Neuropharmacology 74 (2013) 32–40

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Invited review

NMDA receptors and memory encoding

Richard G.M. Morris*

Centre for Cognitive and Neural Systems, Edinburgh Neuroscience, The University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, UK

ARTICLE INFO

Article history:
Received 3 December 2012
Received in revised form
4 April 2013
Accepted 9 April 2013

Keywords:
Learning
Encoding
Storage consolidation
Retrieval
Watermaze
Hippocampus
Amygdala
Neocortex
Glutamate receptor
GRIN

ABSTRACT

It is humbling to think that 30 years have passed since the paper by Collingridge, Kehl and McLennan showing that one of Jeff Watkins most interesting compounds, R-2-amino-5-phosphonopentanoate (D-AP5), blocked the induction of long-term potentiation *in vitro* at synapses from area CA3 of the hippocampus to CA1 without apparent effect on baseline synaptic transmission (Collingridge et al., 1983). This dissociation was one of the key triggers for an explosion of interest in glutamate receptors, and much has been discovered since that collectively contributes to our contemporary understanding of glutamatergic synapses – their biophysics and subunit composition, of the agonists and antagonists acting on them, and their diverse functions in different networks of the brain and spinal cord. It can be fairly said that Collingridge et al.'s (1983) observation was the stimulus that has led, on the one hand, to structural biological work at the atomic scale describing the key features of NMDA receptors that enables their coincidence function to happen; and, on the other, to work with whole animals investigating the contributions that calcium signalling via this receptor can have on rhythmical activities controlled by spinal circuits, memory encoding in the hippocampus (the topic of this article), visual cortical plasticity, sensitization in pain, and other functions. In this article, I lay out how my then interest in long-term potentiation (LTP) as a model of memory enabled me to recognise the importance of Collingridge et al.'s discovery – and how I and my colleagues endeavoured to take things forward in the area of learning and memory. This is in some respects a personal story, and I tell it as such. The idea that NMDA receptor activation is essential for memory encoding, though not for storage, took time to develop and to be accepted. Along the way, there have been confusions, challenges, and surprises surrounding the idea that activation of NMDA receptors can trigger memory. Some of these are described and how they have been addressed and resolved. Last, I touch on some new directions of interest with respect to the functional role of the NMDA receptor in cognition.

This article is part of the Special Issue entitled 'Glutamate Receptor-Dependent Synaptic Plasticity'.

1. The memory encoding idea

1.1. Pharmacological studies

I first became interested in the possibility that activity-dependent synaptic potentiation, such as long-term potentiation (LTP), might be involved in learning and memory at the "Schloss Hippocampus" conference of 1982 held in a Bavarian Castle owned by the Max-Planck Society (Siefert, 1983). Bruce McNaughton and Carol Barnes, who were present, had conducted pioneering studies on this issue by investigating changes in LTP (or what they called 'enhancement') as a function of ageing, while simultaneously investigating if there was any correlation between these LTP changes and age-related changes also observed in an ingenious spatial learning task that Carol Barnes had developed. They reported that LTP was present in old animals but that it decayed much faster over time; and, tantalisingly, they observed faster forgetting in older animals (Barnes, 1983). Lynch had meanwhile conducted important experiments using the then new technique of *in vitro* brain slices to reveal various anatomical and physiological properties of LTP (Lynch et al., 1983, 1977), but had not taken his observations about the associativity of LTP from brain slices to the behavioural level using learning tasks. I decided to go and work with Gary Lynch at U.C. Irvine to do just that and, whilst there in 1984, we begin some behavioural studies investigating whether a calpain inhibitor called leupeptin would block learning. The focus on calpain was linked to Lynch and Baudry's emerging and prescient theory about memory being due to the "insertion of glutamate receptors into the membrane of dendritic spines" (sic) (Lynch and Baudry, 1984), with calpain playing a critical role in alterations

* Tel.: +44 1316503518.
E-mail address: r.g.m.morris@ed.ac.uk.

0028-3908/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2013.04.014

of spine architecture. Thirty years on, similar ideas pertain; *plus ça change, plus c'est la même chose*! We observed some encouraging trends with leupeptin, but little more (Morris et al., 1987).

Whilst in Irvine, my attention was kindly drawn to Collingridge et al.'s (1983) work by Eric Harris, then in Carl Cotman's laboratory, who wondered whether AP5 might have effects on behavioural learning that leupeptin appeared barely to show. I returned to St Andrews (where I held a Lectureship/Assistant Professorship) and began experiments with my research assistant Elizabeth Anderson in late 1984. What emerged was a series of studies investigating the impact of D,L-AP5 (soon after we used D-AP5) on spatial learning in the watermaze (Morris et al., 1982). Collingridge et al.'s (1983) observations suggested that AP5 blocked plasticity while other compounds affected fast synaptic transmission. This intrigued us as, until then, the only kind of dysfunction of the hippocampus in widespread use was a frank lesion – and here was the possibility of realising a much more subtle and functionally significant disruption of its processing.

Our behavioural studies were to reveal that, at a dose at which DL-AP5 blocked the induction of LTP *in vivo* in the dentate gyrus (possibly the first demonstration of its blockade of LTP outside CA1, and the first *in vivo*), it blocked acquisition of the standard hidden platform version of the watermaze without effect on a visual discrimination task also conducted in the same apparatus (Morris et al., 1986). Treated rats swam normally, but they navigated all over the pool in an apparently random manner even after numerous training trials; control rats learned to take relatively direct paths to the hidden platform and to focus their search in a post-training probe test (platform absent) at the target location. However, when trained to discriminate two visibly distinct platforms only one of which enabled escape from the water – a procedural task that is unaffected by hippocampal lesions – AP5 treated rats learned normally.

Mindful that AP5 blocked the induction of LTP but not its expression, our work continued by investigating the impact of not giving AP5 until *after* acquisition. We then observed no effect of the drug, indicating that the deleterious effect of AP5 was not a "performance effect" but something to do with the acquisition of information at the time of learning (Morris, 1989). The analogy to the effect of AP5 on the induction but not the expression of LTP was intriguing, and raised the possibility that NMDA receptors were a 'trigger' for memory, but not involved in either storage or retrieval. This was important because, to observe that a drug blocks or otherwise affects 'learning' leaves open a range of distinct memory processes with which it may interact. It is natural to think of such an effect as on the learning process *per se*. Equally, however, learning may be happening but the information stored may be inaccessible under the influence of the drug because it affects retrieval processing. Another possibility is that information to which the animal is exposed during a learning task is encoded, but for some reason fails to get stored. That AP5 worked when given before learning but not after pointed the finger very directly at a memory encoding process. It was a long time before the contemporary concept of calcium inflow via NMDA receptors activating signal-transduction pathways that alter AMPA receptor trafficking and expression, and thus storage, was to get worked out.

I then moved to Edinburgh to join the Department of Pharmacology. Given this, and encouraged by my new colleagues, the next step was to see whether the dose–response profile for blocking LTP *in vivo* was similar to its profile for impairing learning. To do this thoroughly, and advised by the biochemical pharmacologists Stephen Butcher and Henry Olverman, we set up an HPLC assay to measure AP5 concentration *in vivo* using microdialysis. Subject to a few assumptions, we were able to compare our varied levels of impairment of the behavioural task with the concentrations of drug measured *in vivo* rather than, as is more common in behavioural pharmacology, the dose administered. Moreover, our estimate of the effective concentration could then be compared with concentrations used during *in vitro* brain slice experiments. Somewhat to our amazement, everything fitted (Butcher et al., 1991; Davis et al., 1992). In this work, the primary technique that was used to deliver DL-AP5 and D-AP5 was via osmotic minipumps with a catheter from the subcutaneously located pump to an intracranial cannula implanted into the lateral ventricle of the brain. These pumps have no moving parts, but deliver a steady concentration of drug 24 h/day for varying durations. Their operational principle is to take advantage of osmosis of body-fluids into the pump that literally "squeeze-out" the contents of a pre-filled reservoir within. Extremely high concentrations of drug in the minipump (e.g. 30 mM) associated with a very low flow rate (e.g. typically 0.5 μL/h) result in a steady-state concentration in the extracellular fluid of around 15 μM. Further, by using pumps that operated for 14 days, we were able to design multi-day behavioural training studies that could be completed within that window of time and allow for *in vivo* assessment of LTP in the same animals before the pump was exhausted. This 'steady-state' approach is advantageous over acute infusions in which concentration varies across time. The drawback is that an intraventricular site of infusion results in a global distribution of the drug throughout the forebrain with some spread down to the spinal cord. Consequently, AP5 treated rats are a bit 'flaccid' and display a slower righting-reflex, this being an example of the supra-segmental reflexes that Dick Evans in Bristol had studied electrophysiologically in the early days.

As the behavioural dissociation we had initially seen with DL-AP5 was between a task that is thought to require the integrity of the hippocampus (spatial learning) and one that does not (visual discrimination), it was incumbent upon us to examine the impact of specific intrahippocampal or intracortical sites of administration of D-AP5 upon learning to address further the issue of whether NMDA receptors are involved in some but not all forms of learning. We therefore turned to acute infusions – which have the advantage of targeting a specific brain structure (at the cost of the imprecision of concentration over time before excretion or metabolism). The first part of this study was straightforward and we were able to show that intrahippocampal D-AP5, shown autoradiographically to be localised to the dorsal hippocampus, also impaired memory encoding without effect upon storage or retrieval (Morris et al., 1989). The second aspect was intellectually less satisfactory, for while we showed that sufficient AP5 reached the neocortex in our visual discrimination studies for the task dissociation to be meaningful (Butcher et al., 1991), we never conducted an acute infusion study as it was far from obvious where in the cortex the drug should be infused (and this remains so to this day). We had at least established a partial dissociation between distinct behavioural tasks (spatial learning, visual discrimination) that mapped onto the likely contribution of different brain regions to spatial learning. More recently, we have also used osmotic pumps for chronic delivery of AMPA and NMDA antagonists directly into the hippocampus; not only does this seem to work quite well, it substantially limits the inevitable side-effects of intraventricular infusion (Inglis et al., 2013; Riedel et al., 1999).

An important and parallel body of work led by Mark Bear and Wolf Singer concerned the use of D-AP5 to examine cortical plasticity in developing animals. Their initial work focused on neuromodulatory inputs such as acetylcholine and noradrenaline (Bear and Singer, 1986). However, guided by a neurobiological theory derived from an earlier strictly mathematical formulation (Bear et al., 1987), attention turned to the possibility that the effects of monocular deprivation might be mediated, at least in part, by glutamatergic plasticity. An early study showed that D-AP5 blocked

changes in ocular dominance induced by monocular deprivation (Gu et al., 1989). Similar care to that of my own group was exercised in measuring the concentration of AP5 required to realise this blockade (Bear et al., 1990). Both I and Mark Bear had to overcome skepticism that the effects of D-AP5 infusion on learning and developmental plasticity respectively were a selective consequence of NMDA receptor blockade, and not a non-specific consequence of inactivating cortical circuits. Bear went on to identify a possible role for NMDA receptor-dependent long-term depression in developmental plasticity (Dudek and Bear, 1992, 1993) thereby providing key evidence for bidirectional changes in synaptic efficacy in relation to cortical fine-tuning. This body of work and framework has been reviewed several times (e.g. Bear and Malenka, 1994; Smith et al., 2009).

An important shift in our own focus occurred much later in work establishing that 1-trial spatial learning in the watermaze, using the so-called delayed matching-to-place (DMP) task, was especially sensitive to AP5 – more so than spatial reference memory in which deficits were observed but the treated animals could eventually learn. In DMP, the hidden platform moves location between days, but stays in the same location for several (usually four) trials of each day. Rats rapidly adapt to this scheduling protocol by searching for the platform on trial 1 of each day, and then remembering its location to perform much better on trials 2 onwards. Steele and Morris (1999) observed that AP5 caused a delay-dependent deficit in this task – little impairment at a short delay between the end of trial 1 and the next trial, but a very large impairment at a long delay. Like spatial working-memory tasks, later to be used extensively by Rawlins' group in Oxford (see below), this protocol enables multiple within-subject observations and is, in our hands, the most robust deficit that i.c.v. and intrahippocampal AP5 produces. A similar 1-trial deficit is seen in an analogous task in the new event arena (Day et al., 2003). This work suggests that NMDA receptor-dependent plasticity in hippocampus is critical for episodic-like memory.

Part of the work that followed our initial studies in the 1980s involved examining other behavioural tasks that were differentially sensitive to hippocampal lesions, and of course we were not alone in doing this. For example, in a collaboration with Nick Rawlins in Oxford, we saw a decline in memory with AP5 in curious but interesting operant task called differential responding and low-rates (or 'drl') provided there was a sufficient temporal delay (Tonkiss et al., 1988), a finding that linked into theoretical ideas that he had been developing about a possible function of the hippocampus in mediating temporary memory (Rawlins, 1985). This finding anticipated our later work with the DMP task. Throughout the 1990s, a much larger body of work was done by other laboratories who confirmed that AP5 blocked acquisition in a range of behavioural tasks (Danysz et al., 1995; Riedel et al., 2003), some of which are now used as routinely as the watermaze. These include the radial maze (Caramanos and Shapiro, 1994; Danysz et al., 1988) and context fear conditioning (Fanselow et al., 1994). We did not ourselves work on amygdala-mediated learning, such as fear conditioning, as my lab is not licensed to use aversive stimuli such as electric shock, but the work of others suggested that NMDA receptors contribute to various but not all forms of learning. Pleasingly, the dissociation between acquisition (encoding) and performance (storage and retrieval) was confirmed in several tasks, including rapidly acquired olfactory discriminations (Staubli et al., 1989). Thus, the behavioural profile was realised very early on to be similar to the differential effects of AP5 on the induction and expression of LTP.

One issue that did not occur to us in the late 1980s was the possibility that an activity- and NMDA receptor-dependent *decrease* in synaptic efficacy – long-term depression (LTD) – could be involved in memory. Heterosynaptic depression had been discovered and was considered little more than a 'normalising' influence on total synaptic strength in a neuron, but the discovery of effective protocols for the induction of homosynaptic LTD (Dudek and Bear, 1992) changed the picture. Analyses of LTD suggested it may be much more prominent in juvenile than in adult animals (Dudek and Bear, 1993), raising the possibility that LTD was more to do with fine-tuning of neural connectivity during development than the storage of information, as noted in the work of Mark Bear discussed above. Later work by Bashir and Brown in Bristol was to establish a possible connection between LTD and the stimulus-specific decrease in neural activity seen in recognition memory.

1.2. Theoretical work

In parallel with this strictly experimental work, several groups were trying to think out the relationship between synapse-specific activation of NMDA receptors and distributed-associative models of memory formation. It is worth remembering that what captured attention from the outset was the coincidence detecting function of NMDA receptors, arising from the magnesium block in the resting state and the dual ligand and voltage-dependent characteristics of the receptor (Ascher and Nowak, 1987; Mayer et al., 1984; Nowak et al., 1984). This biophysics enables an association between pre- and post-synaptic activity to be detected.

Distributed-associative models of memory require this coincidence detecting mechanism to function. They are very different to the 'reflex' models of habituation, sensitization and alpha-conditioning in which synaptic changes associated with learning have been studied in *Aplysia* (Kandel, 1978). The key difference is that instead of plasticity augmenting or decreasing the 'throughput' in a reflex circuit that is subject to experience-dependent modification (Hawkins and Kandel, 1984), associative synaptic potentiation in the hippocampus offers the opportunity of combining two items of information (say A and B) such that A becomes associated with B and can then serve as a retrieval cue for B (Morris, 1990). B does not have to be a biologically significant stimulus (such as food) and the change to A is not of its 'reward value' nor the ability to elicit a learned reflex, but of its capacity to predict what stimulus may follow. These distinct forms of associative learning are well understood in the somewhat esoteric world of animal learning theory, but less well recognised in neuroscience where 'associative learning' is not yet fully appreciated as a family of qualitatively distinct learning processes.

In a theoretical paper that explicitly built on the foundations laid by the mathematician David Marr (Marr, 1971), McNaughton and Morris (1987) outlined how the hippocampal formation, supported by NMDA receptor-dependent plasticity, could form A-B type associations (McNaughton and Morris, 1987). This paper, perhaps presciently, discusses pattern separation in the dentate gyrus and the learning of event sequences in area CA3, including the role of feedforward inhibition to normalise outputs during memory retrieval. However, our analysis was preliminary, largely descriptive and did not involve a formal computational model. Now famous work by others, notably Hopfield and Tank, formalised the properties and learning-rule requirements of distributed-associative memories (Hopfield, 1982; Hopfield and Tank, 1985). In addition, work by colleagues in Edinburgh examined the impact of having both up- and down-regulation of synaptic strength on memory storage capacity (Willshaw and Dayan, 1990), bringing long-term depression (LTD) into the picture, while others made major contributions regarding 'attractor networks' (Amit, 1989) and the implications of differential forms of representation such as 'sparse coding' (Rolls and Treves, 1990). There was also a serious computational effort to recognise that specific aspects of the

intrinsic circuitry of the hippocampus has implications for distinct forms of synaptic plasticity that might play a role in learning (Treves and Rolls, 1991). A major obstacle in trying to take this work further was that we, and others, had little idea of the nature and coding of the information entering the hippocampus from the entorhinal cortex. It was many years before grid cells were to be discovered, and the relationship between place cell representations and the encoding of events happening within specific contexts remained elusive.

1.3. Molecular-genetic studies

In the early 1990s, a major new experimental development with respect to investigating the role of the NMDA receptor in learning and memory emerged from molecular-genetics and, specifically, via the new wave of gene 'knock-out' studies. These had been first introduced into the field of learning and memory by two pioneering studies of the impact of a global knock-out of alpha-CaMKII (Silva et al., 1992) and of fyn tyrosine kinase (Grant et al., 1992). The possibility of knocking out the NMDA receptor was high on everyone's wish-list, but it was soon shown that a standard homologous recombination knock-out of NR1 displayed abnormal development (e.g. in barrel cortex) and died soon after birth (Li et al., 1994). The first successful NMDA receptor knock-out study related to learning was conducted by Mishina's group (Sakimura et al., 1995), who showed that deletion of NR2A affected learning in the watermaze much as we had shown earlier with D-AP5 (but see Bannerman et al., 2008 for an apparent failure to replicate). However, the big step forward was the importation into neuroscience of Cre-Lox technology by Tonegawa's group in 1996.

A series of ingenious studies using specific lines of mice showed, first, that it was possible to knock-out the NR1 subunit of the NMDA receptor specifically in area CA1 of the hippocampus by cross-breeding a line of mice expressing Cre downstream of the alpha-CAMKII promoter with a separate line in which the NMDA NR1 coding sequence was flanked by LoxP sites (Tsien et al., 1996a). This was the first paper to show convincingly that a complete gene knock-out can occur in a given region of the brain. Numerous lines were developed and one, T29, showed expression apparently restricted to area CA1 of the hippocampus. These mice were then found to be impaired in learning the watermaze (Tsien et al., 1996b) and displayed abnormal place fields in CA1 (McHugh et al., 1996). Using a kainate promoter rather than αCaMKII, this group has gone on to use this same molecular-genetic approach to dissect differential functions of NMDA receptors in distinct parts of hippocampal circuitry. For example, one study provided evidence that NMDA receptors within area CA3 were essential at memory encoding to enable pattern completion at the time of memory retrieval in a watermaze surrounding by specific sets of cues (Nakazawa et al., 2002) and for what is sometimes called 'one-shot' learning (Nakazawa et al., 2003). This approach to using gene-targeting was reviewed by (Nakazawa et al., 2004), and recently extended to include a selective deficit in pattern separation when NR1 is deleted in the dentate gyrus (McHugh et al., 2007).

Another group led by Peter Seeburg and Bert Sakmann, with behavioural studies led by Nick Rawlins also used 'knock-out' technology to investigate the role of glutamate receptors in learning and memory. They observed that whole brain deletion of GluR1 (GluA1) can cause deficits in LTP at CA3–CA1 synapses but, importantly, a behavioural dissociation between impaired spatial working-memory alongside intact reference memory (Reisel et al., 2002; Schmitt et al., 2003). However, the LTP deficit in these mice may have been over-estimated in the original study of (Zamanillo et al., 1999). Subsequent experiments using different LTP induction protocols revealed considerable LTP in these mice (e.g. Hoffman et al., 2002; Romberg et al., 2009). The claim by Zamanillo et al. (1999) of a clear cut dissociation between impaired LTP coupled to successful spatial reference memory is somewhat undermined by these later findings.

Nonetheless that the deficit in spatial working-memory could be rescued by transgenic expression of GluR1 on the knock-out background (Schmitt et al., 2005) was an important observation. This group also observed that GluR1−/− mice are hyperactive, display a subtle lack of motor coordination, and are sometimes more anxious than wild-type controls. Thus, they may be a very different phenotype to that of selective NMDA receptor knock-outs. More recently, they have turned their attention to NMDA receptors using a cell-type and region-specific strategy, and observed that selective deletion of NR1 (GluN1) in dentate gyrus also causes the same behavioural dissociation (Bannerman et al., 2008; Niewoehner et al., 2007). Given the strikingly similar effects of GluR1 and NR1 deletion in sub-regions of the hippocampus, they suggest that there is a specific role for an NMDAR-dependent signalling pathway that leads to the activation of a GluR-A-dependent expression mechanism for rapidly acquired, flexible forms of spatial memory (Sanderson et al., 2008). This claim is compatible with the 'episodic-like' memory processing hypothesis of Steele and Morris (1999) and Nakazawa et al.'s (2003) one-shot learning results, excepting that the MIT/RIKEN group emphasise CA3 while the Heidelburg group focus on CA1 and the dentate. A very recent paper using a new line of mice in which NR1 is deleted in both CA1 and the dentate gyrus again shows the relative sparing of spatial reference memory in the watermaze, but suggests that deficits can be observed if a beacon task is used which maximizes the opportunity for navigational interference, particularly when a path has to be inhibited (Bannerman et al., 2013).

2. Confusions, challenges, and surprises

2.1. Confusions

There has sometimes been some confusion about how NMDA receptors should be categorised functionally – with occasional reference to NMDA receptors as "learning receptors" at conferences and in discussion. Certainly, the combination of pharmacological, computational, and molecular-genetic studies just described attest to the importance of hippocampal NMDA receptors in mediating the particular forms of synaptic plasticity and memory encoding in which this structure is engaged. It also plays a role in different forms of learning in amygdala, olfactory bulb, pyriform cortex, and striatum. In each of these brain areas, it is now clear that NMDA receptor activation acts as a trigger for encoding but does not mediate trace storage, but even this important though limited contribution to memory did not prevent the "learning receptor" label getting used. That this happened is understandable, even if an oversimplification. Within the region of the brain mediating one-shot, associative episodic-like memory (the hippocampus), the coincidence property of NMDA receptors is ideal for detecting associations and triggering the storage of 'traces' that represent the occurrence of events. In amygdala, it helps mediate fear conditioning, while in pyriform cortex, the association of an odour with a reward. Thus, in different brain regions, NMDA receptors can help mediate diverse forms of learning and memory.

However, any categorisation as a "learning receptor" is misleading because a receptor and its subunits can have certain biophysical properties but the function(s) that these help mediate will depend on the neuron in which they are expressed, the circuits in which this neuron operates, and so on. Other papers in this issue discuss this matter in greater detail, but the primary property of an NMDA receptor is its dual voltage-and ligand dependent activation, a result of

ion-channel block by extracellular Mg2+ ions. Because of this, it can be accurately described as a glutamatergic ligand/voltage receptor that opens an ion channel that is nonselective to cations with an equilibrium potential near 0 mV. Normally, calcium enters upon channel opening, whereupon it can trigger calcium-dependent calcium release from mitochondria and can act on post-synaptically located signal-transduction cascades to bring about lasting changes – such as alterations in AMPA receptor trafficking and thus synaptic potentiation and depression.

However, not all post-synaptic sites may have this 'machinery' and in some, calcium can act directly on other ion-channels and receptors directly. For example, Dale and Roberts, working in Bristol in the early 1980s, discovered the contribution of NMDA receptors to the swimming rhythm in Xenopus embryos (Dale and Roberts, 1985). Grillner and his colleagues went on to show how a calcium-dependent potassium current in lamprey spinal cord was essential for its rhythmical swimming pattern (Grillner et al., 1998). There are numerous other examples of diverse functions that NMDA receptors help perform, including in the sensitization of pain (Dickenson and Sullivan, 1987) and the development of drug tolerance and addiction (Trujillo and Akil, 1991) that are linked to its role in synaptic plasticity. While these represent 'learning' of a kind, they are clearly different from the episodic-like memory in which the hippocampus is engaged.

2.2. Challenges

A separate issue has to do with various challenges to the emerging idea of a role for NMDA receptors in memory. One debate emerged early on when it was noticed that the effects of AP5 were arguably smaller than those of frank lesions to the hippocampus, and the reasons for this then discussed and explained (Keith and Rudy, 1990). A more engaging challenge came from the observation, made simultaneously by Peter Cain and my own Edinburgh lab, that the impact of NMDA antagonists in blocking spatial learning depended on the learning history of the animal. Whereas a clear deficit was apparent in experimentally naïve animals, animals that had previously been trained in a watermaze can sometimes learn relatively normally under the drug (Bannerman et al., 1995; Saucier and Cain, 1995). This dissociation did not appear to have anything to do with the dose or intrahippocampal concentration of the NMDA antagonists being used as there were checks across labs that these were sufficient to block LTP. Cain went on to conduct a number of studies of this phenomenon, arguing that NMDA antagonists cause gross sensorimotor disturbances and that these masquerade as learning deficits for the trivial reason that animals which cannot move around properly cannot demonstrate that they can learn (Cain et al., 1996; Saucier et al., 1996). He also drew attention to the work of Olney, primarily his work with the non-competitive antagonist MK-801, who showed that blocking NMDA receptors can cause pathological changes in the cingulate cortex (Olney et al., 1991).

From the outset, Cain's sensorimotor challenge reflected the point already made above – namely that NMDA receptors do different things in different circuits. MK-801 in particular is an extremely difficult drug with which to work in behaving animals as it rapidly induces stereotopy and other abnormal aspects of behaviour. At concentrations sufficient to block LTP *in vivo,* an MK-801 treated animal is barely testable. However, Cain's argument had two strands to it, namely the additional feature that if the behavioural training is conducted in such a manner than the sensorimotor effects of the drug can be reduced, such as by 'pre-training', the animals can learn normally. A problem with his demonstration of this, however, was that the pre-training was generally done in the same apparatus as that used later to reveal the subsequent lack of effect of the NMDA receptor blockade. This is unfortunate for, despite the use of curtains ostensibly to occlude sight of extramaze cues, it is possible that some elements of contextual learning will proceed during the pretraining that preclude the need for such learning later under the drug. It was to guard against precisely this possibility that Bannerman et al. (1995) used two separate laboratories on two separate floors of the building – the so-called 'upstairs-downstairs' study. We argued that pretraining dissociated distinct components of spatial learning – in some ways anticipating the later molecular-genetic work in hinting that this form of learning is a composite made up of a variety of potentially dissociable components.

Notwithstanding, it has taken some time to address and resolve the important issues raised by Cain (Inglis et al., 2013; Morris et al., 2013). One paradoxical aspect is that the sensorimotor disturbances induced by NMDA antagonists during watermaze training may arise in part from the failure to learn rather than being the sole cause of a failure of learning. That is, there is a chicken-and-egg problem. Using two separate camera systems to monitor the animals, and quantitative scoring, Morris et al. (2013) have revisited this matter and shown that at the lower concentrations of chronic i.c.v. infusions of D-AP5 that are sufficient to impair memory encoding, the phenotype of slower swimming, failure to stay on the platform and other 'disturbances' are mild or even non-existent at the beginning of a series of daily trials but then build up across the session. Further, if a spatial visible platform task is used that limits substantially the expression of sensorimotor abnormalities (because there are visible platforms occupying different locations for the animals to head for), D-AP5 still causes a learning impairment. Finally, if the animals are trained extensively for many days on the more 'episodic-like' delayed matching-to-place version of the watermaze (in which the location of the platform is moved between days), the drug causes little sensorimotor impairment (as Cain predicts) but a massive impairment in learning the location of the platform that day (which he does not). In a companion paper using intrahippocampal D-AP5 infusion, Inglis et al. (2013) now show that the pretraining phenomenon is dose-related. It is apparent as soon as a concentration sufficient to block LTP in the hippocampus is reached, and may indeed reflect dissociable components of spatial learning. Our view is, therefore, that the sensorimotor challenge to the memory encoding idea was an important one but ultimately does not undermine it.

2.3. Surprises

Given the hypothesis that the encoding of episodic-like memory traces in the hippocampus depends on NMDA receptor activation, it has come as a considerable surprise that two molecules which block the associated ion-channel should act as either a cognitive enhancer (magnesium) or as a drug that could be efficacious in Alzheimer's Disease (memantine) of which loss of recent episodic-memory is an early symptom. It is well known that elevating magnesium concentration during *in vitro* brain slice experiments can reduce the induction of LTP. Accordingly, Slutsky et al.'s (2010) observation that chronic administration of magnesium-L-threonate (MgT) should enhance LTP and learning feels paradoxical. A likely explanation is in terms of 'neuronal homeostasis' (Turrigiano and Nelson, 2000), with the chronic availability of MgT causing a compensatory increase in the number of NMDA receptors over time. Indeed, they found that elevation of brain Mg2+ led to significant enhancement of NR2B-containing NMDARs, an enhancement of NMDAR signalling and synaptic plasticity, and at the cellular level, an increase in presynaptic boutons as measured by synaptophysin staining. Parallel work in the domain of ocular dominance plasticity indicates that a reduction in the NR2A/B ratio

during monocular deprivation is permissive for the compensatory potentiation of non-deprived inputs (Cho et al., 2009). Behaviourally, they observed a small but significant enhancement of spatial memory using both a delayed T-maze task and a watermaze. The paradox is resolved by the fact that increasing the magnesium block of the NMDA receptor with MgT under baseline conditions triggers a homeostatic upregulation synaptic NMDARs that precisely counterbalances the increased blockade of NMDAR opening associated with chronic increase in Mg2+, thereby restoring a steady-state of baseline NMDA conductance. However, in this state, while the level of background NMDAR currents remains constant, NMDAR currents during the coincidence of pre- and post-synaptic activity as occurs during learning and LTP is enhanced.

The efficacy of memantine is an analogous but different story. At first sight, the now considerable body of work showing that memantine, a non-competitive NMDA receptor antagonist, is an effective therapy in Alzheimer's Disease (Danysz and Parsons, 2012; Parsons et al., 2007) is also hardly a prediction that the NMDA receptor and memory encoding hypothesis might have made. As Alzheimers Disease (AD), at least in its early stages, is characterised by loss of recent memory, the possible use of a non-competitive NMDA antagonist as a therapy is then no less paradoxical than the use of magnesium as a memory enhancer. The mechanism is different – for NMDA receptors can also trigger calcium entry that is excitotoxic and this may contribute insidiously over time to the neurodegeneration that is characteristic of AD. Studies of its mode of action have revealed that the channel blocking action of memantine is sufficient to limit excitotoxic effects of tonic activation of NMDA receptors while, like magnesium, preserving the phasic effects that arise when pre- and post-synaptic activity co-occur. To the extent that excitotoxicity is a contribution to the gradual neurodegeneration observed in AD, possibly mediated by interactions between Aβ and NMDA receptors, an NMDA antagonist may be helpful. In a scholarly review of its relevance to AD, Danysz and Parsons (2012) contrast memantine with a competitive antagonist such as AP5 or CPP, arguing that only the latter blocks both tonic and phasic activations of the receptor, and thus synaptic plasticity and associated memory formation. They also suggest that memantine may be more tightly bound to the channel than Mg2+, though much less than MK-801. These kinetics are fortuitous for its efficacy.

3. Future directions

In preparing this summary of work on NMDA receptors and memory encoding, Google Scholar revealed over 23,000 hits for the conjunction of "NMDA receptor", "memory" and "learning". There clearly has been and still is a great deal of research going on, and all manner of intriguing new issues (and technologies) have emerged in the last ten years that will refine the concept that activation of these receptors is essential for diverse aspects of memory encoding in different circuits of the brain.

The work discussed so far has focused on rodents – rats and mice. What about other species? The generality of the memory encoding concept has now been firmly established through studies of several species ranging from invertebrates (flies), non-mammalian vertebrates (birds), and in diverse mammals through to humans (Riters and Bingman, 1994; Steele and Stewart, 1993; Xia et al., 2005). For example, song-birds not only fail to learn songs when specific nuclei of the song-system are subject to NMDA antagonists, they also express increased mRNA for the NR2B subunit during the season when they are learning their mating song (Singh et al., 2003). Humans fail to remember the widely used Rey-figure as they study and attempt to remember it under the influence of ketamine (Grunwald et al., 1999), and recent work indicates that chromosome translocation breakpoints in individuals with mental retardation and/or epilepsy in humans are associated with GRIN2B or GRIN2A – genes that encode the NMDA receptor subunits NR2B and NR2A (Endele et al., 2010). There is also growing interest in NMDA receptor function in humans through the development of the glutamatergic theory of schizophrenia (Harrison and Weinberger, 2005).

Another issue for the future concerns the possibility of improving learning by enhancing NMDA receptor signalling. The creation of the so-called "Doogie" mouse by Joe Tsien and his colleagues at Princeton raised the intriguing possibility of doing this by over-expressing NR2B receptors (Tang et al., 1999). There is some way to go from smart mice to smart humans, but the possibility of achieving this is brought into focus by the studies discussed above of the impact of Magnesium Threonate as a cognitive enhancer. The trick for enhancing NMDA receptor function appears either to be the use of a drug that increases their expression; or, alternatively, involves finding a method of enhancing NMDA receptor efficacy under the very specific circumstances of learning while simultaneously limiting the opportunity for compensatory homeostatic downscaling.

One of the challenges to the hypothesis of NMDA receptors and memory encoding came from the observation, discussed above, that experienced animals that have gone through a first-learning task can be unaffected by blockade of NMDA receptors during a similar but distinct second learning task. This effect is not always observed, for example with simple fear conditioning mediated by the amygdala (Lee and Kim, 1998), but is by no means restricted to spatial learning in the watermaze as it is also seen in context fear conditioning (Sanders and Fanselow, 2003). There have been some recent and exacting analyses of this important phenomenon that collectively suggest that, in context fear conditioning, the same neurons must be used for both tasks for the lack of dependence on NMDA receptors to be seen (Tayler et al., 2011; Wiltgen et al., 2010, 2011). This is an intriguing finding, but still challenges the NMDA receptor and encoding hypothesis in its simplest form as, from the perspective of distributed-associative computational models, the pre-supposition is that storage at different synaptic terminals on the same neurons would still require NMDA receptor activation at the time of second learning. In a complication to the way these experiments and the associated drug infusions are carried out, (Wang et al., 2012) have raised the complication that first- and second-learning of similar tasks may sometimes use different parts of the hippocampus (dorsal vs. ventral) and thus the success of second-learning with AP5 may reflect limited diffusion of the drug to the ventral site in the hippocampus where second-learning is mediated. Even so, this switch to different parts of the hippocampus is itself a puzzle as it is unclear how the hippocampus could "metacognitively know" (sic) whether a task on which an animal is being trained is a first or second learning task of a particular category.

One difficulty that we face in taking this fascinating set of puzzles forward is that a specific learning task in humans (e.g. picture recognition) may later enable memory retrieval that has qualitatively distinct characteristics depending on the nature of the encoding that happens at initial learning. Specifically, people may respond positively at retrieval to a stimulus because, having seen it before, it has become 'familiar'. Alternatively, they could do so because they really have the mental experience of 'remembering' when and/or where they saw it. Remembering in this sense entails a deeper association between a stimulus or event and the spatial-temporal context of its occurrence. The dissociation between knowing and remembering is itself controversial, and very difficult to analyse in animals, but it relates directly to systems memory consolidation, and with it the dynamic relationship of the hippocampus and neocortex in storing and later retrieving long-term

memories. Continued work on the puzzle of the necessity for NMDA receptor activation during hippocampal-dependent learning and retrieval may help to shed light on these and other dissociations.

There are host of other issues that could be discussed, but I shall end on a potentially practical note. It is well known that anaesthetics used in children, such as ketamine, are NMDA receptor antagonists (Anis et al., 1983). In adult animals, the use of these drugs appears to be reversible, but is this also the case in developing animals and, in particular, human babies subject to perinatal operations? Recent work in animals has called attention to potential long-term dangers of such anaesthetics (Jevtovic-Todorovic et al., 2003). The key observation is that exposure of the developing brain during the period of synaptogenesis to drugs that block NMDA glutamate receptors or drugs that potentiate $GABA_A$ receptors may trigger widespread apoptotic neurodegeneration. These authors administered a combination of drugs commonly used in pediatric anesthesia to 7-day old infant rats in doses sufficient to maintain a surgical plane of anesthesia for 6 h. They observed widespread neurodegeneration in the developing brain, deficits in hippocampal synaptic function, and persistent memory/learning impairments. The question of whether such anaesthetics are really dangerous for humans is, however, a different matter. Thomas et al. (2011) take a skeptical view of the possibility, but in the context of a carefully written editorial that documents a number of exacting studies that have looked at the issue.

To conclude, we have come a long way since Collingridge et al.'s (1983) first observation of the role of the NMDA receptor in activity-dependent synaptic plasticity. We understand better the NR1, NR2 and NR3 subunit composition of NMDA (GRIN) receptors, their contribution to triggering changes in AMPA receptor expression, and their expression pattern in different regions of the brain and spinal cord. Along this path of adventure, the beneficial and the more dangerous aspects of NMDA receptor signalling have been identified and studied. The coincidence detecting function of NMDA receptors appears, in hippocampus, to play a critical role in episodic-like memory formation. Maintaining this system in a fully functional state is important for the more automatic aspects of day-to-day cognition. Efforts to develop new cognitive-enhancing drugs are likely to continue to include a major focus on glutamatergic receptors and their downstream signalling pathways.

Acknowledgements

This review was prepared during a sabbatical at the Institute for Neurosciences in Alicante, and I am grateful to Professor Juan Lerma for allowing me to visit. My research on NMDA receptors and memory was largely supported by the Medical Research Council by a Programme Grant held from 1988 through to 2011. I am immensely grateful to the MRC for their longstanding support.

References

Amit, D.J., 1989. Modeling Brain Function: The World of Attractor Networks. Cambridge University Press, Cambridge.

Anis, N.A., Berry, S.C., Burton, N.R., Lodge, D., 1983. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by *N*-methyl-aspartate. Br. J. Pharmacol. 79, 565–575.

Ascher, P., Nowak, L., 1987. Electrophysiological studies of NMDA receptors. Trends Neurosci. 10, 284–288.

Bannerman, D.M., Bus, T., Taylor, A., Sanderson, D.J., Schwarz, I., Jensen, V., Hvalby, O., Rawlins, J.N., Seeburg, P.H., Sprengel, R., 2013. Dissecting spatial knowledge from spatial choice by hippocampal NMDA receptor deletion. Nat. Neurosci. 15, 1153–1159.

Bannerman, D.M., Good, M.A., Butcher, S.P., Ramsay, M., Morris, R.G., 1995. Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature 378, 182–186.

Bannerman, D.M., Niewoehner, B., Lyon, L., Romberg, C., Schmitt, W.B., Taylor, A., Sanderson, D.J., Cottam, J., Sprengel, R., Seeburg, P.H., Kohr, G., Rawlins, J.N., 2008. NMDA receptor subunit NR2A is required for rapidly acquired spatial working memory but not incremental spatial reference memory. J. Neurosci. 28, 3623–3630.

Barnes, C.A., 1983. The physiology of the senescent hippocampus. In: Siefert, W. (Ed.), Neurobiology of the Hippocampus. Academic Press, London, pp. 87–108.

Bear, M.F., Cooper, L.N., Ebner, F.F., 1987. A physiological basis for a theory of synapse modification. Science 237, 42–48.

Bear, M.F., Kleinschmidt, A., Gu, Q.A., Singer, W., 1990. Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J. Neurosci. 10, 909–925.

Bear, M.F., Malenka, R.C., 1994. Synaptic plasticity: LTP and LTD. Curr. Opin. Neurobiol. 4, 389–399.

Bear, M.F., Singer, W., 1986. Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 320, 172–176.

Butcher, S.P., Hamberger, A., Morris, R.G.M., 1991. Intracerebral distribution of DL-2-amino-phosphonopentanoic acid (AP5) and the dissociation of different types of learning. Exp. Brain Res. 83, 521–526.

Cain, D.P., Saucier, D., Hall, J., Hargreaves, E.L., Boon, F., 1996. Detailed behavioral analysis of water maze acquisition under APV or CNQX: contribution of sensorimotor disturbances to drug-induced acquisition deficits. Behav. Neurosci. 110, 86–102.

Caramanos, Z., Shapiro, M.L., 1994. Spatial memory and *N*-methyl-D-asparate receptor antagonists APV and MK-801: memory impairments depend on familiarity with the environment, drug dose and training duration. Behav. Neurosci. 108, 30–43.

Cho, K.K., Khibnik, L., Philpot, B.D., Bear, M.F., 2009. The ratio of NR2A/B NMDA receptor subunits determines the qualities of ocular dominance plasticity in visual cortex. Proc. Natl. Acad. Sci. U.S.A. 106, 5377–5382.

Collingridge, G.L., Kehl, S.J., McLennan, H., 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol. 334.

Dale, N., Roberts, A., 1985. Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in Xenopus embryos. J. Physiol. 363, 35–59.

Danysz, W., Parsons, C.G., 2012. Alzheimer's disease, beta-amyloid, glutamate, NMDA receptors and memantine – searching for the connections. Br. J. Pharmacol. 167, 324–352.

Danysz, W., Wroblewski, J.T., Costa, E., 1988. Learning impairment in rats by *N*-methyl-D-aspartate receptor antagonists. Neuropharmacology 27, 653–656.

Danysz, W., Zajaczkowski, W., Parsons, C.G., 1995. Modulation of learning processes by ionotropic glutamate receptor ligands. Behav. Pharmacol. 6, 455–474.

Davis, S., Butcher, S.P., Morris, R.G.M., 1992. The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. J. Neurosci. 12, 21–34.

Day, M., Langston, R., Morris, R.G., 2003. Glutamate-receptor-mediated encoding and retrieval of paired-associate learning. Nature 424, 205–209.

Dickenson, A.H., Sullivan, A.F., 1987. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology 26, 1235–1238.

Dudek, S.M., Bear, M.F., 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc. Natl. Acad. Sci. U.S.A. 89, 4363–4367.

Dudek, S.M., Bear, M.F., 1993. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J. Neurosci. 13, 2910–2918.

Endele, S., Rosenberger, G., Geider, K., Popp, B., Tamer, C., Stefanova, I., Milh, M., Kortum, F., Fritsch, A., Pientka, F.K., Hellenbroich, Y., Kalscheuer, V.M., Kohlhase, J., Moog, U., Rappold, G., Rauch, A., Ropers, H.H., von Spiczak, S., Tonnies, H., Villeneuve, N., Villard, L., Zabel, B., Zenker, M., Laube, B., Reis, A., Wieczorek, D., Van Maldergem, L., Kutsche, K., 2010. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat. Genet. 42, 1021–1026.

Fanselow, M.S., Kim, J.J., Yipp, J., De_Oca, B., 1994. Differential effects of the *N*-methyl-D-aspartate antagonist DL-2-amino-5-phosphonovalerate on acquisition of fear of auditory and contextual cues. Behav. Neurosci. 108, 235–240.

Grant, S.G.N., O'Dell, T.J., Karl, K.A., Stein, P.L., Sorian, O.P., Kandel, E.R., 1992. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. Science 258, 1903–1910.

Grillner, S., Parker, D., El Manira, A., 1998. Vertebrate locomotion-a lamprey perspective. Ann. N. Y. Acad. Sci. 860, 1–18.

Grunwald, T., Beck, H., Lehnertz, K., Blumcke, I., Pezer, N., Kurthen, M., Fernandez, G., Van Roost, D., Heinze, H.J., Kutas, M., Elger, C.E., 1999. Evidence relating human verbal memory to hippocampal *N*-methyl-D-aspartate receptors. Proc. Natl. Acad. Sci. U.S.A. 96, 12085–12089.

Gu, Q.A., Bear, M.F., Singer, W., 1989. Blockade of NMDA-receptors prevents ocularity changes in kitten visual cortex after reversed monocular deprivation. Brain Res. Dev. Brain Res. 47, 281–288.

Harrison, P.J., Weinberger, D.R., 2005. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol. Psychiatry 10, 40–68. image 45.

Hawkins, R.D., Kandel, E.R., 1984. Is there a cell-biological alphabet for simple forms of learning? Psychol. Rev. 91, 375–391.

Hoffman, D.A., Sprengel, R., Sakmann, B., 2002. Molecular dissection of hippocampal theta-burst pairing potentiation. Proc. Natl. Acad. Sci. U.S.A. 99, 7740–7745.

Hopfield, J.J., 1982. Neural networks and physical systems with emergent collective computational abilities. Proc. Natl. Acad. Sci. U.S.A. 79, 3088–3092.
Hopfield, J.J., Tank, D.W., 1985. Neural computation of decisions in optimization problems. Biol. Cybernet. 52, 141–152.
Inglis, J., Martin, S.J., Morris, R.G.M., 2013. Upstairs–downstairs revisited: spatial pretraining-induced rescue of normal spatial learning during selective blockade of hippocampal *N*-methyl-D-aspartate receptors. Eur. J. Neurosci..
Jevtovic-Todorovic, V., Hartman, R.E., Izumi, Y., Benshoff, N.D., Dikranian, K., Zorumski, C.F., Olney, J.W., Wozniak, D.F., 2003. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J. Neurosci. 23, 876–882.
Kandel, E.R., 1978. A Cell-biological Approach to Learning. Society for Neuroscience, Bethesda, MD.
Keith, J.R., Rudy, J.W., 1990. Why NMDA receptor-dependent long-term potentiation may not be a mechanism of learning and memory: reappraisal of the NMDA receptor blockade strategy. Psychobiology 18, 251–257.
Lee, H., Kim, J.J., 1998. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J. Neurosci. 18, 8444–8454.
Li, Y., Erzurumlu, R.S., Chen, C., Jhaveri, S., Tonegawa, S., 1994. Whisker-related neuronal patterns fail to develop in the trigeminal brainstem nuclei of NMDAR1 knockout mice. Cell 76, 427–437.
Lynch, G., Baudry, M., 1984. The biochemistry of memory: a new and specific hypothesis. Science 224, 1057–1063.
Lynch, G., Halpain, S., Baudry, M., 1983. Structural and biochemical effects of high-frequency stimulation in the hippocampus. In: Siefert, W. (Ed.), Neurobiology of the Hippocampus. Academic Press, London, pp. 253–264.
Lynch, G.S., Dunwiddie, T., Gribkoff, V., 1977. Heterosynaptic depression: a post-synaptic correlate of long-term potentiation. Nature 266, 737–739.
Marr, D., 1971. Simple memory: a theory for archicortex. Phil. Trans. Roy. Soc. Lond. B 262, 23–81.
Mayer, M.L., Westbrook, G.L., Guthrie, P.B., 1984. Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature 309, 261–263.
McHugh, T.J., Blum, K.I., Tsien, J.Z., Tonegawa, S., Wilson, M.A., 1996. Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. Cell 87, 1339–1349.
McHugh, T.J., Jones, M.W., Quinn, J.J., Balthasar, N., Coppari, R., Elmquist, J.K., Lowell, B.B., Fanselow, M.S., Wilson, M.A., Tonegawa, S., 2007. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science 317, 94–99.
McNaughton, B.L., Morris, R.G.M., 1987. Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends Neurosci. 10, 408–415.
Morris, R.G., Garrud, P., Rawlins, J.N., O'Keefe, J., 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297, 681–683.
Morris, R.G., Halliwell, R.F., Bowery, N., 1989. Synaptic plasticity and learning. II: do different kinds of plasticity underlie different kinds of learning? Neuropsychologia 27, 41–59.
Morris, R.G.M., 1989. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the *N*-methyl-D-aspartate receptor antagonist AP5. J. Neurosci. 9, 3040–3057.
Morris, R.G.M., 1990. Synaptic plasticity, neural architecture, and forms of memory. In: McGaugh, J.L., Weinberger, N.M., Lynch, G. (Eds.), Brain Organization and Memory: Cells, Systems and Circuits. Oxford University Press, New York, pp. 52–77.
Morris, R.G.M., Anderson, E., Lynch, G.S., 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.
Morris, R.G.M., Hagan, J.J., Nadel, L., Jensen, J., Baudry, M., Lynch, G.S., 1987. Spatial learning in the rat: impairment induced by the thiol-proteinase inhibitor, leupeptin, and an analysis of [3H]glutamate receptor binding in relation to learning. Behav. Neural Biol. 47, 333–345.
Morris, R.G.M., Steele, R.J., Bell, J.E., Martin, S.J., 2013. *N*-methyl-D-aspartate receptors, learning and memory: chronic intraventricular infusion of the NMDA receptor antagonist D-AP5 interacts directly with the neural mechanisms of spatial learning. Eur. J. Neurosci. 37, 700–717.
Nakazawa, K., McHugh, T.J., Wilson, M.A., Tonegawa, S., 2004. NMDA receptors, place cells and hippocampal spatial memory. Nat. Rev. Neurosci. 5, 361–372.
Nakazawa, K., Quirk, M.C., Chitwood, R.A., Watanabe, M., Yeckel, M.F., Sun, L.D., Kato, A., Carr, C.A., Johnston, D., Wilson, M.A., Tonegawa, S., 2002. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. Science 297, 211–218.
Nakazawa, K., Sun, L.D., Quirk, M.C., Rondi-Reig, L., Wilson, M.A., Tonegawa, S., 2003. Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. Neuron 38, 305–315.
Niewoehner, B., Single, F.N., Hvalby, O., Jensen, V., Meyer zum Alten Borgloh, S., Seeburg, P.H., Rawlins, J.N., Sprengel, R., Bannerman, D.M., 2007. Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. Eur. J. Neurosci. 25, 837–846.
Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307, 462–465.
Olney, J.W., Labruyere, J., Wang, G., Wozniak, D.F., Price, M.T., Sesma, M.A., 1991. NMDA antagonist neurotoxicity: mechanism and prevention. Science 254, 1515–1518.
Parsons, C.G., Stoffler, A., Danysz, W., 2007. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system–too little activation is bad, too much is even worse. Neuropharmacology 53, 699–723.
Rawlins, J.N.P., 1985. Associations across time: the hippocampus as a temporary memory store. Behav. Brain Sci. 8, 479–497.
Reisel, D., Bannerman, D.M., Schmitt, W.B., Deacon, R.M., Flint, J., Borchardt, T., Seeburg, P.H., Rawlins, J.N., 2002. Spatial memory dissociations in mice lacking GluR1. Nat. Neurosci. 5, 868–873.
Riedel, G., Micheau, J., Lam, A.G., Roloff, E., Martin, S.J., Bridge, H., Hoz, L., Poeschel, B., McCulloch, J., Morris, R.G.M., 1999. Reversible neural inactivation reveals hippocampal participation in several memory processes. Nat. Neurosci. 2, 898–905.
Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. Behav. Brain Res. 140, 1–47.
Riters, L.V., Bingman, V.P., 1994. The NMDA-receptor antagonist MK-801 impairs navigational learning in homing pigeons. Behav. Neural Biol. 62, 50–59.
Rolls, E.T., Treves, A., 1990. The relative advantage of sparse versus distributed encoding for associative neuronal networks in the brain. Network 1, 407–421.
Romberg, C., Raffel, J., Martin, L., Sprengel, R., Seeburg, P.H., Rawlins, J.N., Bannerman, D.M., Paulsen, O., 2009. Induction and expression of GluA1 (GluR-A)-independent LTP in the hippocampus. Eur. J. Neurosci. 29, 1141–1152.
Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., Sugiyama, H., Mishina, M., 1995. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. Nature 373, 151–155.
Sanders, M.J., Fanselow, M.S., 2003. Pre-training prevents context fear conditioning deficits produced by hippocampal NMDA receptor blockade. Neurobiol. Learn. Mem. 80, 123–129.
Sanderson, D.J., Good, M.A., Seeburg, P.H., Sprengel, R., Rawlins, J.N., Bannerman, D.M., 2008. The role of the GluR-A (GluR1) AMPA receptor subunit in learning and memory. Prog. Brain Res. 169, 159–178.
Saucier, D., Cain, D.P., 1995. Spatial learning without NMDA receptor-dependent long-term potentiation. Nature 378, 186–189.
Saucier, D., Hargreaves, E.L., Boon, F., Vanderwolf, C.H., Cain, D.P., 1996. Detailed behavioral analysis of water maze acquisition under systemic NMDA or muscarinic antagonism: nonspatial pretraining eliminates spatial learning deficits. Behav. Neurosci. 110, 103–116.
Schmitt, W.B., Deacon, R.M., Seeburg, P.H., Rawlins, J.N., Bannerman, D.M., 2003. A within-subjects, within-task demonstration of intact spatial reference memory and impaired spatial working memory in glutamate receptor-A-deficient mice. J. Neurosci. 23, 3953–3959.
Schmitt, W.B., Sprengel, R., Mack, V., Draft, R.W., Seeburg, P.H., Deacon, R.M., Rawlins, J.N., Bannerman, D.M., 2005. Restoration of spatial working memory by genetic rescue of GluR-A-deficient mice. Nat. Neurosci. 8, 270–272.
Siefert, 1983. Neurobiology of the Hippocampus. Academic Press, London.
Silva, A.J., Paylor, R., Wehner, J.M., Tonegawa, S., 1992. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257, 206–211.
Singh, T.D., Heinrich, J.E., Wissman, A.M., Brenowitz, E.A., Nordeen, E.J., Nordeen, K.W., 2003. Seasonal regulation of NMDA receptor NR2B mRNA in the adult canary song system. J. Neurobiol. 54, 593–603.
Slutsky, I., Abumaria, N., Wu, L.J., Huang, C., Zhang, L., Li, B., Zhao, X., Govindarajan, A., Zhao, M.G., Zhuo, M., Tonegawa, S., Liu, G. Enhancement of learning and memory by elevating brain magnesium. Neuron 65, 165–177.
Smith, G.B., Heynen, A.J., Bear, M.F., 2009. Bidirectional synaptic mechanisms of ocular dominance plasticity in visual cortex. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 357–367.
Staubli, U., Thibault, O., DiLorenzo, M., Lynch, G., 1989. Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. Behav. Neurosci. 103, 54–60.
Steele, R.J., Morris, R.G.M., 1999. Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. Hippocampus 9, 118–136.
Steele, R.J., Stewart, M.G., 1993. 7-Chlorokynurenate, an antagonist of the glycine binding site on the NMDA receptor, inhibits memory formation in day-old chicks (Gallus domesticus). Behav. Neural Biol. 60, 89–92.
Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., Tsien, J.Z., 1999. Genetic enhancement of learning and memory in mice. Nature 401, 63–69.
Tayler, K.K., Lowry, E., Tanaka, K., Levy, B., Reijmers, L., Mayford, M., Wiltgen, B.J., 2011. Characterization of NMDAR-Independent Learning in the Hippocampus. Front. Behav. Neurosci. 5, 28.
Thomas, J., Crosby, G., Drummond, J.C., Todd, M., 2011. Anesthetic neurotoxicity: a difficult dragon to slay. Anesth. Analg. 113, 969–971.
Tonkiss, J., Morris, R.G.M., Rawlins, J.N.P., 1988. Intra-ventricular infusion of the NMDA antagonist AP5 impairs performance on a non-spatial operant DRL task in the rat. Exp. Brain Res. Experimentelle Hirnforschung. Experimentation Cerebrale 73, 181–188.
Treves, A., Rolls, E.T., 1991. Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. Hippocampus 2, 189–199.
Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251, 85–87.
Tsien, J.Z., Chen, D.F., Gerber, D., Tom, C., Mercer, E.H., Anderson, D.J., Mayford, M., Kandel, E.R., Tonegawa, S., 1996a. Subregion- and cell type-restricted gene knockout in mouse brain (see comments). Cell 87, 1317–1326.

Tsien, J.Z., Huerta, P.T., Tonegawa, S., 1996b. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell 87, 1327–1338.
Turrigiano, G.G., Nelson, S.B., 2000. Hebb and homeostasis in neuronal plasticity. Curr. Opin. Neurobiol. 10, 358–364.
Wang, S.H., Finnie, P.S., Hardt, O., Nader, K., 2012. Dorsal hippocampus is necessary for novel learning but sufficient for subsequent similar learning. Hippocampus 22, 2157–2170.
Willshaw, D., Dayan, P., 1990. Optimal plasticity from matrix memories: what goes up must come down. Neural Commun., 85–93.
Wiltgen, B.J., Wood, A.N., Levy, B., 2011. The cellular mechanisms of memory are modified by experience. Learn. Mem. 18, 747–750.
Wiltgen, B.J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M.G., Parivash, S.N., Li, W., Silva, A.J., 2010. The hippocampus plays a selective role in the retrieval of detailed contextual memories. Curr. Biol. 20, 1336–1344.
Xia, S., Miyashita, T., Fu, T.F., Lin, W.Y., Wu, C.L., Pyzocha, L., Lin, I.R., Saitoe, M., Tully, T., Chiang, A.S., 2005. NMDA receptors mediate olfactory learning and memory in Drosophila. Curr. Biol. 15, 603–615.
Zamanillo, D., Sprengel, R., Hvalby, O., Jensen, V., Burnashev, N., Rozov, A., Kaiser, K.M., Koster, H.J., Borchardt, T., Worley, P., Lubke, J., Frotscher, M., Kelly, P.H., Sommer, B., Andersen, P., Seeburg, P.H., Sakmann, B., 1999. Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. Science 284, 1805–1811.