processes no doubt underlie cold-adaptation in free-living populations, but the extent to which the changes we have recorded are general in such populations remains to be determined.

To maternal effects our present findings add an enhanced paternal performance. Maynard Smith[12] asks why male mammals that look after their young do not lactate. Perhaps the versatile house mouse, if bred for long enough in a cold climate, could achieve even this.

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1. Barnett, S. A. *Biol. Rev.* **40**, 5-51 (1965).
2. Barnett, S. A. *Biol. Rev.* **48**, 477-508 (1973).
3. Barnett, S. A. & Dickson, R. G. *J. Zool.* **203**, 163-180 (1984).
4. Barnett, S. A. & Neil, A. C. *J. Physiol., Lond.* **215**, 655-678 (1971).
5. Barnett, S. A. & Dickson, R. G. *J. Physiol., Lond.* **346**, 409-417 (1984).
6. Barnett, S. A. & Burn, J. *Nature* **213**, 150-152 (1967).
7. Barnett, S. A. & Foster, K. A. *Physiol. Behav.* **26**, 839-843 (1981).
8. Gandelman, R., Paschke, R. E., Zarrow, M. X. & Denenberg, V. H. *Devl Psychobiol.* **3**, 245-250 (1970).
9. Noirot, E. *Anim. Behav.* **12**, 442-445 (1964).
10. Priestnall, R. & Young, S. *Devl Psychobiol.* **11**, 23-30 (1978).
11. Hartung, T. G. & Dewsbury, D. A. *Behav. neural Biol.* **26**, 466-478 (1979).
12. Maynard Smith, J. *Anim. Behav.* **25**, 1-9 (1977).
13. Kleiman, G. D. *Q. Rev. Biol.* **52**, 39-69 (1977).
14. Pelikán, J. *Symp. zool. Soc. Lond.* **47**, 205-229 (1981).
15. Bronson, F. H. *Q. Rev. Biol.* **54**, 265-299 (1979).
16. Berry, R. J. *Symp. zool. Soc. Lond.* **47**, 395-425 (1981).
17. Barnett, S. A. & Dickson, R. G. *J. Zool.* (in the press).

Synaptic connectivity of a local circuit neurone in lateral geniculate nucleus of the cat

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Although receptive fields of relay cells in the lateral geniculate nucleus of the cat nearly match those of their retinal afferents[1,2], only 10–20% of the synapses on these cells derive from the retina and are excitatory[3,4]. Many more (30–40%) are inhibitory and largely control the gating of retinogeniculate transmission[3–7]. These inhibitory synapses derive chiefly from two cell types: intrinsic local circuit neurones and cells in the adjacent perigeniculate nucleus[5–7]. It has been difficult to study the functional organization of these inhibitory pathways; most efforts have relied on indirect approaches[6–12]. Here we describe the use of direct techniques to study a local circuit neurone by iontophoresing horseradish peroxidase (HRP) into it, which completely labels the soma and processes of cells for subsequent light- and electron microscopic analysis. Although the response properties of the labelled cell are virtually indistinguishable from those of many relay cells[6], its morphology is typical of 'class 3' neurones[13] (see Fig. 1 legend), which are widely believed to be interneurones[8–11] (but see ref. 12). Here, we refer to the cell as a 'local circuit neurone', which allows for the possibility of a projection axon, rather than as an 'interneurone', a term that commonly excludes a projection axon. We find that the labelled cell has a myelinated axon, but that the axon loses its myelin within 50 μm of the soma and has not yet been traced further. The dendrites of the labelled cell possess presynaptic terminals that act as intrinsic sources of inhibition on geniculate relay cells. We also characterize other morphological aspects of this inhibitory circuitry.

Our methods have been described previously[4,12]. Briefly, a cat was anaesthetized, paralysed and prepared for neurophysiological recording with stimulating electrodes positioned across the optic chiasm. We used a bevelled micropipette, filled with HRP and KCl, to record the response properties of a geniculate cell in lamina A, impale the cell and iontophorese HRP into it. Two neuronal classes, X and Y cells, reside in laminae A and A1[1,2,12]. The labelled neurone responded on all physiological tests like a typical X relay cell (see Fig. 1 legend). Several hours after HRP iontophoresis into the cell, the cat was deeply anaesthetized and perfused with fixative. We coronally sectioned the lateral geniculate nucleus at 50 μm and reacted the sections with diaminobenzidine and 1% CoCl. The four sections containing the labelled neurone were osmicated, dehydrated and embedded in plastic resin. We fully reconstructed the neurone from the light microscope (Fig. 1) and then obtained a continuous series of thin sections for electron microscopy from the two 50-μm-thick sections containing the labelled soma plus most of its dendritic arborization.

To chracterize synaptic terminals in the lateral geniculate nucleus, we adopted Guillery's criteria and nomenclature[3]. These terminal types include: RLP (*r*ound vesicles, *l*arge profile and *p*ale mitochondria), which derive from retina, form asymmetrical contacts and are excitatory; RSD (*r*ound vesicles, *s*mall profile and *d*ark mitochondria), which mostly derive from cortex, form asymmetrical contacts and are excitatory; and F (*f*lattened or pleomorphic vesicles), which are thought to derive from local circuit neurones and/or neurones of the perigeniculate nucleus, form symmetrical contacts and are inhibitory. F terminals can be subdivided into F1, which have a darker matrix, are never postsynaptic to other terminals and possess consistently flattened and densely packed vesicles; and F2, which have a lighter matrix, are both pre- and postsynaptic to other terminals and possess more pleomorphic and sparsely distributed vesicles. Finally, we recognized the relatively rare RLD (*r*ound vesicles, *l*arge profile and *d*ark mitochondria) terminals[14], which form asymmetrical contacts, are probably excitatory, and may derive from intrinsic collaterals of relay cell axons (refs 4, 12, 14 and our unpublished observations).

The labelled dendritic terminals contain sparsely distributed synaptic vesicles and form symmetrical synapses clearly distinguishable from the asymmetrical synapses made by nearby RLP or RSD terminals (Figs 2*c*, 3*a*). Thus, although the vesicle morphology is often indistinct because of the HRP (Figs 2*c*, 3*a–e*), the labelled terminals are clearly F terminals. Evidence presented here indicates that they are F2 terminals. Output from the dendritic terminals focuses on dendritic appendages of other geniculate neurones (Figs 2*c*, 3*a*). Figure 4 illustrates the innervation pattern from a labelled terminal cluster to the eight dendritic appendages of one postsynaptic cell. Labelled terminals provide nine synapses to varicosities from five of these appendages and contact no other adjacent structure. In this region, the postsynaptic cell receives 40 synapses from unlabelled F terminals, 23 of which clearly derive from F2 terminals (Fig. 2*c*). Synapses from several local circuit neurones can thus converge onto single cells. Inputs to labelled terminals originate primarily from RLP, F and occasional RSD terminals (Figs 3, 4*a*). Some also originate from RLD terminals (Fig. 3*e*), which

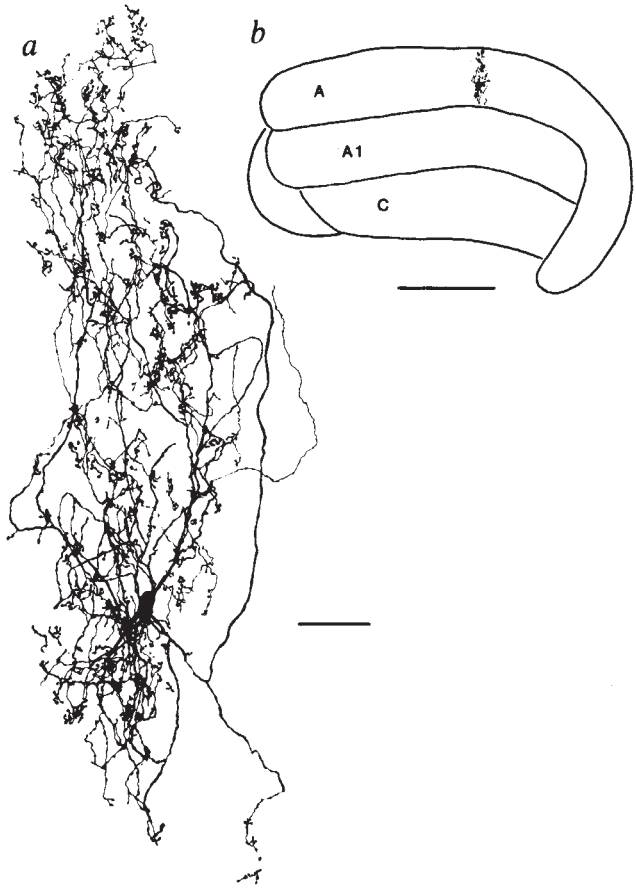

Fig. 1 Light microscopic reconstruction of a HRP-labelled neurone. The cell exhibited response properties typical of X relay cells, including linear summation in response to visual stimuli, a small on-centre receptive field (0.7° centre diameter at an eccentricity of 8° from the area centralis), and a monosynaptic response latency of 1.9 ms to stimulation of the optic chiasm[1,2]. *a*, High-power drawing of neurone showing class 3 morphological features[10]. These include several thin, sinuous dendrites that radiate from a small soma (180 μm^2 in cross-sectional area) and fine processes that issue from the dendrites to arborize into multi-lobed swellings. These swellings have presynaptic specializations and are thus dendritic terminals. Scale bar, 50 μm. *b*, Low-power drawing, showing the laminar location of the injected local circuit neurone in a coronal section through the lateral geniculate nucleus. The dendritic arborization of the cell, which is oriented perpendicular to the laminar boundaries, spans the entire depth (550 μm) of lamina A but extends only 150 μm in the mediolateral and rostrocaudal axes. Scale bar, 1.0 mm.

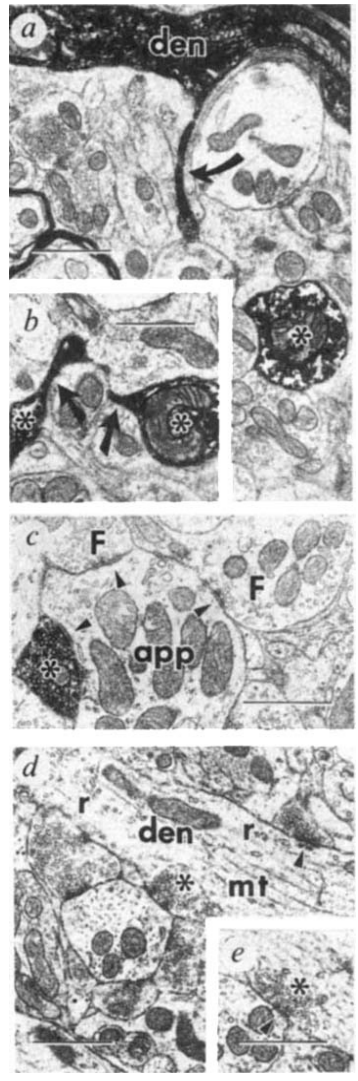

Fig. 2 Comparison of dendritic terminals from the labelled local circuit neurone with a presynaptic dendrite from an unlabelled neurone. *a*, Electron micrograph of a fine process (arrow) emanating from a secondary dendrite (den) of the local circuit neurone. In nearby sections, this process arborizes into dendritic terminals (asterisk; see also *b*, *c*). *b*, Section next to *a*, indicating branches (arrows) emanating from the process in *a* and leading to vesicle-filled terminals (asterisks). *c*, Section near *a* and *b*. The labelled terminal (asterisk) is vesicle-filled and forms a synaptic contact (arrowhead) onto a dendritic appendage (app) of an unlabelled geniculate neurone. The symmetrical contact formed by the labelled terminal is indistinguishable from those of other unlabelled F2 terminals (F) formed onto the same postsynaptic appendage (arrowheads). *d*, Electron micrograph of an unlabelled process (that is, not from the labelled neurone) with features of a presynaptic dendrite[16]. Note the cluster of synaptic vesicles (asterisk; see also *e*), ribosomes (r), microtubules (mt) and the site of a synaptic input (arrowhead) to the dendrite (den; other synaptic inputs are evident in adjacent sections). *e*, Section next to *d*, indicating symmetrical synapse from the presynaptic dendrite (arrowhead) to another unlabelled, medium-sized dendrite. Such dendro-dendritic contacts have been described previously for the cat lateral geniculate nucleus[16] and they clearly differ from those formed by the labelled neurone reported here. Scale bars, 1.0 μm.

contact three different clusters (among the 12 analysed) of labelled dendritic terminals. This is a remarkable density of RLD terminals given their general scarcity in laminae A and A1 (unpublished observations).

Many labelled dendritic terminals enter into complex synaptic circuits, such as synaptic triads, in which a labelled terminal is postsynaptic to an RLP terminal and both terminals contact the same postsynaptic appendage (Fig. 3*a*). An RLD terminal occasionally replaces the RLP terminal in these triads. Some labelled dendritic terminals participate in serial synapses, in which the labelled terminal is both postsynaptic to an unlabelled F1 terminal and presynaptic to a dendritic appendage.

We identified the labelled dendritic terminals as F2 terminals for several reasons. These include their sparse distribution of vesicles, their symmetrical contacts and their synaptic relationships, particularly their triadic relationships[3,4,8,15]. This is the first definitive evidence that F2 terminals are of dendritic origin, a widely hypothesized conclusion[8] that has not been unambiguously demonstrated until now (see, for example, ref. 15). Furthermore, the relatively common F2 terminals are morphologically distinct from another type of quite rare dendro-dendritic synapse previously documented for the cat's lateral geniculate nucleus[16]. For these other dendro-dendritic contacts, the presynaptic element is a vesicle-filled dendritic shaft that contacts other dendritic shafts in simple synaptic arrangements (Fig. 2*d*, *e*).

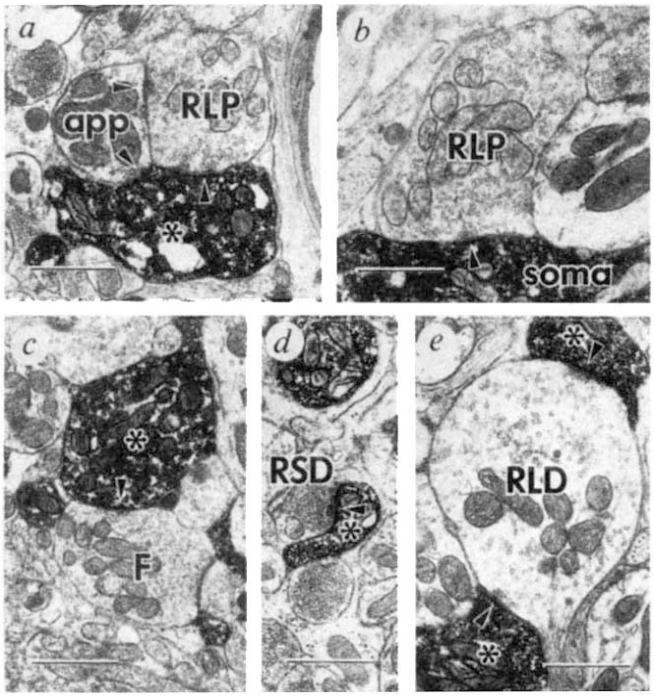

Fig. 3 Electron micrographs of synapses involving the local circuit neurone. *a*, Triadic synaptic arrangement involving a retinal terminal (RLP), an unlabelled dendritic appendage (app), and a labelled dendritic terminal (asterisk). The retinal terminal forms synapses on both the labelled dendritic terminal and an adjacent unlabelled dendritic appendage (arrowheads). This is the most common form of retinal input to the local circuit neurone. The labelled terminal contacts the appendage with a symmetrical synapse (arrowhead) and is thus equivalent to the F2 component of a synaptic triad (see text). *b*, Retinal terminal (RLP) forming a synapse (arrowhead) directly onto the soma of the local circuit neurone. This synapse plus three retinal inputs to spine-like protrusions from the soma (not illustrated) represent the only examples to date of retinal inputs contacting the local circuit neurone in a region other than a dendritic terminal. *c–e*, Other examples of synaptic inputs (arrowheads) to labelled dendritic terminals. Each is less common than retinal input (for example, in *a*) and are shown in descending relative frequency, from F terminals (*c*; most commonly from the F1 variety), RSD terminals (*d*) and RLD terminals (*e*). Scale bars, 1.0 μm.

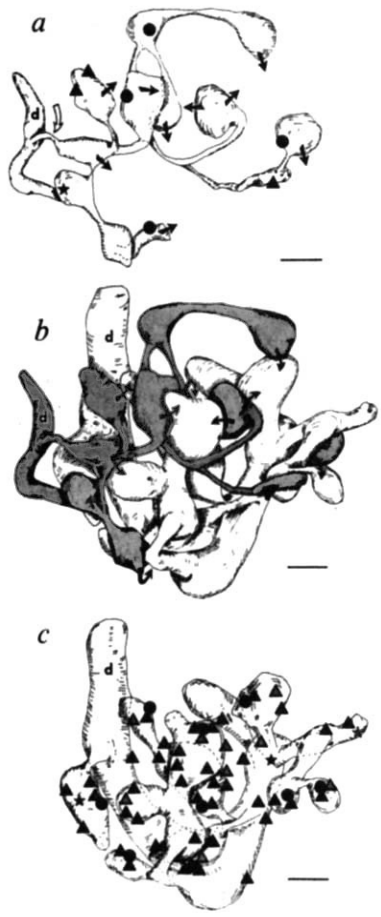

Fig. 4 Three-dimensional reconstructions from serial electron micrographs of a labelled cluster of dendritic terminals, including the stem dendrite and the single unlabelled group of dendritic appendages postsynaptic to these terminals. *a*, Labelled processes from the local circuit neurone. A small dendrite (d) from the labelled neurone emits a fine process (open arrow) that arborizes into 12 swellings, which are the dendritic terminals. The terminals receive inputs from RLP terminals (circles), unlabelled F terminals (triangles) and an RSD terminal (star); the labelled terminals also form nine synaptic outputs (closed arrows). *b*, Combined reconstruction of the labelled presynaptic processes (from *a*; stippled) and unlabelled postsynaptic dendritic appendages (from *c*; open). The synapses from the terminals (closed arrows as in *a*) are formed onto five appendages from a single unlabelled neurone presumed to be an X relay cell (see text). *c*, Unlabelled postsynaptic dendrite (d) with its eight appendages. These dendritic structures receive nine synapses from RLP terminals (circles), nine from labelled F terminals (stippled triangles; these correspond to arrows in *a* and *b*), 40 from unlabelled F terminals (filled triangles) and three from RSD terminals (stars). Triadic synapses ($n = 16$; see text) are indicated by overlapping pairs of symbols for RLP and F synapses. Scale bars, 1.0 μm.

The postsynaptic targets of the labelled dendritic terminals (for example, Fig. 4*c*) are almost certainly X relay cells rather than Y relay cells or other local circuit neurones. X relay cells have many dendritic appendages that receive many inhibitory (F) and retinal (RLP) inputs, whereas Y relay cells do not exhibit such features[4,12]. Also, synaptic triads and innervation from F2 terminals are common to X relay cells and rare for Y relay cells[4]. Finally, the dendritic morphology and pattern of synaptic inputs of the postsynaptic cells differ markedly from such features of the labelled cell.

The labelled dendritic terminals are connected to one another and to dendritic shafts by long, slender processes (Figs 2*a*, *b*; 4). Processes from the dendritic shafts are 0.08–0.25 μm in diameter and extend for 1.0–7.4 μm before arborizing into the terminal clusters. Processes connecting the terminals are 0.03–0.33 μm in diameter and extend up to 4 μm. Moreover, portions of the dendritic shafts between clustered terminals are also quite long and slender. Because of the expected high impedance of such connecting processes, the synaptic circuits of dendritic terminals may be electrically isolated from one another and from the dendritic shaft. As postsynaptic potentials derive from conductance changes in the postsynaptic membrane, the high impedance of the terminal and its connecting processes would severely restrict the amount of synaptic current flowing through the terminals[17]. The limitation of current flow and the attenuating properties of the connecting processes would dramatically limit the amplitude of the postsynaptic potentials conducted to the dendritic shaft and soma. Calculations based on our morphometric measurements and a passive electrical model of the postsynaptic membranes suggest that activation of an excitatory synapse located on a dendritic terminal would produce a postsynaptic potential seen at the dendritic shaft and soma that would be 10–100 times smaller than one produced from activity of an equivalent synapse located on a nearby dendritic shaft[17]. Such electrical isolation implies that the clusters of dendritic terminals operate in very local microcircuits. It is thus interesting that the cell exhibits action potentials. An action potential could passively depolarize the entire dendritic arbor, including the

dendritic terminals, thereby changing the gain of transmission through these terminals in response to their synaptic inputs. Hence, the firing rate of the parent cell could control the state of these microcircuits.

However, electrical isolation of the dendritic terminal clusters is difficult to reconcile with certain properties of the labelled cell. We reconstructed the pattern of retinal inputs onto two-thirds of the labelled soma and on two of its three primary dendrites plus their daughter branches up to 60 μm from the soma. This represents the most dense region of retinal synapses found on relay cells[4]. All but four of the retinal synapses contact the labelled dendritic terminals as described above. The exceptions include one retinal synapse formed directly onto the soma (Fig. 3*b*) and three retinal synapses on short spine-like extensions of the soma (data not shown). Such somatic sites of retinal synapses are extremely rare in geniculate neurones (refs 3, 4; our unpublished observations). This pattern of retinal inputs contrasts sharply with that seen on X relay cells, for which hundreds of retinal synapses are formed proximally on dendritic shafts and short appendages[4]. Despite the presumed electrical isolation of the vast majority of retinal inputs to the labelled cell, it responded vigorously to visual stimulation and optic chiasm shock, much like an X relay cell.

That the labelled cell and identified X relay cells exhibit such striking morphological differences despite similar response properties seems particularly interesting and deserves further investigation. We suggest at least three explanations: (1) Many retinal synapses may be located on unreconstructed regions of the labelled neurone. (2) The few somatic inputs, particularly those on spine-like processes, may activate the cell effectively. Miller *et al.*[18] recently suggested that active processes in spines may significantly amplify postsynaptic potentials, so that a few retinal synapses strategically located on somatic spines might effectively discharge the postsynaptic neurone. (3) The dendritic terminals may not be isolated from each other, because our assumption that dendrites and their processes have passive electrical properties is invalid.

Here, we have described some aspects of intrinsic circuitry for the X pathway. The response properties of the labelled local circuit neurone indicate that it is innervated and vigorously driven by retinal X axons. The labelled dendritic terminals are F2 terminals and the profiles postsynaptic to them seem to derive from X relay cells. In the synaptic triads, the local circuit neurone and X relay cells are innervated by some of the same retinal X axons. This is a plausible morphological substrate of feed-forward inhibition, for which there is physiological evidence[5–7]. Because of other synaptic inputs found on clusters of labelled dendritic terminals, we further conclude that the feed-forward circuit is partially under the control of cortical (RSD), other inhibitory (F) and other relay cell (RLD) inputs. Important features that remain to be determined include a comparison of the dendritic and axonal synaptic outputs of the cell, the extent to which clusters of dendritic terminals from the same local circuit neurone are electrically isolated and the effect of action potentials in the parent cell on transmission in the dendritic terminals. Similar circuitry for the Y pathway has not yet been described, and it may be qualitatively different. Inhibitory inputs to Y relay cells are predominantly associated with F1 terminals[4], which may derive from extrinsic sources, such as the perigeniculate nucleus[19].

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1. Stone, J., Dreher, B. & Leventhal, A. *Brain Res. Rev.* **1**, 345–394 (1979).
2. Sherman, S. M. & Spear, P. D. *Physiol. Rev.* **62**, 738–855 (1982).
3. Guillery, R. W. *Vision Res. Suppl.* **3**, 211–227 (1971).
4. Wilson, J. R., Friedlander, M. J. & Sherman, S. M. *Proc. R. Soc.* **B221**, 411–436 (1984).
5. Singer, W. *Physiol. Rev.* **57**, 386–420 (1977).
6. Dubin, M. W. & Cleland, B. G. *J. Neurophysiol.* **40**, 410–427 (1977).
7. Lindstrom, S. *Brain Res.* **234**, 447–453 (1982).
8. Famiglietti, E. V. Jr & Peters, A. *J. comp. Neurol.* **144**, 285–334 (1972).
9. LeVay, S. & Ferster, D. *J. comp. Neurol.* **172**, 563–584 (1977).
10. Sterling, P. & Davis, T. L. *J. comp. Neurol.* **192**, 737–749 (1980).
11. Fitzpatrick, D., Penny, G. R. & Schmechel, D. E. *J. Neurosci.* **4**, 1809–1829 (1984).
12. Friedlander, M. J., Lin, C.-S., Stanford, L. R. & Sherman, S. M. *J. Neurophysiol.* **46**, 80–129 (1981).
13. Guillery, R. W. *J. comp. Neurol.* **128**, 21–50 (1966).
14. Ide, L. S. *J. comp. Neurol.* **210**, 317–334 (1982).
15. Rapisardi, S. C. & Miles, T. P. *J. comp. Neurol.* **223**, 515–534 (1984).
16. Famiglietti, E. V. Jr *Brain Res.* **20**, 181–191 (1970).
17. Koch, C. & Poggio, T. *Proc. R. Soc.* **B218**, 455–477 (1983).
18. Miller, J. P., Rall, W. & Rinzel, J. *Brain Res.* **325**, 325–330 (1985).
19. Cucchiaro, J. B., Uhlrich, D. J., Hamos, J. E. & Sherman, S. M. *Soc. Neurosci. Abstr.* **11**, 231 (1985).

Presynaptic control of synaptic channel kinetics in sympathetic neurones

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The kinetic properties of synaptically activated ion channels are an important determinant of the duration of the synaptic currents that produce postsynaptic potentials in autonomic neurones[1–3] and skeletal muscle[4–6]. In the two types of principal neurones in frog sympathetic ganglia, B and C cells, a twofold difference in the mean open time of the nicotinic acetylcholine (ACh-gated) ion channels accounts for the twofold difference in the decay rate of their fast excitatory postsynaptic currents (e.p.s.cs)[3]. The B and C cells are selectively innervated by two distinct classes of cholinergic preganglionic axons called B and C fibres, respectively[7]. The present study examined the influence of the preganglionic nerve on the expression of synaptic ion channel properties in sympathetic neurones. B cells were denervated surgically and allowed to become innervated solely by preganglionic C fibres. These B cells, innervated by C fibres, acquired slowly decaying e.p.s.cs and long channel open times, characteristics normally seen in C cells only. These findings provide the first evidence that the kinetic properties of postsynaptic channels can be determined by the particular class of axon innervating a neurone.

Adult bullfrogs (*Rana catesbeiana*) were anaesthetized by immersion in a 0.1% solution of tricaine for 1 h. A 1–2-mm section of the interganglionic trunk between the 6th and 7th paravertebral ganglia was removed bilaterally. This surgery interrupted only the preganglionic B axons because the preganglionic C axons enter the sympathetic chain at the more distal 7th and 8th spinal segments[7,8]. For electrophysiological examination, the 7th–10th sympathetic ganglia and associated spinal nerves were removed and pinned in a recording chamber filled with Ringer's solution[3]. Suction electrodes were applied to the 7th and 8th spinal nerves to stimulate preganglionic C fibres, to the sympathetic chain above the 7th ganglion to stimulate the preganglionic B fibres, and to the sciatic nerve for antidromic stimulation of ganglion cell axons. Nerve conduction velocity was estimated by the ratio of the nerve length to the response latency (time between stimulus and response). To ensure accurate identification of neurones, experiments were limited to cells possessing antidromic conduction velocities exceeding 1 m s^{-1} for B cells and less than 0.25 m s^{-1} for C cells. This avoided the possibility of confusing some more slowly conducting B cells with C cells[9].

Neurones in the 9th and 10th ganglia of operated and normal frogs were impaled with two microelectrodes under visual control, and voltage clamped[3] at −50 mV. In the traces shown in Fig. 1, an antidromic shock for cell identification is followed 70 ms later by an orthodromic stimulus. Figure 1*a* shows representative anti- and orthodromic responses recorded from a normal B cell; a short-latency antidromic response is followed by a short-latency orthodromic e.p.s.c. evoked by stimulating the fast-conducting preganglionic B fibres. A typical recording from a C cell (Fig. 1*b*) is easily distinguished from that of a B