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## reviews

# Spatial memory and adaptive specialization of the hippocampus

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*The hippocampus plays an important role in spatial memory and spatial cognition in birds and mammals. Natural selection, sexual selection and artificial selection have resulted in an increase in the size of the hippocampus in a remarkably diverse group of animals that rely on spatial abilities to solve ecologically important problems. Food-storing birds remember the locations of large numbers of scattered caches. Polygynous male voles traverse large home ranges in search of mates. Kangaroo rats both cache food and exhibit a sex difference in home range size. In all of these species, an increase in the size of the hippocampus is associated with superior spatial ability. Artificial selection for homing ability has produced a comparable increase in the size of the hippocampus in homing pigeons, compared with other strains of domestic pigeon. Despite differences among these animals in their histories of selection and the genetic backgrounds on which selection has acted, there is a common relationship between relative hippocampal size and spatial ability.*

Natural selection produces changes in behavior and in the brain that make animals better adapted to the environment in which they live. However, identifying evolutionary adaptations and attributing them unambiguously to the action of natural selection is not always easy. The vertebrate brain provides a good example. Its structure has changed enormously over an evolutionary timespan, but it can be difficult to determine precisely what selective forces have acted on it, and which of its features are adaptations and which are non-adaptive consequences of evolutionary change.

Comparative methods exist, however, for discovering whether a seemingly adaptive feature occurs consistently in the presence of the same selective pressure<sup>1</sup>, and these techniques can be used to analyse evolutionary change in the brain. If a particular neuroanatomical feature occurs in different animals exposed to the same selective pressure, and is better accounted for by this selective pressure than by the phylogenetic relations among the species, then it is reasonable to conclude that the feature is indeed an adaptation – the result of convergent evolution in response to natural selection.

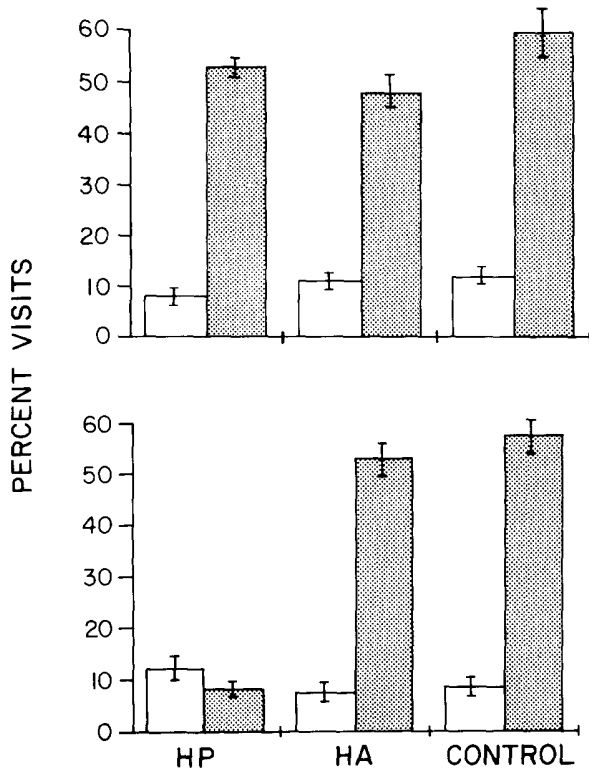
This review is primarily concerned with adaptive modifications of the hippocampus that occur in food-storing birds and polygynous male rodents, and with similar modifications produced by artificial selection in domesticated pigeons.

## Memory and the hippocampus in food-storing birds

Black-capped chickadees, and most other chickadees and tits in the family *Paridae*, store food in cache sites scattered through their home range. They store only one food item per cache and never reuse the same cache site. They can create several hundred caches of seeds, nuts and invertebrate prey in a typical winter's day, and retrieve their stored food after a few days by remembering the precise spatial locations of their caches, as shown in a variety of experimental studies (for recent reviews see Refs 2, 3).

Cache recovery by black-capped chickadees is disrupted by aspiration lesions of the hippocampus, without any obvious effect on caching, feeding or other behavior<sup>4</sup> (Fig. 1). Birds possess a hippocampal complex, comprising the hippocampus and area parahippocampalis, that has little anatomical resemblance to the mammalian hippocampus (Fig. 2). However, the avian hippocampal complex is homologous with the mammalian hippocampus both ontogenetically<sup>5</sup> and neuroanatomically<sup>6,7</sup>. Hippocampal lesions in chickadees also disrupt their ability to solve spatial problems unrelated to food storing, and produce deficits in working memory<sup>8</sup> on both spatial and non-spatial tasks.

*Comparative studies.* In addition to chickadees, two other groups of passerine birds regularly store food: nuthatches (in the family *Sittidae*) and jays (in the family *Corvidae*). Comparison of the size of the hippocampus in North American representatives of these three food-storing families with that of the hippocampus in ten non-food-storing North American families and subfamilies shows that food storers possess a hippocampus more than twice the size expected for birds of their telencephalon size and body weight<sup>9</sup> (Fig. 3). A similar comparison of European representatives of the three food-storing



**Fig. 1.** The effect of bilateral hippocampal aspiration on cache recovery accuracy in black-capped chickadees. Tinted bars show visits to cache sites as a percentage of visits to all sites while searching for caches (mean  $\pm$  1 SE). Open bars show visits to these sites before food was stored and indicate the random level of site visiting. An increase in the number of site visits after food is stored over the number before storage indicates a greater accuracy of cache recovery than expected by chance or by biases to search preferred sites. Upper panel shows the mean accuracy for birds in each of three groups prior to surgery. Lower panel shows mean accuracy following either bilateral aspiration of the hippocampus (HP), bilateral aspiration of the same volume of tissue from the hyperstriatum accessorium (HA), or no surgical procedure (Control). All differences between the number of visits before and after food storage are statistically significant (Tukey's  $HSD_6 > 16.93$ ,  $p < 0.01$ ), except for the post-aspiration HP group. (Copyright 1989 by the American Psychological Association. Adapted by permission from Ref. 4.)

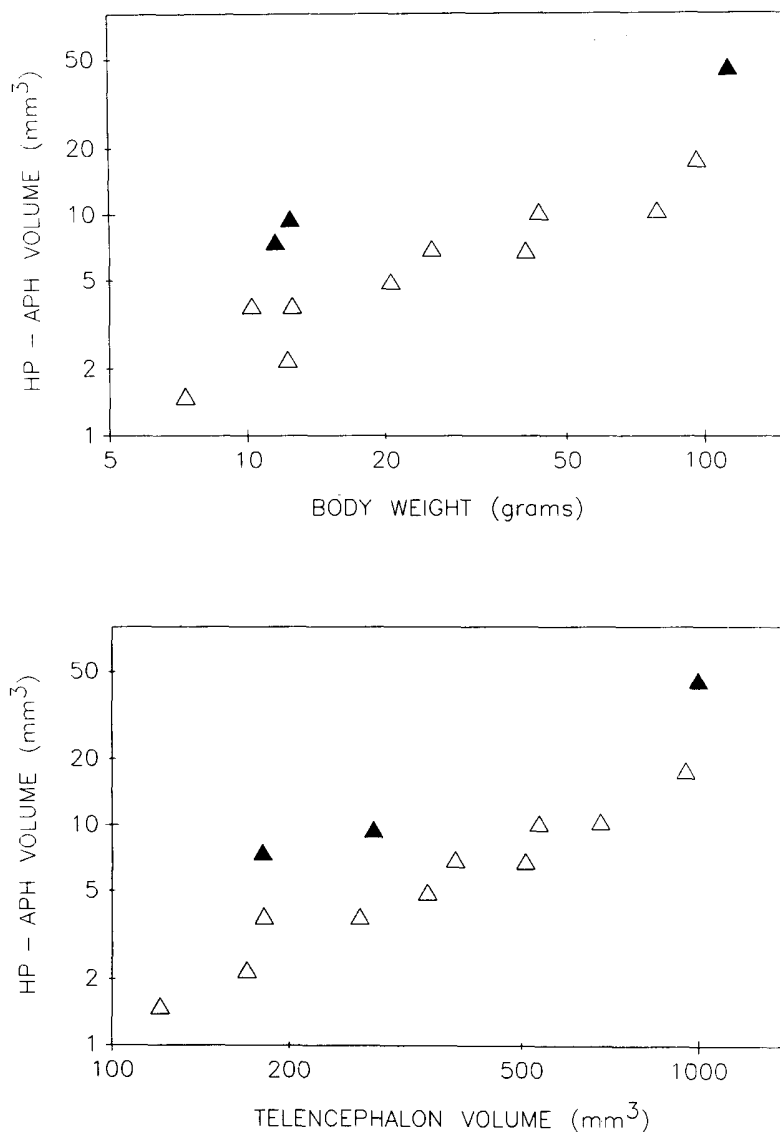
families with non-food-storing European passerines produced the same result<sup>10</sup>. Further analyses of these data showed that differences among the various groups of birds in migratory behavior, social organization, diet, mode of development, nest dispersion and habitat could not account for the differences observed in hippocampal size<sup>9,10</sup>. The ecological selection pressures that have produced food storing have thus also led to an increase in the size of a brain structure that plays a critical role in remembering the spatial locations of food caches. It is worth noting that the three food-storing families are no more closely related to each other than they are to other non-food-storing birds, and that the observed increase in hippocampal size is an evolutionary response to food storing, not a consequence of descent from a common ancestor that both stored food and possessed a large hippocampus. It may be assumed that an association between

migratory behavior and hippocampal size should exist, especially since migration must make demands on spatial memory. However, further comparative analyses have shown no relation between hippocampal size and either migratory behavior or migratory path length in passerines<sup>9,11</sup>.

*Artificial selection for homing ability.* Homing pigeons are able to return to their home loft from distant release sites using a variety of navigational strategies. There is evidence for the use of celestial, geomagnetic, olfactory and other types of information in homing. Lesions of the hippocampus produce no impairment in homeward orientation by pigeons at distant release sites, but they do impair homing ability. Although lesioned birds correctly orient themselves shortly after release, few actually reach home<sup>12</sup>. In fact, even when released near home, in view of their home loft, hippocampally lesioned homing pigeons often fail to reach their loft. The deficit produced by the hippocampal lesions is in the use of familiar local cues to locate the home loft, not in the use of navigational information to select a homeward bearing at distant release sites. Comparative analysis of the hippocampi of homing and non-homing domestic breeds of pigeons has produced a result very similar to the findings with food-storing and non-food-storing birds. The hippocampus of homing pigeons is larger than that of non-homing breeds<sup>13</sup>. Results such as these with homing pigeons may provide an explanation for the absence, noted earlier, of any association between migratory behavior and hippocampal size in passerines. If migratory birds select and maintain bearings using the same mechanisms that homing pigeons use to select a homeward bearing at a release site, then there may be little hippocampal involvement in long-distance orientation.



**Fig. 2.** The avian hippocampus, shown in a coronal section of the right dorsomedial telencephalon of a black-capped chickadee, stained for acetylcholinesterase. APH, area parahippocampalis; HA, hyperstriatum accessorium; HP, hippocampus; HV, hyperstriatum ventrale; V, ventricle. (Photomicrograph by Gwen Ivy and Moshe Khurgel.)



**Fig. 3.** The volume of the hippocampal complex (HP-APH) in three food-storing and ten non-food-storing families and subfamilies of passerine birds. In the upper panel HP-APH volume is shown relative to body weight; in the lower panel it is shown relative to telencephalon volume. All axes are logarithmic. The three food-storing families (filled triangles), in increasing order of body weight, are: Sittidae, Paridae and Corvidae. The ten non-food-storing families and subfamilies (open triangles), in increasing order of body weight, are: Sylviinae, Troglodytidae, Parulinae, Fringillidae, Emberizinae, Passeridae, Mimidae, Cardinalinae, Turdinae and Sturnidae. (Reprinted by permission of S. Karger AG, Basel, from Ref. 9.)

The increase in size of the homing pigeon hippocampus may suggest pigeons use familiar local cues for orientation to a greater extent than migratory birds.

Work by Bingman *et al.*<sup>14</sup> has also shown that the hippocampus plays an important role in the ontogeny of navigational ability in pigeons. The hippocampus is necessary for a pigeon to acquire the ability to home from distant release sites, although it is not needed by experienced birds for the later use of release-site information to orient homewards<sup>14</sup>. One interpretation of this result is that the hippocampus plays a central role during development, organizing the long-term storage of navigational information used by pigeons for homing.

### Sexual selection

Just as natural selection produces differences in the hippocampus among species of passerine birds, and artificial selection produces differences among breeds of pigeons, so sexual selection produces hippocampal differences between the sexes. Sexual selection is the mechanism that Darwin proposed to account for traits that would seem to reduce survival but increase mating success, either by increasing intra-sexual competitive ability or by increasing attractiveness to the opposite sex. Intra-sexual selection is strongest in species with polygamous mating systems, where the exaggeration of competitive traits in one sex, typically males, permits a monopolization of the reproductive potential of the other sex. Sexual selection is weak in most monogamous species because, under monogamy, neither sex can monopolize mating opportunities<sup>15,16</sup>.

Sex differences evolve slowly. Within any species, male and female genotypes are assembled from the same gene pool. Thus, if an autosomal allele augments reproductive success in one sex only, it will nevertheless be distributed to both sexes in the next generation. In this way, selection for a given trait in one sex, for example males, will tend to have a very similar effect on females, even if the trait does not augment female reproduction. No analogous process operates between species - alleles favored in one species are not automatically distributed to any other species. It requires more time, or stronger selection, for sexual selection to produce a sex difference than for natural selection to produce a species difference of similar magnitude<sup>17</sup>. Precisely because sex differences evolve slowly, any findings of sex differences in neuroanatomy and related cognitive function are especially strong evidence of adaptive modification of the brain.

Males and females of a given species generally have similar ecologies, and thus experience similar natural selection for spatial ability. However, certain mating systems can trigger sexual selection that favors sex differences in spatial skills. Under some kinds of polygyny, females have small localized ranges, while males travel more widely, competing to include in their home range as many female ranges as possible<sup>18</sup>. In these systems, mobility confers greater reproductive benefits on males than on females. Males would therefore profit more than females from any increase in spatial skills that could facilitate mobility. In contrast, male and female partners in monogamous species typically have the same range, and travel together on a permanent basis<sup>19</sup>. In a fully monogamous system, neither sex would benefit differentially from augmented spatial skills.

A male advantage in spatial tasks has been documented in laboratory rodents<sup>20,21</sup> and in humans<sup>22,23</sup>. Laboratory rats and mice have recent evolutionary histories of polygyny<sup>24,25</sup>. Furthermore, because 85% of described human societies overtly practice polygyny, and because the sexual dimorphism in anatomy, physiology and behavior found in humans conforms to that generally observed in polygynous mammals, it is reasonable to conclude that humans are polygynous or, at the least, have a very recent evolutionary history of polygyny<sup>25</sup>. More definitive evidence on the effect of sexual selection on spatial ability and the brain comes from comparisons

of closely related species that have experienced different regimes of sexual selection for spatial skills.

**Sexual selection for spatial ability and hippocampal size.** Voles, which are North American rodents of the genus *Microtus*, exhibit a wide range of mating systems. At least two species, prairie voles and pine voles (*Microtus ochrogaster* and *Microtus pinetorum*), are monogamous. Radiotelemetry in the field has confirmed the predicted isomorphic ranges of males and females in these monogamous species<sup>26,27</sup>. In contrast, the meadow vole (*Microtus pennsylvanicus*) is highly polygynous. In this species, males have ranges 4–7 times larger than female ranges<sup>26,27</sup>. This sex difference does not emerge until sexual maturity and disappears outside the breeding season, further suggesting that the larger range of the male vole is a reproductive tactic.

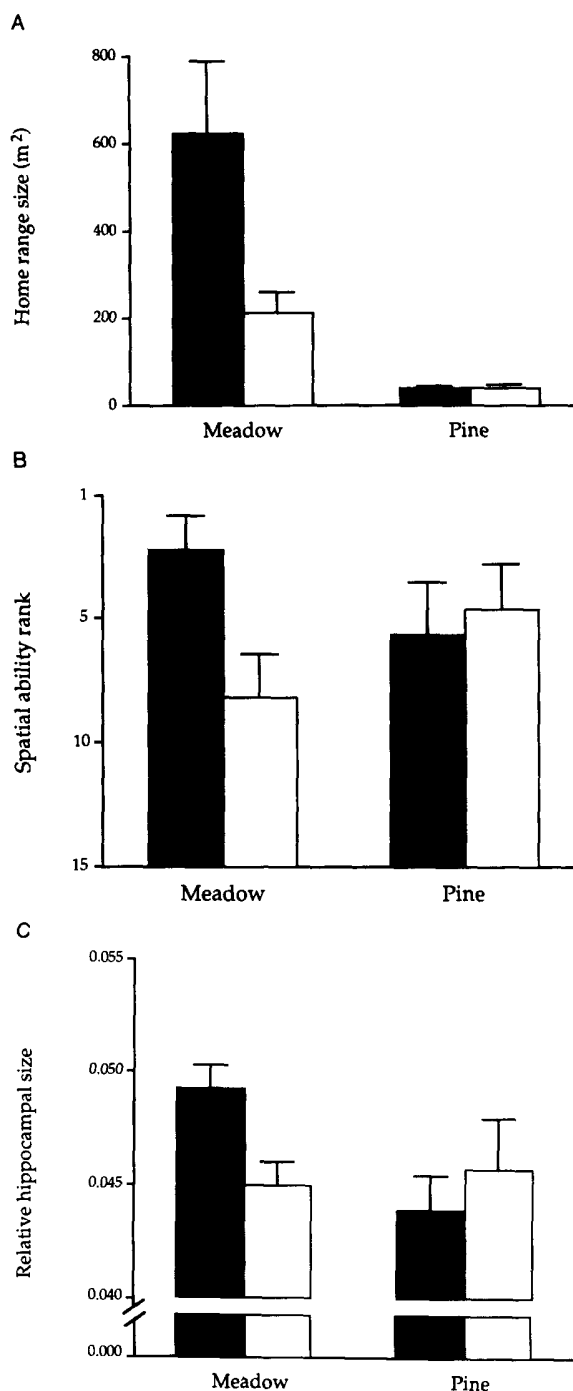
Laboratory studies have shown that this pattern of sex differences in range size is accompanied by a similar pattern of sex differences in spatial ability. Males of the polygynous meadow vole show superior performance to females in both 'place-learning' and 'route-learning' tasks. Monogamous prairie voles and pine voles exhibit no sex difference in these spatial tasks<sup>26,27</sup> (Fig. 4).

These behavioral results lead to a straightforward prediction. If, as in birds, increased selection for spatial ability leads to an increase in hippocampal size, then patterns of hippocampal size should follow patterns of spatial behavior. Hippocampal size should be sexually dimorphic in polygynous species and monomorphic in monogamous species. For species in which this issue has been examined, this is found to be true. Polygynous male meadow voles, trapped as adults during the breeding season, have a significantly larger hippocampus, relative to the size of the whole brain, than do conspecific females. There is no sexual dimorphism in hippocampal size in monogamous pine voles<sup>28</sup> (Fig. 4).

**Interaction between natural and sexual selection for hippocampal size.** Since most mammalian species are polygynous, like the meadow vole, and are sexually dimorphic in their use of space, hippocampal size should be dimorphic in most species. Many mammals, particularly small rodents, also store food. Hippocampal size in these animals should be affected by the kind of selective pressure that affects passerine birds – natural selection for the ability to efficiently retrieve caches. Food-storing mammals that rely on spatial memory to relocate scattered caches should have larger hippocampi than species that do not store food, or that store food in only one central place.

This hypothesis can be tested with data from kangaroo rats, desert rodents of the genus *Dipodomys*. Kangaroo rats are small (30–150 g) nocturnal granivores, whose bipedal locomotion enables them to cover large distances in search of seeds. All species store food and all of the well-studied species are polygynous. One would therefore predict a relatively large hippocampal size for this genus as a whole, because of its mode of foraging and storing food, and a greater hippocampal size for males relative to females within each species, because of polygyny.

The bannertail kangaroo rat (*Dipodomys spectabilis*) hoards seeds in its burrow<sup>29</sup>, and cache retrieval thus requires no specialized spatial memory. Merriam's kangaroo rat (*Dipodomys merriami*), in contrast,



**Fig. 4.** Sex differences in behavior and hippocampal size in two species of vole, the polygynous meadow vole and the monogamous pine vole. Tinted bars, males; open bars, females. Range sizes are given as means  $\pm$  1 SE. (A) Home range, computed as a minimum area polygon from telemetric observations of individual voles tracked under natural conditions during the breeding season. (B) Spatial ability on the sunburst maze. Ranks were determined within each species separately, because the species differed in the number of trials required to reach training criterion on the maze. A low rank indicates superior performance (note reversal of y-axis scale). (C) Relative hippocampal size (the volume of hippocampus relative to the volume of the entire brain) in breeding adults; data collected from the same populations as behavioral data. Sample sizes for studies of home range (A) and spatial ability (B) vary from 9 to 13 individuals; some provided both home range and spatial ability data. Hippocampal size (C) was determined from an additional sample of 10 males and 10 females from each species.

hoards food in scattered locations and uses spatial memory to relocate its caches<sup>30</sup>. Despite this difference in foraging strategy, both species are polygynous, and males increase their use of space during the breeding season<sup>31,32</sup>.

As expected, hippocampal size, relative to brain size, is significantly larger in the scatter-hoarding species, Merriam's kangaroo rat, than in the larder-hoarding species, the bannertail kangaroo rat. Furthermore, males have larger hippocampi than females in both Merriam's kangaroo rat and bannertails<sup>33</sup>. This pattern suggests that, as in passerine birds, hippocampal size is determined partially by the cognitive demands of foraging and food storing, and, as in voles, partially by the cognitive demands imposed by the mating system.

### The significance of hippocampal size

The observation that hippocampal size is correlated with a species' reliance on spatial information supports Jerison's principle of proper mass: "The mass of neural tissue controlling a particular function is appropriate to the amount of information processing involved in performing the function"<sup>34</sup>.

What exactly is the significance of this increased hippocampal size? Until recently, studies of hippocampal size in mammals were limited to searching for evolutionary progressions – progressive increases in the size of the hippocampus in more recently evolved taxa. Such an approach has demonstrated, among other things, that hippocampal size is greater in primates than in insectivores<sup>35</sup>. However, deducing evolutionary progressions from extant species may be misleading. On a large scale, such as between taxonomic orders, a simple ratio of hippocampal size to brain size will be confounded by changes in other brain regions. The relative size of the hippocampus, for example, is smaller in humans than in any of the insectivores or primates, primarily because of the relatively large size of the human neocortex<sup>36</sup>. It is far more informative to determine the correlation between a structure and its known function in a monophyletic group of animals<sup>37</sup>.

Within closely related groups, it is reasonable to assume that brain space is allocated in proportion to the function of each structure. Studies of short-term changes in the size of brain structures have revealed possible proximate causes of the evolutionary change in size. Laboratory rats raised in complex environments have greater cortical weights than rats raised in standard laboratory cages<sup>38</sup>. Increases in the volume of visual cortex of the rats from complex environments are associated with increases in dendritic and axonal processes, the development of new synaptic connections<sup>39</sup>, and increases in metabolic support systems such as capillaries and mitochondria<sup>40</sup>. Given such developmental plasticity, natural selection may act on heritable differences in development to produce the differences in hippocampal size observed in nature, both within and between species. A series of elegant studies has confirmed the existence of such heritable differences in the development of the hippocampus in strains of laboratory rats and mice, and, most importantly, has shown correlations between hippocampal morphology and learning abilities, including spatial learning, among these strains<sup>41</sup>. In rat strains selected for good or poor acquisition of a

non-spatial shock-avoidance task, the size of the terminal fields of intra- and infrapyramidal mossy fiber projections is negatively correlated with acquisition. However, among mouse strains, the size of these projections is positively correlated with spatial learning in a water maze<sup>41,42</sup>.

### Concluding remarks

Comparative studies have shown that the size of the hippocampus is affected by similar selective pressures in birds and mammals, namely the degree to which the animal relies on spatial information to locate ecologically important resources, such as food and mates. The hippocampus seems remarkably responsive to natural selection, sexual selection and artificial selection for spatial abilities. The challenge for future research is to identify the changes in cell populations and projections that are responsible for the observed increases in hippocampal size, and to determine how these changes lead to more effective hippocampal function and augmented spatial ability.

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## Calcium-binding proteins in the nervous system

K. G. Baimbridge, M. R. Celio and J. H. Rogers

*Among the many calcium-binding proteins in the nervous system, parvalbumin, calbindin-D28K and calretinin are particularly striking in their abundance and in the specificity of their distribution. They can be found in different subsets of neurons in many brain regions. Although it is not yet known whether they play a 'triggering' role like calmodulin, or merely act as buffers to modulate cytosolic calcium transients, they are valuable markers of neuronal subpopulations for anatomical and developmental studies.*

Calcium ions ( $\text{Ca}^{2+}$ ) play a key role in transmembrane signalling and the intracellular transmission of signals. However,  $\text{Ca}^{2+}$  does not act alone. Many cells contain a variety of cytosolic calcium-binding proteins (CaBPs) which either modulate or mediate the actions of this ion<sup>1</sup>. The more notable of these are listed in Box 1 and include several ubiquitous proteins that mediate biochemical responses to intracellular  $\text{Ca}^{2+}$  signals. In this review, however, we will concentrate on the CaBPs that have more restricted distributions in neurons, and are abundant but of unknown function: parvalbumin, calbindin-D28K and calretinin.

These proteins are members of the 'EF-hand' family of CaBPs (Ref. 2), which is defined by an amino acid sequence similar to the consensus sequence shown in Fig. 1A. This sequence folds up into a helix-loop-helix pattern, known as the EF hand, in which hydrophilic sidechains bind one calcium atom (Fig. 1B). For didactic purposes, two groups of EF-hand CaBPs are defined, the 'trigger' and the 'buffer' proteins<sup>3</sup>. Trigger proteins (calmodulin and troponin-C) change their conformation after binding  $\text{Ca}^{2+}$ , and can then modulate the activity of various enzymes<sup>4</sup> and ion channels<sup>5</sup>. The buffer CaBPs, such as parvalbumin in muscle or the 9-kDa calbindin in the mammalian gut enterocyte, are believed to make up a more passive system, which may limit a stimulated rise in intracellular free calcium concentration. The 28-kDa calbindin (calbindin-D28K) replaces the smaller form in the chick gut enterocyte; it is only this larger form that is found in neurons of the central and peripheral nervous systems, including the enteric nervous system, of birds, mammals and other species.

Parvalbumin, calretinin and calbindin-D28K occur in distinct subpopulations of neurons that may therefore be distinguished by specific calcium-dependent pro-

cesses<sup>6–8</sup>. Although the function of these CaBPs in neurons is unknown, antibodies against these proteins have been employed as neuroanatomical markers<sup>8–13</sup>. The CaBPs have an advantage over other neuronal markers in that, by virtue of their high solubility, they are usually present throughout the cytosol, even in the thin processes of neurons, therefore facilitating studies of neuronal shape and connectivity. In general, different CaBPs are segregated to separate subpopulations of complementary systems in the brain, but there are many cases of cells harbouring two or more types of CaBP; for example, Purkinje cells<sup>8</sup> and many neurons within the spinal cord<sup>14,15</sup> contain both parvalbumin and calbindin-D28K. However, some nerve cells, including most pyramidal cells of the cerebral cortex and hippocampus<sup>16,17</sup>, do not contain any of the known members of the EF-hand family of CaBPs, with the exception, perhaps, of calmodulin.

Antibodies to CaBPs have been used to follow the ontogeny of various functional systems<sup>18–21</sup>. In addition, they have aided the elucidation of highly

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### Box 1. Major calcium-binding proteins in the nervous system

Present in most cell types, including neurons	Present in certain cell types in CNS
<i>EF-hand family</i>	<i>EF-hand family</i>
Calmodulin (ubiquitous calcium-dependent modulator of protein kinases and other enzymes)	Parvalbumin (in some neurons)
Calpains (calcium-dependent proteases)	Calbindin-D28K (in some neurons)
$\alpha$ -Actinin	Calretinin (in some neurons)
<i>Other families</i>	Recoverin, visinin (in photoreceptors; regulate guanylyl cyclase)
Annexins ( $\text{Ca}^{2+}$ -phospholipid-binding proteins; of unknown function, but implicated in exocytosis)	S100 $\alpha,\beta$ (in glia; effects on phosphorylation and neurite outgrowth)
Protein kinase C	
Gelsolin (and other cytoskeleton-associated proteins)	