CMLS, Cell. Mol. Life Sci. 55 (1999) 593–600
1420-682X/99/040593-08 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Making more synapses: a way to store information?

M. B. Moser

Department of Psychology, Norwegian University of Science and Technology, N-7491 Trondheim (Norway), Fax + 47 73 59 19 20, e-mail: maybm@svt.ntnu.no

Abstract. Although information may be stored in the brain as changes in the strength of existing synapses, formation of new synapses has long been thought of as an additional substrate for memory storage. The identification of subcellular structural changes following learning in mammals poses a serious 'needle-in-the-haystack' problem. In most attempts to demonstrate structural plasticity during learning, animals have been exposed for prolonged periods to complex environments, where they are confronted with a variety of sensory, motor and spatial challenges throughout the exposure period. These environments are thought to promote several forms of learning. Repeated exposure to such environments has been shown to increase the density of spines and dendritic complexity in relevant brain structures. The number of neurons has also been reported to increase in some areas. It is not clear, however, whether the new synapses emerging from these forms of plasticity mediate specific information storage, or whether they reflect a more general sophistication of the excited parts of the network.

Key words. Spatial learning; memory; hippocampus; pyramidal cells; CA1; spines; synaptic plasticity; complex environment; enriched environment.

The problem of identifying structural changes in adult mammals

It has long been assumed that the brain stores information by changing the efficacy of connections within the cellular network involved in the storage process [1–3]. Synaptic transmission may be facilitated by enhancement of transmission across existing synapses, or by formation of new synapses or new cells. These possibilities are not mutually exclusive.

The search for structural changes following information storage is difficult. Such changes are expected to be tiny and distributed [2, 3], implying a serious 'needle-in-the-haystack' problem. The problems can be overcome to some extent in invertebrates, which have ganglia with only a few thousand cells. In the marine snail *Aplysia*, the cellular network responsible for certain types of learning is well described and easily accessible. Nonassociative modification of the gill-siphon withdrawal reflex in this species is accompanied by structural changes in the network mediating the reflex behaviour [4, 5]. Following long-term habituation, in which the snail learns to ignore a neutral stimulus, the number of synapses between sensory and motor neurons decreases. Following sensitization, in which the snail reacts stronger to a noxious stimulus with repeated presentations, new synapses are formed between these neurons. Is there structural plasticity also in the nervous system of adult mammals? Several lines of evidence suggest that synaptic and dendritic structure may be subject to continuous modification. For example, it has been reported that circulating hormones have huge effects on spine density in CA1 pyramidal neurons of the adult hippocampus. Dendritic spine density increases dramatically (30%) when estradiol levels increase from estrus to proestrus [6–8]. Exogenous administration of estradiol causes a similar increase of spine density [8–10]. Morphological changes induced by stress provide another example of detectable structural plasticity in mammals. Restraint stress and psychosocial stress both cause atrophy in dendrites of pyramidal neurons in CA3 [11, 12]. To identify structural changes associated with learning, it would make sense to search in brain regions that are known to be involved in mnemonic functions. The hippocampus is such a structure [13, 14]. The hippocampus has a role in spatial memory [15, 16],

although its function may be broader [14, 17, 18]. The hippocampus is probably responsible primarily for fairly recent memory, as events acquired months or years ago can be retrieved independently of hippocampal circuits [19].

Several observations suggest that the hippocampus is somehow involved in the storage of the acquired information for a certain length of time. Hippocampal synapses are capable of long-lasting modifications [20, 21]. Blockade of such modifications interferes with memory formation [22–24], suggesting that memory formation is accompanied by modifications in hippocampal synapses. It is not clear what is stored in these synapses. The hippocampus may store specific information [25], or it may store only pointers or indexes to neocortical ensembles where the specific information is stored [26]. Structural plasticity may be beneficial to both forms of information storage.

Neurogenesis in response to environmental demands

One form of structural change that may subserve information storage is neurogenesis. This is particularly clear in the avian hippocampus. Food-storing birds use spatial memory for the accurate retrieval of food stored in numerous storage sites. As in mammals, such spatial memory requires the integrity of the hippocampus. Bilateral damage to the hippocampus disrupts memory for the location of food caches, but does not affect caching and feeding behaviour per se [27, 28]. Food-storing birds have larger hippocampi (relative to total brain size) with more neurons than related nonstoring species [29, 30]. Similar differences in hippocampal volume have been reported in mammals. Polygamous voles have more dispersed home ranges than a monogamous species, and home range size correlates with the volume of the hippocampus [31]. Scatter-hoarding kangaroo rats have larger hippocampal volumes than closely related rats, which cache all their food in one larder [32].

The increased hippocampal volume (and neuronal number) in animals confronted with extensive spatial challenge may either be an effect of natural selection to fit environmental requirements or an effect of the individual animal's experience. In birds, hippocampal growth seems to be experience dependent. The hippocampal volume of hand-raised marsh tits allowed to store and recover food catches at different ages was compared with that of inexperienced age-matched controls [33]. Experience with food storing and retrieval resulted in hippocampal enlargement and more neurons in the hippocampus. Inexperienced birds had a higher proportion of apoptotic cells in the hippocampus, suggesting that programmed cell death was reduced in experienced birds. It has also been reported that the rate of cell proliferation in the ventricular zone adjacent to the hippocampus is elevated in experienced birds [34]. Thus, the increase in hippocampal volume may reflect an increase of both birth and survival of neurons, triggered by environmental demands.

In mammals, in contrast to birds, the final number of nerve cells is generally reached before birth [35], suggesting that hippocampal volume may not increase as a function of birth of new neurons. One exception to this rule is the neurogenesis of granule cells in the dentate gyrus that occurs throughout life in rats [36–38], although it decreases with increasing age [39]. The neurogenesis is sensitive to environmental challenge. Enriched housing leads to a larger granule cell layer in the dentate gyrus and a 15% increase of the number of granule cells compared with controls housed in standard cages [40]. Proliferation of progenitor cells appears to be unaffected by environmental stimulation. Thus, enriched housing seems to have a survival-promoting effect on granule neurons.

Subcellular structural changes

To store information by increasing neuronal number alone may not be adaptive. A more flexible mechanism would be to increase the number of synapses. Synapses are so tiny and so numerous that a huge amount of information could be stored without affecting the limited space of the brain. In order to maximize the chances to detect subcellular structural changes following training, animals have often been exposed to enriched environments, and then been compared with animals housed alone or in groups in stereotyped environments. The assumption is that exposure to a changing, complex environment is accompanied by larger changes than acquisition of a single learning task.

The choice of methods for detecting subcellular changes is critical. The Golgi technique is not suitable for quantitative comparisons, because it stains incompletely. Estimates of spine density and dendritic length based on Golgi-stained material are generally smaller than estimates based on intracellular staining [41, 42]. The unknown factor interfering with the staining may bias the selection of cells and spines in some of the treatment groups. Due to these limitations, the majority of attempts to identify environmentally induced structural changes in the mammalian brain have employed light microscopy to detect large-scale changes in dendritic morphology and electron microscopy to detect smaller changes at the level of the synapse.

Structural plasticity detected with light and electron microscopy

Rearing from weaning in an enriched environment increases higher-order branching of basal pyramidal-cell dendrites in the visual and temporal cortex [43–45]. Such changes may be detectable after only 4 days of differential experience [46]. The effect is expressed only in selected cortical regions [45], implying that it may not be due to nonspecific hormonal or nutritional variables only.

Rearing also affects the density of synapses. It has been reported that rearing in an enriched environment increases the number of spines per neuron in the visual cortex [47]. Spine density was increased in the dendrites of occipital cortical neurons [48]. Mice trained in several motor tasks, including swimming, rope climbing and running wheel activity, had Purkinje cells with more spines than mice reared in a small cage in which movement was severely restricted [49]. Cats reared from 6 weeks to 8 months of age in an enriched environment had more asymmetric (excitatory) synapses and fewer symmetric (inhibitory) synapses than isolated cats [50]. The interpretation of these data is not straightforward. There are two reasons for this. The first is that most of the data were collected from young and immature animals. The development of the brain is not finished at the time of birth. Although neurogenesis and apoptosis are usually complete, the formation of connections between the cells continues postnatally. New synapses are formed, and some existing synapses are retracted. In the macaque striate cortex, between 10,000 and 40,000 synapses are formed per second during the first two postnatal months [51]. During puberty, synaptic density decreases rapidly to reach the adult level [51]. A longitudinal study carried out in the cat suggests that the period of plasticity in response to monocular deprivation lasts longer than previously thought and actually ends during puberty [52]. Thus, developmental structural changes may confound the detection of changes induced by learning per se. In studies where differential rearing started at adult age, the reported effects are essentially limited to gross dendritic morphology. Enriched housing caused increased dendritic branching of basal dendrites in the visual and cerebellar cortex [53, 54], and extensive training in a Hebb-Williams maze led to more extensively branched apical dendrites of pyramidal neurons in the visual cortex [55].

The second problem relates to the use of electron microscopy to detect changes in density of spines or synapses. Although electron microscopy provides high synaptic detail, it is an inconvenient method for whole cell reconstruction. Dendritic length comparisons are virtually impossible, implying that undetected differential shrinking or expansion of the tissue (e.g. altered glia/neuron ratios) may account for observed increases in spine density. Moreover, the parent dendrite can only be identified if electron microscopy is combined with serial reconstruction of the dendrite. This is time-consuming and results in very small sample sizes. Due to small samples and limited whole-cell reconstruction, it is difficult to separate changes caused by the treatment from the normal variance.

Increased spine density in hippocampal pyramidal cells after extensive spatial training

Confocal microscopy combined with intracellular staining with a fluorescent dye may be viewed as a compromise between Golgi and electron-microscopic techniques. The resolution is higher than in light microscopes, and spines lying close to each other can be detected by studying sections through the dendritic segment one by one. The three-dimensional reconstruction obtained by the confocal method makes it possible to measure dendritic length and identify the parental cell and dendrite of each spine. The reconstruction process is automated and less time-consuming than serial reconstructions in electron microscopy, allowing measurements on a higher number of cells. Thus, confocal microscopy is well suited for detecting small structural changes in relatively large samples of cells.

We have measured changes in dendritic morphology and spine number following extensive spatial training in a complex environment (fig. 1A) in adult rats with confocal microscopy [56, 57]. To stimulate explorative behaviour, a number of objects of different texture and form were placed in the environment. The rats were food-deprived to increase searching for hidden food and water. The number, position and angle of the floors, and the ladders that connected them, was changed before each daily exposure. Over sessions, the difficulty in reaching all the floors was increased by making the ladders fewer, narrower and steeper, and by introducing obstacles that had to be passed to reach a new floor. The rats soon visited all floors within the first hour of exposure, in spite of complete rearrangement of the environment (fig. 1B). Nontrained rats were either housed in pairs in transparent cages (paired group) or individually in opaque cages in a quiet room (isolated group). CA1 pyramidal cells from the hippocampus of adult rats trained in these environments were stained with Lucifer yellow. To achieve a homogenous sample, only cells from the middle of CA1 (not close to CA3 or subiculum) of the dorsal hippocampus were sampled for analysis with the confocal microscope. For spine detection, dendritic segments were sampled only from the mid-proximal parts of the basal and apical dendrites, and explicit criteria for spine counting were used.

Basal dendrites of CA1 pyramidal cells in the trained group had 10% more spines per micrometer than corresponding dendritic segments in the control groups (fig. 2). Total dendritic length was not different, suggesting that the increased spine number on some of the segments was due to insertion of new spines. Excitatory synapses are almost exclusively located on spines in hippocampal pyramidal cells, whereas inhibitory synapses are generally located on the dendrites themselves [58]. Thus, the increase in spine density probably reflects an increased number of excitatory synapses per neuron.

Relation between structural plasticity and learning

An important question is the extent to which changes in spine density following exposure to complex environments are related to memory formation. A general increase in spine number on all dendritic segments in the trained group might indicate that general metabolic factors led to the changes in the mean spine density. However, in the confocal-microscopy study [57, 58], spine density was changed only in the basal dendrites of the CA1 pyramidal cells. There was no significant change in spine density or dendritic features on the apical side, even though apical dendrites are capable of dramatic alterations in spine number in response to changed estrogen concentrations [6–10]. The distribution of spine densities in the basal dendrites in the trained group had an enlarged tail to the right (fig. 3), suggesting a specific increase in the number of segments with high spine density. Moreover, in animals with split hemispheres, morphological (dendritic) changes in the visual cortex following enriched housing were restricted to the hemisphere that received visual input [59]. Acrobatic learning induced synaptogenesis in the cerebellar cortex of adult rats, whereas activity per se increased only the density of the blood vessels [60]. Finally, training on a reaching task increased dendritic branching selectively in neurons of the sensory-motor forelimb cortex in the trained hemisphere [61]. Thus, structural changes in the cortex may be causally related to the training.

The question of causality in the hippocampus could be addressed more specifically by selectively preventing information storage during exposure to the enriched

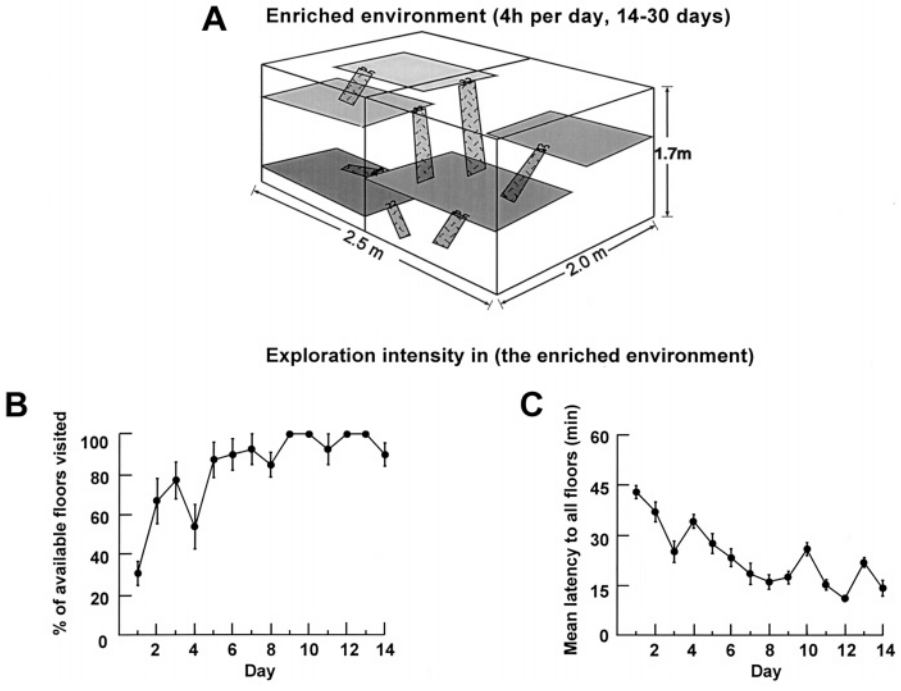

Figure 1. Spatial training and rate of exploration in a complex environment. (*A*) Outline of the basic features of the complex environment. In addition to floors and ladders, a variety of familiar and unfamiliar objects were placed on each of the floors. Floors and ladders were moved between each exposure to the environment. (*B*, *C*) Development of exploration intensity during the first hour of daily 4-hr exposures in the complex environment, expressed as the mean percentage of available floors visited during the first hour by all rats (*B*) and the mean latency to visit all of these floors (*C*). If a rat failed to enter all floors during the first hour, it was assigned a latency of 60 min. Adapted, with permission, from [56]: © (1994) National Academy of Sciences, USA.

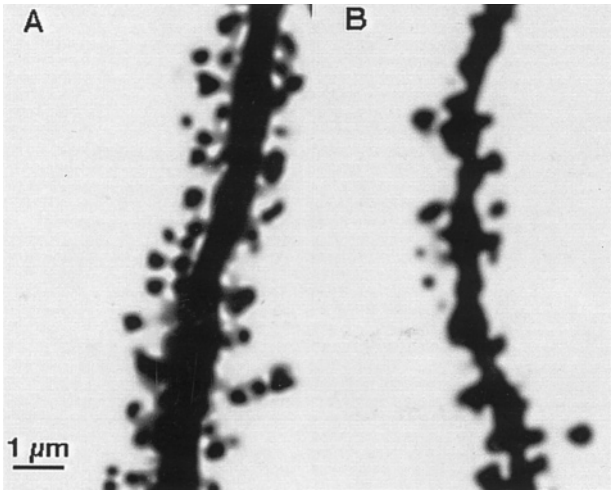

Figure 2. Examples of basal dendritic segments from CA1 pyramidal cells in a rat trained in the complex environment (*A*) and in an isolated rat (*B*). The spine densities for these two segments were 2.71 and 1.75 spines per micrometer, respectively. Spine densities in isolated rats were usually < 2.0 spines per micrometer and never exceeded 2.4 spines per micrometer. Reprinted with permission from [56]: © (1994) National Academy of Sciences, USA.

environment. This could most easily be done with conditional targeted mutations [62], although repeated intrahippocampal infusions of, for example, blockers of N-methyl-D-aspartate receptors are also feasible. If similar structural changes are found in the enriched group with synaptic plasticity blocked as in an enriched control group, the changes in the hippocampus may not be due to the spatial training but rather some nonspecific effect of being in the enriched environment.

It is not clear whether spine formation following extensive training mediates information storage specifically. An alternative possibility is that selected hippocampal synaptic populations, possibly restricted to specific segments of the principal cell dendrites, grow as a whole in response to the increased complexity of the input. In hippocampal cultures, the estradiol-induced increase of spine number on pyramidal cells is likely to be mediated through a reduction of inhibition by aspiny gamma-aminobutyric acid (GABA)ergic interneurons [63–65]. This means that the estradiol-induced increase in spine density probably correlates with a general increase in excitatory drive. A similar effect, restricted to specific segments innervated by specific interneurons, may be responsible for the increase of spine density in rats exposed for long periods to complex environments or complex learning tasks.

A general increase in the number of available synapses on segments of cells involved in memory formation

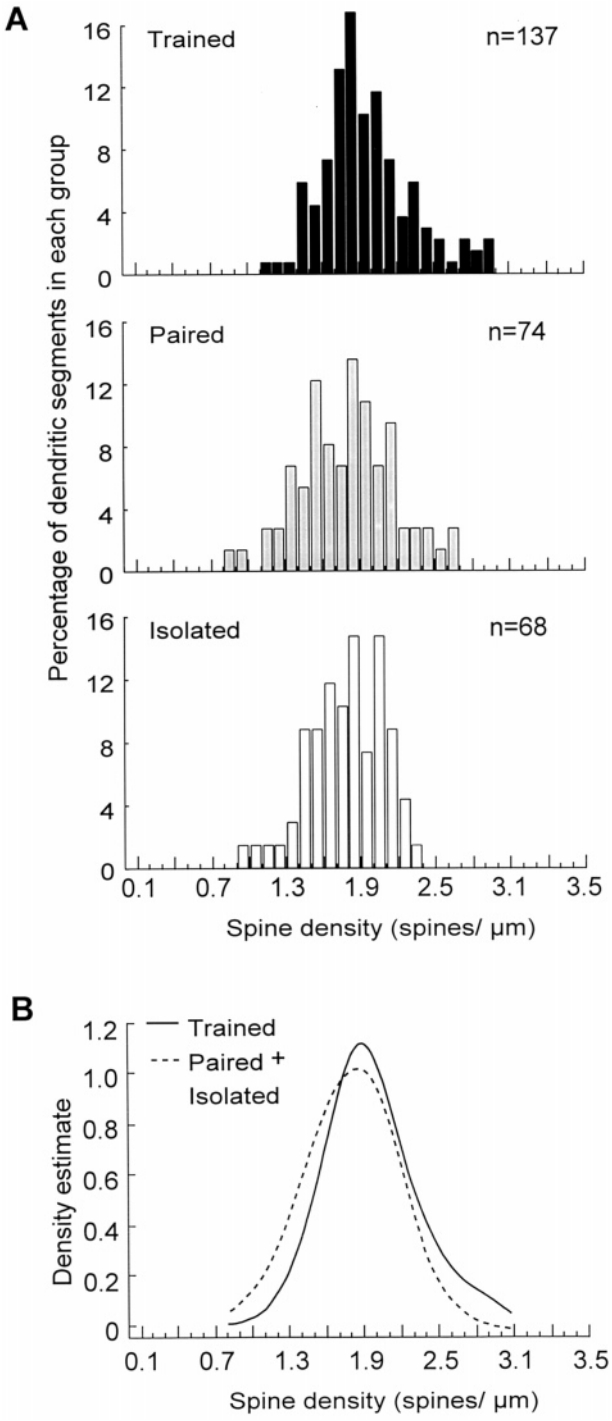

Figure 3. The distribution of basal dendritic spine densities in the rats that were trained in the complex environment and in rats that were housed in pairs or in isolation. (*A*) The values from all segments were divided into bins of 0.1 spine/μm. The number of spine densities in each category is expressed as the percentage of the total spine density count. (*B*) The probability density distribution of spine densities in the group that was trained in the complex environment (solid line) and in the control groups, with paired and isolated animals combined to a single group (dashed line). Reprinted with permission from [57]: Wiley-Liss Inc., a subsidiary of John Wiley and Sons, Inc. © (1997) John Wiley and Sons.

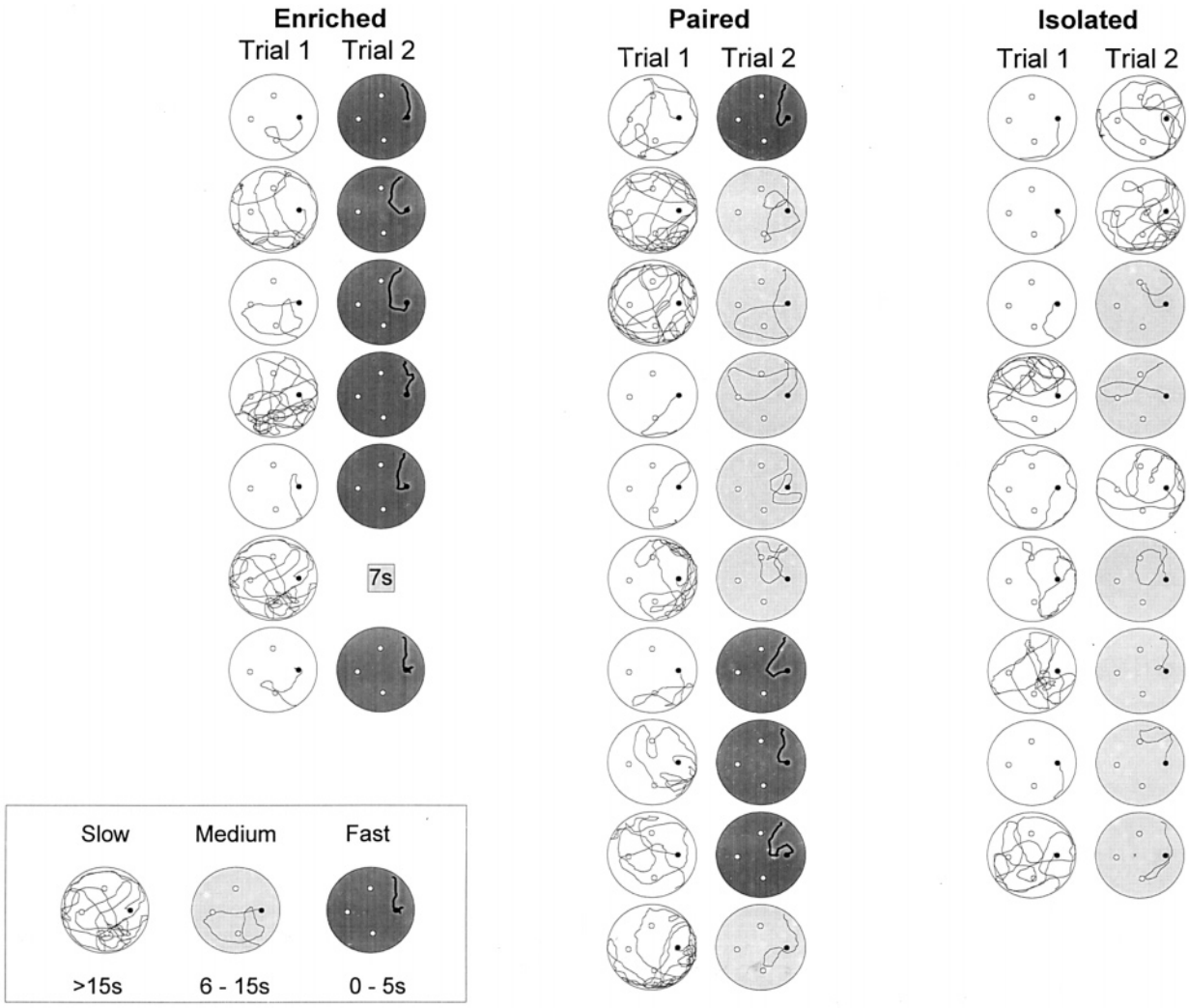

Figure 4. Swim paths of enriched rats and rats housed in pairs or in isolation in a delayed-matching task in a water maze. One-trial learning was enhanced in rats exposed to the complex environment, with 6/7 animals swimming directly to the new platform position on the second trial with this position (escape latencies < 5 s). Only 4/10 paired animals showed such behaviour. None of the isolated animals swam directly to the new target position. Inset: Categories for slow, medium and fast learning. Adapted, with permission, from [56]. Copyright (1994) National Academy of Sciences, USA.

would increase the capacity of the animal to cope with a variety of new environmental challenges. Thus, we investigated whether exposure leading to increased spine density also improved new learning. Animals trained in the complex environment (fig. 1A) showed slightly faster acquisition of a water-maze task than paired or isolated animals [56]. The effect was small and consistent with previous results showing either moderate enhancement or no change [66–68]. The weak effects may reflect a ceiling effect. The reference version of the water-maze task may often be too simple to detect an improvement of learning. Thus, we subsequently trained the differentially reared animals in a more challenging delayed-matching version of the water-maze task. The enriched animals were clearly superior and showed efficient one-trial learning [56]. On the second trial with the platform in a new position, they swam directly towards the target, whereas the control rats had several detours (fig. 4).

These observations suggest that increased spine density is associated with increased capacity for new learning. It is conceivable that the new synapses do not primarily store specific information from the complex environment, although such a role cannot be excluded. The increased spine density may reflect a general sophistication of parts of the network, which after training in the

complex environment may embody new or more elaborated programmes that increase the general capacity of hippocampal circuits to handle memory.

Conclusions

Information may be stored in the brain not only as changes in the strength of existing synapses but also by formation of new synapses. It is difficult to identify such changes, and most attempts to demonstrate structural plasticity as a substrate for memory storage are based on correlations. Although structural changes have been observed repeatedly in trained animals, it is not known whether they are causally related to memory formation. Assessment of learning following interventions that specifically block growth of new synapses or cells in relevant neuronal populations will be important in this respect.

Acknowledgments. I thank Per Andersen and Edvard Moser for discussion. The author is supported by grants from the Norwegian Research Council (no. 115013/310, 115015/310, 122512/310).

1 Hebb D. O. (1949) The Organization of Behavior, Wiley, New York
2 Marr D. (1971) A theory of archicortex. Proc. R. Soc. Lond. Ser. B **262:** 23–81
3 McNaughton B. L. and Morris R. G. M. (1987) Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends Neurosci. **10:** 408–415
4 Bailey C. H. and Chen M. (1983) Morphological basis of long-term habituation and sensitization in Aplysia. Science **220:** 91–93
5 Bailey C. H. and Chen M. (1988) Long-term memory in *Aplysia* modulates the total number of varicosities of single identified sensory neurons. Proc. Natl. Acad. Sci. USA **85:** 2373–2377
6 Woolley C. S., Gould E., Frankfurt M. and McEwen B. S. (1990) Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J. Neurosci. **10:** 4035–4039
7 Woolley C. S. and McEwen B. S. (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J. Neurosci. **12:** 2549–2554
8 Woolley C. S. and McEwen B. S. (1993) Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. J. Comp. Neurol. **336:** 293–306
9 Gould E., Woolley C. S., Frankfurt M. and McEwen B. S. (1990) Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J. Neurosci. **10:** 1286–1291
10 Murphy D. D. and Segal M. (1996) Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. J. Neurosci. **16:** 4059–4068
11 Watanabe Y., Gould E. and McEwen B. S. (1992) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res. **588:** 341–345
12 Magarinos A. M., McEwen B. S., Flugge G. and Fuchs E. (1996) Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J. Neurosci. **16:** 3534–3540
13 Squire L. R. (1992) Memory and the hippocampus: a synthesis of findings from rats, monkeys and humans. Psychol. Rev. **99:** 195–231
14 Eichenbaum H. (1997) Declarative memory: insights from cognitive neurobiology. Annu. Rev. Psychol. **48:** 547–572
15 O'Keefe J. and Nadel L. (1978) The Hippocampus as a Cognitive Map, Clarendon, Oxford
16 Nadel L. (1991) (with commentaries) Is the hippocampal formation preferentially involved in spatial behavior? Hippocampus **1:** 221–292
17 Rawlins J. N. P. (1985) Associations across time: the hippocampus as a temporary memory store. Behav. Brain Sci. **8:** 479–528
18 Wallenstein G. V., Eichenbaum H. and Hasselmo M. E. (1998) The hippocampus as an associator of discontiguous events. Trends Neurosci. **21:** 317–323
19 Squire L. R. and Alvarez P. (1995) Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr. Opin. Neurobiol. **5:** 169–177
20 Bliss T. V. P. and Lømo T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. J. Physiol. (Lond.) **232:** 331–356
21 Bliss T. V. P. and Collingridge G. L. (1993) A synaptic model of memory—long-term potentiation in the hippocampus. Nature **361:** 31–39
22 Morris R. G. M., Anderson E., Lynch G. S. and Baudry M. (1986) Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. Nature **319:** 774–776
23 Tsien J. Z., Huerta P. T. and Tonegawa S. (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell **87:** 1327–1338
24 Moser E. I., Krobert K. A., Moser M.-B. and Morris R. G. M. (1998) Impaired spatial learning after saturation of long-term potentiation. Science **281:** 2038–2042
25 Nadel L. and Moscovitch M. (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. Curr. Opin. Neurobiol. **7:** 217–227
26 Teyler T. J. and DiScenna P. (1985) The role of hippocampus in memory: a hypothesis. Neurosci. Biobehav. Rev. **9:** 377–389
27 Sherry D. F. and Vaccarino A. L. (1989) Hippocampus and memory for food caches in black-capped chickadees. Behav. Neurosci. **103:** 308–318
28 Patel S. N., Clayton N. S. and Krebs J. R. (1997) Hippocampal tissue transplants reverse lesion-induced spatial memory deficits in zebra finches (*Taeniopygia guttata*). J. Neurosci. **17:** 3861–3869
29 Krebs J. R., Sherry D. F., Healy S. D., Perry V. H. and Vaccarino A. L. (1989) Hippocampal specialization of food-storing birds. Proc. Natl. Acad. Sci. USA **86:** 1388–1392
30 Sherry D. F., Vaccarino A. L., Buckenham K. and Herz R. S. (1989) The hippocampal complex of food-storing birds. Brain Behav. Evol. **34:** 308–317
31 Jacobs L. F., Gaulin S. J., Sherry D. F. and Hoffman G. E. (1990) Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. Proc. Natl. Acad. Sci. USA **87:** 6349–6352
32 Jacobs L. F. and Spencer W. D. (1994) Natural space-use patterns and hippocampal size in kangaroo rats. Brain Behav. Evol. **44:** 125–132
33 Clayton N. S. and Krebs J. R. (1994) Hippocampal growth and attrition in birds affected by experience. Proc. Natl. Acad. Sci. USA **91:** 7410–7414
34 Patel S. N., Clayton N. S. and Krebs J. R. (1997) Spatial learning induces neurogenesis in the avian brain. Behav. Brain Res. **89:** 115–128
35 Rakic P., Bourgeois J. P. and Goldman-Rakic P. S. (1994) Synaptic development of the cerebral cortex: implications for learning, memory and mental illness. Prog. Brain Res. **102:** 227–243

36 Altman J. and Das G. D. (1965) Autoradiographic and histologic evidence of postnatal neurogenesis in rats. J. Comp. Neurol. **124:** 319–335
37 Kaplan M. S. and Hinds J. W. (1977) Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science **197:** 1092–1094
38 Cameron H. A., Woolley C. S., McEwen B. S. and Gould E. (1993) Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. Neurosci. **56:** 337–344
39 Kuhn H. G., Dickinson-Anson H. and Gage F. H. (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. **16:** 2027–2033
40 Kempermann G., Kuhn H. G. and Gage F. H. (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature **386:** 493–495
41 Amaral D. G., Ishizuka N. and Claiborne B. (1990) Neurons, numbers and the hippocampal network. Prog. Brain Res. **83:** 1–11
42 Trommald M., Jensen V. and Andersen P. (1995) Analysis of dendritic spines in rat CA1 pyramidal cells intracellularly filled with a fluorescent dye. J. Comp. Neurol. **353:** 260–274
43 Volkmar F. R. and Greenough W. T. (1972) Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science **176:** 1445–1447
44 Greenough W. T. and Volkmar F. R. (1973) Pattern of dendritic branching in occipital cortex of rats reared in complex environments. Exp. Neurol. **40:** 491–504
45 Greenough W. T., Volkmar F. R. and Juraska J. M. (1973) Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. Exp. Neurol. **41:** 371–378
46 Wallace C. S., Kilman V. L., Withers G. S. and Greenough W. T. (1992) Increases in dendritic length in occipital cortex after 4 days of differential housing in weanling rats. Behav. Neural Biol. **58:** 64–68
47 Turner A. M. and Greenough W. T. (1985) Differential rearing effects on rat visual cortex synapses. I. Synaptic and neuronal density and synapses per neuron. Brain Res. **329:** 195–203
48 Globus A., Rosenzweig M. R., Bennett E. L and Diamond M. C. (1973) Effects of differential experience on dendritic spine counts in rat cerebral cortex. J. Comp. Physiol. Psychol. **82:** 175–181
49 Pysh J. J. and Weiss G. M. (1979) Exercise during development induces an increase in Purkinje cell dendritic tree size. Science **206:** 230–232
50 Beaulieu C. and Collonnier M. (1987) Effects of the richness of the environment on the cat visual cortex. J. Comp. Neurol. **266:** 478–494
51 Bourgeois J. P. and Rakic P. (1993) Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. J. Neurosci. **13:** 2801–2820
52 Daw N. W., Fox K., Sato H. and Czepita D. (1992) Critical period for monocular deprivation in the cat visual cortex. J. Neurophysiol. **67:** 197–202
53 Uylings H. B. M., Kuypers K., Diamond M. C. and Veltman W. A. M. (1978) Effects of differential environments on plasticity of dendrites of cortical pyramidal neurons in adult rats. Exp. Neurol. **62:** 658–677
54 Green E. J., Greenough W. T. and Schlumpf B. E. (1983) Effects of complex or isolated environments on cortical dendrites of middle-aged rats. Brain Res. **264:** 233–240
55 Greenough W. T., Juraska J. M. and Volkmar F. R. (1979) Maze training effects on dendritic branching in occipital cortex of adult rats. Behav. Neural Biol. **26:** 287–297
56 Moser M.-B., Trommald M. and Andersen P. (1994) An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc. Natl. Acad. Sci. USA **91:** 12673–12675
57 Moser M.-B., Trommald M., Egeland T. and Andersen P. (1997) Spatial training in a complex environment and isolation alter the spine distribution differently in rat CA1 pyramidal cells. J. Comp. Neurol. **380:** 373–381
58 Andersen P., Blackstad T. W. and Lømo T. (1966) Location and identification of excitatory synapses on hippocampal pyramidal cells. Exp. Brain Res. **1:** 236–248
59 Chang F. F. and Greenough W. T. (1982) Lateralized effects of monocular training on dendritic branching in adult split brain rats. Brain Res. **232:** 283–292
60 Black J. E., Isaacs K. R., Anderson B. J., Alcantra A. A. and Greenough W. T. (1990) Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. Proc. Natl. Acad. Sci. USA **87:** 5568–5572
61 Greenough W. T., Larson J. R. and Withers G. S. (1985) Effects of unilateral and bilateral training in a reaching task on dendritic branching of neurons in the rat motor sensory forelimb cortex. Behav. Neural Biol. **44:** 301–314
62 Tsien J. Z., Chen D. F., Gerber D., Tom C., Mercer E. H., Anderson D. J. et al. (1996) Subregion- and cell type-restricted gene knockout in mouse brain. Cell **87:** 1317–1326
63 Papa M. and Segal M. (1996) Morphological plasticity in dendritic spines of cultured hippocampal neurons. Neurosci. **71:** 1005–1011
64 Weiland N. G., Orikasa C., Hayashi S. and McEwen B. S. (1997) Distribution and hormone regulation of estrogen receptor immunoreactive cells in the hippocampus of male and female rats. J. Comp. Neurol. **388:** 603–612
65 Murphy D. D., Cole N. B., Greenberger V. and Segal M. (1998) Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. J. Neurosci. **18:** 2550–2559
66 Whishaw I. Q., Zabrowski J. and Kolb B. (1984) Postsurgical enrichment aids adult hemidecorticate rats on a spatial navigation task. Behav. Neural Biol. **42:** 183–190
67 Whishaw I. Q., Sutherland R. J. and Kolb B. (1986) Effects of neonatal forebrain noradrenaline depletion on recovery from brain damage: performance on a spatial navigation task as a function of age of surgery and postsurgical housing. Behav. Neural Biol. **46:** 285–307
68 Mohammed A. K., Winblad B., Ebendal T. and Lärkfors L. (1990) Environmental influence on behaviour and nerve growth factor in the brain. Brain Res. **528:** 62–72