PREFACE

E. CHERUBINI and G. NISTICO'

As every year since the departure of Rita Levi-Montalcini (30 December 2012), EBRI Foundation has organized in her memory a magistral lecture on her birthday anniversary (22 April) with a one-day Workshop on a special topic.

This year the EBRI Rita Levi-Montalcini Lecture was delivered by professor Susumu Tonegawa (Ricken-MIT, USA) on: "Engrams for genuine and false memories in memory of Rita Levi-Montalcini". This was followed by an International Workshop entitled "Engrams and memory traces".

"Engrams" are memory traces stored in our brain in response to experience. Episodic memories, essential for day-to-day living, are made of associations of several elements, including objects, space and time. These associations are encoded by enduring structural changes at the level of synapses and specialized neuronal circuits in selective brain areas such as the hippocampus and the cortex.

Tonegawa and colleagues, have elegantly demonstrated that specific neuronal ensembles involved in the representation of memory engrams cannot be only visualized but also reactivated using optogenetic tools that allow cells to be selectively turned on or off by light.

Outstanding scientists from Europe and USA participated with their original works in the Workshop which represented a great success. Therefore, as a tribute to Rita Levi-Montalcini we have decided to prepare a special issue of the European Journal of Neurodegenerative Diseases, a new Journal published during the first 2 years by EBRI Foundation and of which Rita was the Honorary President. Unfortunately, due to the short time available before the deadline, we have missed some contributions, but luckily we have managed to integrate these with other invited papers.

In particular, after an Introduction by Antonino Cattaneo, K. Cowansage (from the Scripps Research Institute of La Jolla, CA, USA) discusses how new genetic tools allow manipulating selective neuronal populations in the anterior cingulate, retrosplenial, medial prefrontal and posterior parietal cortices at early stages of memory consolidation.

J. Campi and R. Quian Quiroga (from the Centre for Systems Neuroscience, University of Leicester, Leicester, UK) describe the advantages and disadvantages of experiments aimed at elucidating the mechanisms by which memory traces are stored and recalled in both animals and humans.

J. Csicsvari (from the Institute of Science and Technology, Austria) highlights the functional role of network synchronization underlying sharp waves/ripples activity in the hippocampal formation and beyond, not only in learning and memory consolidation but in also planning and short term memory processes.

A particular form of network synchronization occurring in the hippocampus immediately after birth is represented by the so-called Giant Depolarizing Potentials or GDPs. E. Cherubini (from the International School for Advanced Studies, Trieste, Italy) provides evidence that GDPs act as coincident detector signal for enhancing synaptic efficacy at emerging glutamatergic and GABAergic synapses.

Synaptic dysfunction and memory loss have devastating consequences in the daily life of patients affected by Alzheimer Disease (AD) or other forms of Dementia.

P. Regan, E. Hogg, D. J. Whitcomb and K. Cho (from the School of Clinical Sciences, Faculty of Medicine and Dentistry, and the Centre for Synaptic

Mailing address:
European Brain Research Institute (EBRI)
Via del Fosso di Fiorano, 64
00143 Rome, Italy
Tel: +39 06 501703024
Email: scientific.assist@ebri.it

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Plasticity, University of Bristol, UK), suggest that an aberrant LTD-like downscaling of synaptic transmission, due to AMPA receptor endocytosis and synapse elimination, may trigger cognitive deficits in AD.

In the final article, M. Richter, V. Varela, E. Trias and L. Barbeito (from the Institut Pasteur and the Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay) review recent data showing that the neuro-inflammatory response associated with neurodegenerative diseases leads to activation of glial cells and increased levels of nitrated NGF species, which may potentially trigger p75NTR-dependent apoptosis in target cells.

We wish to express our gratitude to all scientists who rendered possible this special issue. In this way EBRI keeps alive the extraordinary figure of Rita Levi-Montalcini, a true giant in Science. Her discovery of NGF, the progenitor of other neurotrophic factors have greatly contributed to understanding the basic molecular mechanisms of synaptic plasticity, thus allowing to open new avenues for the treatment of Alzheimer's and other neurodegenerative diseases.

INTRODUCTION

A. CATTANEO

European Brain Research Institute, Rome, Italy
Scuola Normale Superiore, Pisa, Italy

The 2014 Rita Levi Montalcini Lecture is part of an annual series established by the European Brain Research Institute to commemorate the life and legacy of a woman whose outstanding scientific contributions have had a significant impact in the field of Neurosciences and beyond. The Lectures, delivered by distinguished scientists, open an international scientific event. The first edition of the Rita Levi-Montalcini Lecture was delivered in 2013 by Nobel Laureate Aaron Ciechanover. This year's Lecture was held by Nobel Laureate Susumu Tonegawa and was followed by a Workshop on "Engrams and memory traces".

It is with great pleasure and honour that I introduce Susumu Tonegawa, Picower Professor of Biology and Director of the RIKEN-MIT Center for Neural Circuit Genetics at the Massachusetts Institute of Technology.

Tonegawa represents a truly remarkable and outstanding scientist, whose scientific achievements are landmarks in both immunology and neurosciences. Susumu Tonegawa's contributions were recognized with a Nobel Prize in Physiology or Medicine in 1987 for his discovery on how the immune system generates its highly diverse repertoire of antibodies. He later continued to make fundamental contributions to science in an entirely different field: neuroscience. Using advanced techniques of gene manipulation, Tonegawa has made fundamental discoveries to unravel the molecular, cellular and neural network mechanisms that underlie learning and memory. His studies have broad implications for the understanding of human memory.

After studying Chemistry at Kyoto University, Tonegawa became interested in the then blossoming science of molecular biology and his mentor Itaru Watanabe advised him to enroll in an American graduate school. Following his Ph.D on transcriptional control of phage lambda with Masaki Hayashi at UCSD, he continued his postdoctoral research with Renato Dulbecco at Salk Institute, working on eukaryotic molecular biology of tumour viruses. By the end of 1970 his US visa was to expire and close to the end of that year Dulbecco wrote him a letter from Rome, where he was travelling, suggesting that he send an application letter to the newly established Basel Institute for Immunology in Switzerland. "Thanks partly to this remarkably prophetic letter and partly to the US immigration law, that prevented me from remaining in the US, in February 1971 I found myself in this cosy Swiss town almost completely surrounded by immunologists", recalls Tonegawa. Despite having little knowledge of immunology, he soon became intrigued by the question that had long puzzled immunologists: how does the immune system generate the multitude of antibodies to attack virtually any virus or pathogen, from only a limited number of genes? He was in his 30s when he discovered the solution of the GoD problem (Generation of Diversity), demonstrating that antibody diversity is the result of the combinatorial shuffling of genes that are rearranged to produce specific antibodies.

Tonegawa's scientific interest switched to neurobiology in the early 1990s, when he began exploring the molecular underpinnings of learning

Mailing address:
European Brain Research Institute (EBRI)
Via del Fosso di Fiorano, 64
00143 Rome, Italy
Tel: +30 06 501703024
Email: a.cattaneo@ebri.it

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

and memory in mice. His recent work, that will be described in his 2014 Levi-Montalcini Lecture on "Engrams for genuine and false memories", addresses questions which lie at the core of our current framework thinking on the mechanisms of memory. How memories are represented at the neuronal level in the brain is a central question in neurosciences. It is thought that a learning experience is encoded by a sparse population of neurons, forming a memory trace. The idea that memories are represented as physical traces inside our brain goes back as far as Plato (Figure 1), the great Greek philosopher who in the *Thaetetus* formulated the theory with a very vivid and dynamic metaphor of memory as an image inscribed on a wax tablet. Many centuries later, this metaphor was expressed in more scientific terms by the German neuropsychologist Richard Semon (1921), who coined the word "engram": *'Its result, namely, the enduring though primarily latent modification in the irritable substance produced by stimulus, I have called an Engram…'*. Searching the engram, and defining its nature and properties is one of the big questions in modern neurosciences. The engram theory of memory posits that when a memory is formed, a subpopulation of neurons will be excited and stay excited latently. When part of the total information at the time of storage is subsequently available, it will re-excite the engram for recall. A crucial test for the engram theory of memory, providing experimental evidence for memory engrams, requires at least three steps: i) the identification of neuronal populations that could represent candidate engrams in a certain brain region, ii) blocking or interfering with those cells should abolish a given memory and, finally, iii) artificially activating that cell population should mimic the memory process. The last two points correspond to addressing questions of *necessity* and *sufficiency* for a cell population being defined as a memory engram.

The activity-dependent expression of immediate early genes (IEG) such as c-fos and Arc in synaptically activated neurons has been used by many studies, in the past 15 years, as an indicator of recent neural activity. For instance, the cellular expression pattern of these IEGs in the hippocampus is different for different spatial contexts, but remains stable upon re-exposure to the same context, providing molecular signatures of memories. Therefore, these cells may represent a component of the stored memory engram.

The necessity question, for a candidate memory trace, was first addressed in 2009 (Han et al 2009; Zhou et al 2009) by a novel loss-of-function approach to perturb a component of the memory engram. By selectively ablating or inhibiting sparse population of cells in the amygdala that are preferentially recruited into the representation of a fear memory, it was possible to selectively interfere with the recall of that memory in mice, proving the necessity question for that memory trace.

Against this background, the mimicry experiments (to address the sufficiency condition) for memory engram studies remained a considerable challenge, because of the lack of tools that could precisely label and control selected neurons involved in a particular memory. Demonstrating the existence of memory engrams at the cellular level requires a system that can selectively label and activate the memory engram-bearing cells to induce the predicted behavioural changes caused by learning. To selectively activate a cell population bearing an engram for a particular memory, one needs to be able to isolate and label these cells for future manipulation.

This is precisely what Tonegawa's recent papers (Liu et al 2012; Ramirez et al 2013) demonstrated, and what has been illustrated and discussed by Tonegawa in his EBRI Levi-Montalcini Lecture. Tonegawa exploited transgenic mice in which the optogenetic probe Channelrhodopsin 2 (ChR2) could be expressed specifically in activated dentate gyrus granule cells. Activation of ChR2 by light causes the cells that express ChR2 to fire. The expression of ChR2 could be prevented by feeding the mice doxyclin (Dox), allowing to temporally restrict the expression of the optogenetic probe. A group of Dox fed mice were allowed to habituate to a neutral environment context (context A). The mice were then taken off Dox, to open a window for activity-dependent labelling with ChR2 and subsequently underwent fear conditioning in another spatial context (context B). After this conditioning, the animals were given Dox again (to prevent further ChR2 expression) and, on the following day, tested for their fear response in the original, non fearful context A. Fear response was initially low but dramatically increased when the ChR2 expressing

Fig. 1. *The School of Athens by Raphael 1509, Stanza della Segnatura, The Vatican Museums.*

cells (i.e. cells associated to the "engram" of context B) were stimulated by light. This, the fear memory for context B could be artificially induced in context A, by activating a synthetic memory trace. This study proved the sufficiency question for the engram theory, that is, that reactivation of neurons that encode a fear memory is sufficient to induce recall of that memory. Building on the previous finding, that DG hippocampal neurons recruited during learning define an active cell population that is sufficient for memory recall upon subsequent activation, Tonegawa and collaborators went on to ask the question (Ramirez et al 2013): can an artificially activated contextual memory engram serve as a conditioned stimulus (CS) and become associated with an unconditioned stimulus (US) to form an artificial CS-US association? To test this, mice were taken off Dox, so that cells active during exploration of neutral context A would be labelled with ChR2. Mice were then put back on Dox and fear conditioned in context B, while optically activating labelled cells (i.e. cells labelled in context A) with light. The hypothesis was that light-activated context A engram cells could produce an artificial CS while the mice were simultaneously administered a US to form an artificial associative fear memory. Indeed, the experiments revealed that when placed back in context A, the mice displayed increased fear responses, in a context in which they were never actually shocked. Importantly, when placed in a novel context C, the animals showed no fear, indicating that the fear response is context specific and not the result of generalization. The optogenetic inception of a false memory provides further demonstration for the engram theory and may perhaps parallel some types of false recognition memories in humans.

It is well recognized that the way we feel about a past experience can change over time, while other details of the memory remain intact. The malleability of the valence of memories has been used clinically to treat maladaptive behaviours. The neronal mechanisms and brain circuits that enable the switching of the valence of memories are well characterized in a recent optogenetic-based study (Redondo et al 2014). Tonegawa applied their memory engram cell-manipulation technique and artificially altered the emotional valence of a memory in mice. The results showed that in the DG

Fig. 2. *Susumu Tonegawa and Rita Levi-Montalcini at EBRI, 17 September 2007.*

of hippocampus, the neurons carrying the memory engram of a given neutral context have plasticity such that the emotional valence of a conditioned response evoked by their reactivation can be reversed by re-associating this ciontextual memory engram with a new unconditioned stimulus of an opposite valence.

The results presented and discussed by Tonegawa in the Rita Levi-Montalcini Lecture, held at the Accademia Nazionale dei Lincei, provide the foundation for an experimental and conceptual bridge between animal behaviour and human cognitive neuroscience, in the study of memory. The ability to identify and manipulate engram-encoding cells allows looking into the future of memory engram research, with new questions arising and becoming now amenable to experimental interrogation: what is happening at the synapse level in the engram bearing cells? what is the minimum cell population required to activate a memory? how reproducible is the particular combination of memory engram cells on repeated exposure to a similar stimulus? what occurs to these cells during memory consolidation and extinction? And, finally, probably the question that is more directly relevant to a dramatic human disease: how do these studies connect to the memory loss occurring during the progression of Alzheimer's disease, and how can the knowledge acquired help in finding new therapeutic strategies for this devastating condition?

The Lecture delivered by Susumu Tonegawa most fittingly opened the workshop on "Engrams and Memory Traces", which displays cutting-edge research from outstanding scientists worldwide; the best way to honour the memory of the great neuroscientist (Fig. 2).

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COHERENT NETWORK-DRIVEN OSCILLATIONS AS COINCIDENT DETECTORS FOR ENHANCING SYNAPTIC EFFICACY IN THE DEVELOPING HIPPOCAMPUS

E. CHERUBINI

European Brain Research Institute (EBRI), Roma, Italy and International School for Advanced Studies (SISSA), Trieste, Italy

Coherent membrane oscillations are a hallmark of developmental networks. In the hippocampus, network-driven giant depolarizing potentials or GDPs are generated by the interplay of glutamate and GABA that in the immediate postnatal period is depolarizing and excitatory. Here, some recent data are reviewed concerning the mechanisms by which GDPs are generated and their functional role in shaping synaptic currents at emerging mossy fiber (MF)-CA3 and Schaffer collateral-CA1 synapses. Using a pairing procedure consisting in correlating GDPs-associated calcium rise in the postsynaptic cell with stimulation of afferent inputs we were able to persistently enhance synaptic strength at both synapses. This associative type of learning caused the appearance of synaptic responses or persistently enhanced the number of successes in "presynaptically" silent or low probability synapses, respectively. The induction of LTP was postsynaptic and was mediated by calcium rise via voltage-dependent calcium channels activated by the depolarizing action of GABA during GDPs since it was prevented by the calcium chelator BAPTA and by nifedipine. However, the expression of LTP was presynaptic, as assessed in double pulse experiments that unveiled a significant reduction in the paired-pulse ratio and a significant increase in the inverse squared value of the coefficient of variation of response amplitude, all indices of presynaptic release probability. The postsynaptic induction and presynaptic expression of LTP suggest the involvement of a retrograde messenger that, at least at Schaffer collateral-CA1 synapses, was identified as BDNF. Acting on pre and postsynaptic TrkB receptors, respectively, BDNF enhanced the probability of glutamate release and activated the MAPK/ERK signalling pathway, leading to transcriptional regulation and new protein synthesis. In conclusion, GDPs would translate specific patterns of pre and postsynaptic activity into long-lasting changes in synaptic strength and would stabilize synaptic connections, thus contributing to the structural refinement of the hippocampal circuit.

Coherent network oscillations represent a hallmark of developmental networks. They can be detected at late embryonic or early postnatal ages in several CNS structures, including the retina (Feller et al, 1997), the neocortex (Garaschuk et al, 2000), the hippocampus (Ben-Ari et al, 1989), the hypothalamus (Chen et al, 1996), the cerebellum (Eilers et al, 2001) and the spinal cord (O'Donovan, 1999). This activity, which may differ in its specific pattern among different brain regions, is crucial for synaptic wiring and refinement of local neuronal circuits according to the Hebbian rule "neurons that fire together wire together". In the developing hippocampus, during the first week of postnatal life, the so-called Giant Depolarizing Potentials or GDPs (Ben-Ari et al, 1989; Ben-Ari et al, 2007),

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Corresponding Author:
Enrico Cherubini,
European Brain Research Institute (EBRI)
Via del Fosso di Fiorano 64, 00143 Roma,
Tel.: +39 06 50 170 3024
Fax: +39 06 50 170 3335
e-mail: e.cherubini@ebri.it

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constitute a primordial form of synchrony between neurons, preceding more organized forms of activity such as theta and gamma rhythms, thought to be associated with higher cognitive functions (Buzsáki and Draguhn, 2004).

GDPs consist in recurrent long lasting membrane depolarisations (up to 50 mV) giving rise to bursts of action potentials, separated by long-lasting quiescent intervals (Ben-Ari et al, 1989). This discontinuity pattern is reminiscent of the "tracė discontinu" firstly described by Dreyfus-Brisac in the electroencephalogram of immature babies and characterized by intermittent bursts separated by periods of virtually complete suppression of activity (Dreyfus-Brisac and Larroche, 1971). A similar pattern has been described in the hippocampus of fetal macaque during the second half of gestation (Khazipov et al, 2001) suggesting that, at least in vertebrates, this activity is well preserved during evolution. The in vivo counterparts of GDPs are the sharp waves that can be observed in rat pups during distinct behavioural states such as immobility, sleep and feeding (Leinekugel et al, 2002).

IN THE IMMATURE HIPPOCAMPUS, THE DEPOLARIZING ACTION OF GABA AND GLUTAMATE ACCOUNT FOR NETWORK-DRIVEN GDPs

GDPs are generated by the synergistic action of glutamate and GABA that, at this developmental stage, is depolarizing and excitatory (Ben-Ari et al, 1989; Cherubini et al, 1991; Bolea et al, 1999). Electrophysiological recordings associated with fura-2-based fluorometric calcium imaging have demonstrated that within a particular hippocampal area, GDPs involve the synchronous discharge of principal cells and interneurons (Garaschuk et al, 1998). GABAergic interneurons usually drive principal cells, as demonstrated by the temporal relationship of glutamatergic and GABAergic inputs during GDPs. GABA, released from local interneurons binds to GABAA receptor channels and triggers conformational changes that facilitate the inflow or outflow of chloride (Cl-) inside the cell, depending on the equilibrium potential for this anion (E_{Cl-}). In adult neurons, the level of intracellular chloride is relatively low and E_{Cl-} is below the resting membrane potential (V_m). Therefore, GABA hyperpolarizes the membrane and inhibits neuronal firing through an inwardly directed flux of chloride (Cherubini, 2012). In immature neurons, E_{Cl-} is above V_m. Therefore, GABA depolarizes the membrane through an outwardly directed efflux of chloride. To be excitatory, GABA-mediated membrane depolarization should reach the threshold for action potential generation. This usually occurs via the activation of a persistent, non-inactivating sodium conductance that by amplifying subthreshold synaptic responses (Valeeva et al, 2010) bring them to threshold (Cherubini et al, 1991; Ben-Ari, 2002; Owens and Kriegstein, 2002; Ben-Ari et al, 1997; Mohajerani and Cherubini, 2005). However, it is worth mentioning that GABA can depolarize its targets and still exert an inhibitory effect through "shunting" inhibition (Mohajerani and Cherubini, 2005; Banke and McBain, 2006). The intracellular chloride concentration is under control of two main cation-Cl- co-transporters the NKCC1 and KCC2 that import and export $[Cl-]_i$, respectively (Rivera et al, 1999; Payne et al, 2003). The unbalance between these two transporters is responsible for the high $[Cl-]_i$ found early in postnatal life. GDPs disappear towards the end of the second postnatal week when GABA becomes inhibitory (Ben-Ari et al, 1989). The developmentally up-regulated expression of the K+/Cl- co-transporter KCC2 is responsible for this modification (Rivera et al, 1999). The depolarizing action of GABA during GDPs results in calcium influx through the activation of voltage-dependent calcium channels and N-methyl-D- aspartate (NMDA) receptors (Leinekugel et al, 1997; Garaschuk et al, 1998).

The appearance of GDPs is preceded by a well defined sequence of events. At late embryonic stages of development, uncorrelated spontaneous activity consisting in calcium action potentials occurs in cortical structures. Calcium signalling plays a crucial role in regulating a variety of developmental processes including neurogenesis, neuronal migration and differentiation (Owen and Kriegstein, 2002; Komuro and Rakic, 1996). Synchronous activity emerges at birth under the form of spontaneous plateau assemblies or SPAs. SPAs involve small groups of neurons coupled by gap-junctions and consist in non-synaptic membrane oscillations generated by

the activation of intrinsic membrane conductances (Crepel et al, 2007). SPAs are modulated by oxytocin, a maternal hormone essential for the induction of labor, known to transiently converts GABA action from excitatory to inhibitory during parturition (Tyzio et al, 2006). In addition, SPAs are facilitated by the depolarizing action of GABA which activates voltage-dependent calcium channels and facilitates the relief of the voltage-dependent magnesium block from NMDA receptors, thus allowing calcium entry and activation of second messengers.

Before synapse formation, GABA can be released in a calcium- and SNARE-independent way by non-conventional release sites such as growth cones and astrocytes and can diffuse away to activate extrasynaptic receptors in a paracrine fashion (Demarque et al, 2002). The absence of an efficient uptake system will enable GABA to accumulate in the extracellular space and to reach a concentration sufficient to exert its depolarizing and excitatory effects on distal neurons. As the network matures and the density of functional synapses increases, synaptic-driven network oscillations such as ENOs and GDPs replace SPAs. A down regulation in the expression of connexins via CREB signalling, following activation of NMDA receptors, may contribute to SPAs silencing (Arumugam et al, 2005). ENOs, initially thought to constitute the cortical counterpart of hippocampal GDPs, have been shown to precede and coexist during a restricted period of time with GDPs (Allene and Cossart, 2010). ENOs differ from GDPs not only because they involve a different neurotransmitter (glutamate instead of GABA) but also because they exhibit different spatio-temporal dynamics (Allene et al, 2008). ENOs are low frequency oscillations dysplaying slow kinetics that gradually involve the entire network whereas GDPs are recurrent oscillations that repetitively synchronize local neuronal assemblies. In the neocortex, ENOs are critically dependent on the activation of NMDA receptors (Garaschuk et al, 2000) and NMDA signaling may contribute to recruit AMPA receptors to the synapses and to convert postsynaptically silent conenctions into active ones (Voronin and Cherubini, 2004).

HOW ARE GDPs GENERATED?

It is known that to be generated GDPs need the synchronous activation of a relatively small number of cells. At least in the CA3 area, they can be still detected in small islands comprising ~ hundreds of neurons, isolated from the rest of the hippocampus (Khazipov et al, 1997; Garaschuk et al, 1998; Bolea et al, 1999). They involve the activation of both principal cells and interneurons (Leinekugel et al, 1997; Garaschuk et al, 1998; Bonifazi et al, 2009) which by releasing GABA and glutamate activate $GABA_A$ and AMPA receptors, respectively. Interestingly, at single cell level, $GABA_A$ and AMPA receptors are already present at birth. However, these receptors are sequentially activated being the degree of synaptic connections correlated with the level of dendritic arborization (Tyzio et al, 1999). Three stages of develoment can be identified: i. silent neurons with no apical dendrites; ii. GABA-only neurons with small apical and not basal dendrites; iii. GABA-and glutamate neurons with extensive apical and basal dendrites. These observations suggest that GABAergic synapses form prior to glutamatergic ones. At the network level however, GDPs express both components, although the magnitude of the GABAergic conductance exceeds that of the glutamatergic one (GDPs reversal is close to EGABA; Ben-Ari et al, 1989; Bolea et al, 1999). The glutamatergic component can be unveiled by blocking the GABAergic one by loading the cell with an intracellular solution containing potassium fluoride, which only poorly permeates $GABA_A$ receptor channels. In this condition, GDPs reverse polarity at a membrane potential close to the equilibrium potential for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated responses (E_{AMPA}; Bolea et al, 1999). The two components of GDPs can be examined by holding two neighbouring pyramidal neurons at E_{GABA} (fixed at -70 mV) and E_{AMPA} (~0 mV), respectively. With this procedure it became clear that the GABAergic component precedes always the glutamatergic one by several ms suggesting that principal cells are driven by GABAergic interneurons (Mohajerani and Cherubini, 2005). Furthermore, using network dynamics imaging, online reconstruction of functional connectivity and targeted whole-cell recordings from immature hippocampal slices, it was recently demonstrated that functional hubs composed of subpopulations of GABAergic interneurons with

large axonal arborizations are able to synchronize large ensembles of principal cells (Bonifazi et al, 2009).

In analogy with the synchronized activity generated in the disinhibited hippocampus (Menendez de la Prida et al, 2006), it seems likely that GDPs emerge when a sufficient number of cells fire and the excitability of the network attains a certain threshold within a restricted time window. Simultaneous recordings from pairs of CA3 pyramidal neurons have shown a concurrent increment in the instantaneous firing frequency previous to GDPs onset which correlates with an increased frequency of spontaneously occurring synaptic events (Menendez de la Prida and Sanchez-Andres, 1999). Although the entire hippocampal network possesses the capacity to generate GDPs, for its anatomical characteristics, including extensive glutamatergic connections via recurrent collaterals, the CA3 area is particularly well equipped to generate synchronized oscillations and can be considered as the pacemaker region. In addition, the CA3 area of the hippocampus is able to initiate, upon membrane depolarization, intrinsic bursts which by virtue of their spontaneous discharges and large spike output can drive other neurons to fire (Sipila et al, 2005; Safiulina et al, 2008). Burst firing is facilitated by a persistent slow sodium current (Sipila et al, 2006) whose activation threshold can be easily reached by the depolarizing action of synaptically released GABA and glutamate. In addition, the activation of extrasynaptic GABAA receptors by ambient GABA (accumulated in the extracellular space by spillover from neighboring synapses or by release from non-conventional release sites), generates a tonic GABAA-mediated conductance that contributes to depolarize the neurons (bringing them to threshold for activating the persistent sodium current, Sipila et al, 2005), and to further enhance cell excitability and the glutamatergic drive to principal cells (Marchionni et al, 2007). Interestingly, intrinsic bursting activity is facilitated by the low expression, of Kv7.2 and Kv7.3 channels responsible for the non-inactivating, low-threshold M current (I_M), which in adulthood controls spike after-depolarization and burst generation (Yue and Yaari, 2004). The low density of I_M at birth contributes to produce intrinsic bursts that, in comparison to adults, are more robust, last longer and recur more regularly (Safiulina et al, 2008).

In thalamo-cortical neurons, a striking contribution to rhythmogenesis is given by pacemaker neurons, characterized by an unstable behaviour caused by the interplay of depolarizing and hyperpolarizing conductances (Pape, 1996). Pacemaker cells are typically depolarized by the slow hyperpolarization-activated cation current Ih, carried by HCN channels. In these neurons, the oscillatory period last from tens of milliseconds to seconds. In neonates, the intervals between GDPs is much longer and therefore the oscillatory period could not be set solely by this conductance. By interacting with other membrane conductances and network properties, Ih may contribute to network synchronization as suggested by the possibility to disrupt GDPs in principal cells and interneurons, known to be endowed since birth with the HCN1 isoform of HCN channels, with extracellular cesium (Strata et al, 1997) or with the selective I_h blocker ZD 7288 (Bender et al, 2005). It should be stressed however, that I_h is not essential for GDPs generation since synchronous oscillations may occur also in neurons lacking HCN channels (Bender et al, 2005). GDPs can be also facilitated by cell-coupling via gap junctions, whose expression early in development has been well documented (Rozental et al, 2000). This type of signaling which plays a crucial role in SPAs generation may persist at later developmental stages when SPAs are replaced by GDPs (Strata et al, 1997). It would be of interest to know whether in connexin-36 knock-out mice, which exhibit disrupted gamma oscillations (Hormuzdi et al, 2001) and severe impairment in spatial coding and cognition (Allen et al, 2011), GDPs develop normally.

GDPs typically terminate by a slow afterhyperpolarization (AHP), lasting few sec and mediated by calcium-activated potassium conductances (Ben-Ari et al, 1989; Sipila et al, 2006). Blockade of the AHP decreases the interval between GDPs, suggesting that the frequency of these spontaneous events is set by the kinetics of this conductance. However, other factors may contribute to the observed periodicity including a delayed activation of GABAB receptors by GABA released during interneuron firing, as suggested for interictal population events generated in the CA3 area by blocking GABAA-mediated inhibition (Menendez

de la Prida et al, 2006). Preventing the activation of the GABAB conductance with the selective antagonist significantly prolong the duration of GDPs suggesting that GABA via these receptor types contribute to their termination (McLean et al, 1996; Fiorentino et al, 2009).

PERSISTENT INCREASE IN SYNAPTIC STRENGTH INDUCED BY PAIRING GDPs WITH MOSSY FIBER ACTIVATION

Mossy fibres (MF) are the axons of dentate gyrus granule cells. They convey information from the dentate gyrus to the CA3 area of the hippocampus proper. These fibres have been originally called "mossy" by Ramon y Cajal because of their particular appearance at the light microscopic level that reminds, as the mossy fibres in the cerebellum, the shape of the moss on trees (Ramon y Cajal, 1911). MF not only project to the CA3 area but they make synaptic contacts with basket cells in the dentate gyrus (providing the local recurrent inhibition) and with pyramidal-like neurons, the mossy cells, in the hilus (Johnston and Amaral, 2004). MF give rise to large en passant swellings (up to 5 mm in diameter) and terminal expansions on CA3 principal neurons or mossy cells seen as giant boutons at the electron microscopic level. These giant boutons develop gradually during the first 21 days (Amaral and Dent, 1981): at postnatal (P) day 3, immature axons terminate in very small, spherical expansions, which establish both symmetric and asymmetric contacts with pyramidal cell somata, apical dendrites and presumed growth cones. These contacts are made several days before the development of thorny excrescences (Stirling and Bliss, 1978; Amaral and Dent, 1981). Expansions markedly increase in size by day 9 while maintaining a relatively spherical shape.

While in adults, MF are glutamatergic and integrate the classical tri-synaptic circuit, at birth, they release into CA3 principal cells and GABAergic interneurons GABA (Safiulina et al, 2006). In addition, in particular conditions (i.e. seizures). they can co-release glutamate and GABA (Gutierrez and Heinemann, 2001; Münster-Wandowsi et al, 2013; but see Uchigashima et al, 2007; Cabezas et al, 2012; Caiati, 2013). Evidence has been provided that during postnatal development MF constitutively express GAD67 and its product GABA (Schwarzer and Sperk, 1995; Sloviter et al, 1996) as well as the mRNA for the vesicular GABA transporter VGAT (Gomez-Lira et al, 2005). Post embedding immunogold double labelling have revealed the coexistence in MF terminals of VGAT with VGLUT (the vesicular transporters for GABA and glutamate respectively) further suggesting that GABA can be co-released with glutamate (Zander et al, 2010). Moreover, hippocampal pyramidal neurons are able to express not only glutamate but also "mistargeted" GABAA receptors which, in particular conditions may become functional (Rao et al, 2000). This suggests that MF can use GABA as a neurotransmitter since they posses all the machinery for synthesising, storing, releasing and sensing it.

Like adults, immature MF undergo activity-dependent modification of synaptic efficacy. The immature brain is characterized by an elevated number of "silent" synapses (Durand et al, 1996; Gasparini et al, 2000). These are synapses that do not conduct at rest either because the neurotransmitter is not released when the presynaptic terminal is invaded by an action potential (presynaptically silent) or because they are unable to detect the release of the neurotransmitter due to the lack of the respective receptors on the subsynaptic membrane (postsynaptically silent). Conversion of silent synapses into active ones represents the most common mechanism for LTP induction (Voronin and Cherubini, 2004). Using a ''pairing'' procedure consisting in stimulating for a short period of time (5 min) mossy fibres with the rising phase of spontaneously occurring GDPs, in order to associate these events with the activation of afferent inputs, we were able to persistently enhance synaptic strength leading in some cases to synapses un-silencing (Kasyanov et al, 2004; Spitzer, 2004). In double pulse experiments, pairing-induced increase in successes rate was associated with a significant reduction in the paired-pulse ratio and a significant increase in the inverse squared value of the coefficient of variation. This suggests that an increased probability of transmitter release accounts for the persistent increase in synaptic efficacy. By introducing a delay between GDPs and afferent stimulation, synaptic responses progressively declined and regained the

control level in about 3 s. In the absence of pairing no significant changes in synaptic efficacy occurred. Pairing-induced synaptic potentiation could be prevented by loading the cell with the calcium chelator BAPTA, or by adding nifedipine (but not D-APV) to the extracellular solution, indicating that calcium rise via voltage-dependent calcium channels, opened by the depolarizing action of GABA during GDPs is a common trigger for activity-dependent changes in synaptic efficacy (Kasyanov et al, 2004). Interestingly in previous reports (Caillard et al, 1999, Gubellini et al, 2001; Gubellini et al, 2005), it was clearly shown that repeated bursts of action potentials, applied at low frequency to CA3 principal cells, are able to potentiate GABAA-mediated synaptic currents in a NMDA-independent and BDNF-dependent way. However, in these studies the origin of GABAergic inputs was not identified.

GDPs AS COINCIDENT DETECTORS FOR ENHANCING SYNAPTIC EFFICACY AT POORLY DEVELOPED SCHAFFER COLLATERAL-CA1 SYNAPSES

Similar experiments carried out at Schaffer collateral-CA1connections demonstrated that calcium transients associated with GDPs act as coincident detector signals for enhancing synaptic efficacy also at glutamatergic synapses. Here, the pairing procedure produced a persistent increase in synaptic strength of AMPA-mediated spontaneous and evoked excitatory postsynaptic currents. As for MF, LTP induction was postsynaptic while LTP expression was presynaptic, as suggested by pairing-induced decrease in failure rate, in paired pulse facilitation and increase in the inversed square of the coefficient of variation, all traditional indices of presynaptic changes in release probability. This implies a cross-talk between the post and the presynaptic sites of the synapse. We hypothesised that the retrograde signal is brain-derived neurotrophic factor (BDNF), possibly secreted from the postsynaptic neuron during GDPs. Using scavengers of endogenous BDNF or tropomyosin-related kinase B (TrkB) receptor antagonists we were able to prevent LTP induction. Blocking TrkB receptors in the postsynaptic cell did not prevent the effects of pairing suggesting that BDNF, acting on TrkB receptors localized on presynaptic neurons increases the probability of glutamate release (Mohajerani et al, 2007). BDNF may also act on postsynaptic TrkB receptors to activate the MAPK/ERK signalling pathway. Thus, the block of synaptic potentiation with ERK inhibitors present into the patch pipette validated this hypothesis (Mohajerani et al, 2007). Therefore, activation of postsynaptic TrkB receptors by BDNF would lead to transcriptional regulation and new protein synthesis required for the enduring forms of synaptic plasticity.

CONCLUSIONS

It is clear from this review that, at early stages of postnatal development, genetically built neuronal networks are very plastic and undergo activity-dependent changes in their synaptic efficacy, through adaptive processes that involve experience. Thus, during the first postnatal week, an associative type of learning is able to persistently enhances synaptic strength at both GABAergic MF-CA3 and glutamatergic CA3-CA1 connections. This effect requires a transient rise of calcium in the postsynaptic cell. This will be ensured by GDPs-associated calcium transients and the activation of voltage-dependent calcium channels following GABA-induced membrane depolarization. Hence, GDPs would exert an instructive role in shaping synaptic connections in the hippocampus during a critical period of postnatal development, a process that would lead to the structural refinement of synaptic connections and to the establishment of the adult neuronal circuit.

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SHARP WAVE RIPPLES IN THE HIPPOCAMPUS

J. CSICSVARI

Institute of Science and Technology, Klosterneuburg, Austria

During sharp wave/ripple (SWR) patterns large numbers of hippocampal cells fire action potentials together and, in parallel, CA1 neurons engage in transient 200Hz ripple oscillations. SWRs are initiated in the CA3 recurrent collateral system triggering the activation of CA1 neurons and further downstream it synchronises neurons in other brain regions. Emerging evidence has linked SWRs to learning and memory consolidation and recent work has demonstrated a role in planning and short-term memory as well.

Oscillations are omnipresent in the brain: the activity of neurons is influenced by a wide variety of macroscopic network oscillatory patterns (Buzsaki and Draguhn, 2004). Although the most recognizable forms of network oscillations keep on for longer periods of time, over numerous oscillatory cycles [e.g. theta-band (4-10Hz) oscillations], other types of oscillations occur as transient packets of oscillatory waves. Such transient oscillations synchronise neuronal populations as they phase lock to the same oscillations. Moreover, neurons tend also to increase transiently their firing rate during these intermittent oscillations. Possibly one of the best studied example of such transient network oscillatory patterns is the sharp wave/ripple event that occurs in the hippocampus, usually during sleep and rest periods (Buzsaki et al., 1983, 1992; Csicsvari et al., 1999). This review will provide a concise overview about the mechanism and function of SWRs.

DEFINITION OF SWRs

Early studies of rodents by Vanderwolf first identified that hippocampal field oscillations are governed by the behavioural state of the animal (Vanderwolf, 1969). Regular theta-band oscillations are observed during locomotor activity and rapid eye movement sleep. In the absence of theta oscillations, during slow wave sleep and waking immobility, field potentials are less regular - a state referred to as large irregular activity. During large irregular activity, intermittent negative waves (sharp waves) of 40-120ms duration are observed in the CA1 stratum radiatum (Buzsaki, 1986). In conjunction with these sharp waves, 150-250Hz fast oscillatory patterns (ripples) are present in the CA1 region, (O'Keefe and Nadel, 1978; Suzuki and Smith, 1988; Buzsaki et al., 1992). Such sharp wave/ripple patterns have been observed in inactive waking and slow wave sleep periods but they also occur during consummatory behaviour, grooming and in brief pauses in exploratory activity (Buzsaki et al., 1983; O'Neill et al., 2006). Although SWR patterns were originally observed in rodents (rats and mice), recent work has reported their existence in other mammalian species including bats, primates and humans (Skaggs et al., 2007; Ulanovsky and Moss, 2007; Le Van et al., 2008).

MECHANISM OF SWRs

During SWRs two interrelated processes take place: hippocampal CA1 and CA3 neurons

Key words: Network oscillations, local field potentials, plasticity, memory consolidation, temporal code

Corresponding Author:
Jozsef Csicsvari,
Institute of Science and Technology Austria,
Am Campus 1 A-3400 Klosterneuburg, Austria
Tel.: +43-2243 9000-4301
e-mail: jozsef.csicsvari@ist.ac.at

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transiently increase their firing rate and consequently many neurons fire action potentials together. At the same time CA1 neurons engage in a synchronised oscillatory activity leading to ripple oscillations in the CA1 region. Data suggests that SWRs patterns are generated internally within the hippocampus, and are initiated in the CA3 region. It was shown that SWRs remain in animals in which the entorhinal cortex (EC) is lesioned, demonstrating that SWRs can be generated in the absence of extrahippocampal input from the EC (Bragin et al., 1995). Moreover, fimbria fornix lesions demonstrated that subcortical inputs are also not necessary for SWR-generation, although in these conditions SWR events are of larger in amplitude, suggesting an overall repressive role for subcortical inputs (Buzsaki, 1986). In knockout animals in which activity of CA3 neurons is largely suppressed sharp waves can be no longer seen although transient ripples oscillations can be still identified (Nakashiba et al., 2009).

Precise temporal analysis of the SWR-related firing of CA3 pyramidal cells revealed that many CA3 pyramidal cells fire together during SWRs, and they start to fire earlier than CA1 pyramidal cells (Csicsvari et al., 2000). Amongst CA3 cells, those proximal to CA1 (CA3a) start to fire first, 15ms earlier than CA1 pyramidal cells while those proximal to the dentate gyrus (CA3c) fire last, preceding CA1 cells only by 5ms. In the dorsal hippocampus, CA3 recurrent connectivity is stronger in the CA3a-CA3b regions than in the CA3c region (Li et al., 1994; Witter and Moser, 2006). By contrast, the CA1 region receives far more projections from the CA3c region than from CA3a/b regions. Therefore, anatomical connectivity may explain why SWRs tend to initiate in CA3a/b regions and propagate first to the CA3c region and then to CA1.

It is not fully understood how the SWR burst is initiated. It has been suggested that SWRs are triggered by initiator pyramidal cells in the CA3 region that have strong connections with other CA3 cells (Buzsaki et al., 1989;O'Neill et al., 2006). These initiator cells will recruit the first group of cells to fire during SWRs, which in turn will recruit an even larger group of cells. This process will lead to the build-up of SWRs as gradually more and more cells are recruited. However, build-up of such excitatory activity depends largely on the network state, because of the variable levels of subcortical neuromodulation in each state. Acetylcholine has been suggested to have the strongest effect in controlling the emergence of SWRs because it suppresses excitatory neurotransmission through presynaptic muscarinic receptors (Hasselmo, 1999). Therefore, during waking periods, when cholinergic levels are high, the CA3 recurrent collateral system and CA3-CA1 Schaffer collaterals are suppressed, by which action SWRs are ultimately suppressed. Nevertheless sharp waves are not completely blocked because acetylcholine and other non-specific subcortical neurotransmitters that are present during active waking periods also make pyramidal cells more excitable by the action of postsynaptic receptors. This can compensate for the reduced effectiveness of the CA3 inputs (O'Neill et al., 2006).

ROLE OF SWRs IN PLASTICITY

The frequency of ripple oscillations is similar to that used to induce LTP during tetanic stimulations. Therefore, it has been suggested that SWR may facilitate synaptic plasticity (Buzsaki, 1989). Such plastic changes have indeed been demonstrated by stimulating CA1 pyramidal cells during SWRs: it was shown that the SWR responses of cells are potentiated if these cells were stimulated during SWRs before (King et al., 1999). These experiments suggest that synaptically-connected CA3 and CA1 pyramidal cells that repeatedly fire together during SWRs potentiate their synapses. It has been shown before that dendritic spiking takes place during SWRs (Kamondi et al., 1998). Moreover, highly synchronised input from CA3 cells during SWRs can evoke localised dendritic spikes on the apical dendrites of CA1 pyramidal cells. These dendritic spikes could potentially facilitate synaptic plasticity (Losonczy et al., 2008).

However, there have been opposite suggestions as well, proposing that SWRs may trigger LTD. Because SWRs tend to occur at low frequencies similar to LTD stimulation frequency, it has been proposed that SWR may have a role in reducing synaptic weights. Indeed in ventral hippocampal slices, in which SWR-like activity can spontaneously occur, the potentiation following LTP induction gradually reduced (Colgin et al., 2004). Moreover, in dorsal hippocampal slices, where SWRs were not present, LTP was stable; however, stimulation following ventral SWR patterns

led to LTD. The idea that SWRs reduce synaptic weights is in agreement with the presumed role of sleep in down-scaling synapses that were excessively potentiated during waking activity (Tononi and Cirelli, 2006).

Perhaps both of these effects may take place in parallel. Accordingly, SWR can cause the facilitation of synapses if both the post- and the presynaptic pyramidal cells fire frequently during SWRs while synaptic strengths will be reduced when these cells tend not to fire together during SWRs. Such a rule is similar to that which governs how the joint firing tendency of cells changes during reactivation due to waking firing patterns (O'Neill et al., 2008).

REACTIVATION OF WAKING EXPERIENCES DURING SWRs

It had been predicted early on that the waking activity patterns of neuronal assemblies may spontaneously recur in the hippocampus in offline periods when it is not processing external inputs (Marr, 1971). It has been also suggested that such reactivation may have a role in memory consolidation: reactivated neuronal patterns might represent memory traces, which are transferred to extrahippocampal locations for consolidation (Buzsaki, 1989; McClelland et al., 1995). Finally, it has been put forward that such replay could take place during SWRs (Buzsaki, 1989). Strong network synchronization during SWRs has been proposed to facilitate the transfer of reactivated patterns to extrahippocampal locations where those might eventually be stored (Buzsaki, 1989; Kudrimoti et al., 1999). Additionally, by facilitating synaptic plasticity, they may also strengthen downstream connections to extrahippocampal locations during reactivation (Buzsaki, 1996).

Electrophysiological recording confirmed many aspects of this theory. It was shown that waking activity patterns of hippocampal neurons do recur during sleep (Wilson and McNaughton, 1994). Moreover, place cells with similar place fields that fire frequently together during waking exploration also often fire together in subsequent sleep periods. This suggests that reactivation during sleep represents places that the animal visited before. Hence, it was put forward that place cells encoding similar locations form cell assemblies, whose joint activity is reinstated in sleep. The sequential firing patterns of place cells are also reactivated, primarily in cases when the animal follows stereotyped movement paths on tracks and simple mazes (Lee and Wilson, 2002). Importantly, it was confirmed that reactivation tends to occur during SWRs. In the case of reactivated patterns encoding places, it was shown that the increase in firing rates during SWRs is assembly-specific, enabling wider separation of cell assemblies encoding different locations, and thus a more precise coding of reactivated places (O'Neill et al., 2008). Reactivation of firing sequences tends to occur during SWRs as well (Lee and Wilson, 2002). Moreover, reactivation of sequences in neighbouring SWRs is interrelated: long movement paths tend to recur during consecutive SWRs (Davidson et al., 2009).

There are two independent evidence linking SWRs in sleep to memory consolidation.

Firstly, correlative evidence showed the frequency of reactivation of newly-learned reward locations predicts the future memory retention performance of the animals (Dupret et al., 2010). In this work animals were trained to learn the location of food rewards on a cheeseboard maze. Following learning, in sleep and immobile rest periods it was measured how frequently the learned reward locations were reactivation. It was shown that the frequency in which a given reward location was reactivated predicts the subsequent memory performance of the animal. Secondly, in order to demonstrate a causal link, studies were performed using SWR blockade to test role of SWR in learning and memory stabilization (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010). In these studies animals learned locations of rewards in complex mazes over several days. The blockade of SWRs following the learning trials delayed the speed of the animal to reach optimal memory performance to locate the rewards, although the impairment was mild. However, it is possible that electrical stimulation during SWRs may itself cause alterations in synaptic strength, which could explain the learning deficits (King et al., 1999).

SWRs DURING WAKING PERIODS

SWRs that occur during active waking periods may have different roles to those in sleep because neuronal activity in these SWRs can be influenced by ongoing sensory experiences. Initially, it was shown

that SWRs that occur in long immobility periods exhibit different network firing patterns to those that take place when the animal stops only for a short while (O'Neill et al., 2006). As in sleep, SWR firing patterns in long immobility periods represent previous exploration-associated patterns. By contrast, SWR network activity during brief immobility periods is influenced by the current location of the animal: place cell firing is stronger when the animal is located inside the cell's place field as compared to outside.

Sequence reactivation has also been observed during waking SWRs. Surprisingly, these SWR firing sequences were originally reported in a reverse temporal order to that of waking patterns (Foster and Wilson, 2006). Subsequently, similar reverse order reactivation has been seen in open field exploration but only during those SWRs that occurred in brief immobility periods i.e. those with place-selective activity (Csicsvari et al., 2007). Later on, it was shown on the linear track that both forward and reverse order reactivation can take place during waking immobility SWRs (Diba and Buzsaki, 2007; Davidson et al., 2009).

More recent studies suggest a different behavioural role for waking SWRs. Firstly, the blocking of waking SWR caused spatial working memory deficit while trained rats repeatedly visited the arms of a 'W' maze (Jadhav et al., 2012) and were able to differentiate correct and error trials (Singer et al., 2013). Moreover, a study have found that firing sequences correlated with the future 2D path of the animal in reaching goal locations in a 2D environment (Pfeiffer and Foster, 2013).

CONCLUSIONS

SWR network activity highlights the functional importance of network burst events in the brain in which wide populations of neurons synchronise their activity together. These SWRs synchronise neurons within short time windows appropriate for neuronal plasticity. These can also coordinate the activation of distributed cell assemblies in the hippocampal formation and beyond. Reactivation of cell assemblies during SWRs may have dual role: while SWRs during sleep may participate in memory consolidation, waking SWRs may have a role in planning and short-term memory processes.

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LONG-TERM DEPRESSION: A NEW CONCEPTUAL UNDERSTANDING OF ALZHEIMER'S DISEASE

P. REGAN[1,2], E. HOGG[1], D.J. WHITCOMB[1] and K. CHO[1,2]

[1]School of Clinical Sciences, Faculty of Medicine and Dentistry, University of Bristol, United Kingdom; [2]Centre for Synaptic Plasticity, University of Bristol, United Kingdom

The cognitive deficits that characterise Alzheimer's disease (AD) are critically linked to progressive neurodegeneration and synaptic dysfunction in the brain. Previous research into key risk factors for the onset of AD focused heavily on abnormal protein aggregations and regulation of pathogenes. However, a gene-centric view of AD manifestation has generated limited translation into the understanding of pathophysiology, at least in relation to progressive decline of synaptic function. Evidence from a variety of experimental models demonstrates that aberrantly enhanced downscaling of synaptic transmission, of an LTD-like nature, is at the heart of AD pathology; driving AMPA receptor endocytosis and synapse elimination. Inhibition of LTD signals prevents neurotoxic effects from AD pathogens. Therefore, we highlight the importance of 'Synaptic Biology' in AD pathophysiology and discuss a new conceptual understanding of the roles of functional and synaptic structural plasticity in AD and its links to therapeutic intervention.

Alzheimer's disease (AD) is the leading form of dementia, characterized in its late stages by significant neuronal death linked to plaques of amyloid-β and tangles of tau protein (Ballard et al, 2011). This devastating disease remains poorly understood and without an efficacious treatment strategy (Fig. 1). It is, however, increasingly evident that AD has its origins embedded at the synapse (Selkoe, 2002). Its prodromal phase, mild cognitive impairment (MCI), is strongly correlated with subtle but significant loss of synapses (Scheff et al, 2006; Scheff et al, 2007). This is consistent with the notion that synapses, and their innate plasticity, provide the major physiological basis of such cognitive functions as learning and memory (Silva, 2003).

Most established approaches to understanding AD aetiology have focused heavily on investigating genetic influences to disease incidence. Such approaches have revealed variation in *APP, PS1*, and *APOE* (among other genes) as key risk factors for early and late-onset AD respectively (Goate et al, 1991; Corder et al, 1993; Sherrington et al, 1995), and the very latest GWAS studies have also pinpointed a number of other genetic loci with late-onset AD association (Harold et al, 2009; Lambert et al, 2009; Naj et al, 2011; Hazrati et al, 2012). However, translating these findings into the fully elucidated biochemical pathways that drive AD pathogenesis, particularly with regard to synaptic dysfunction has proved difficult. How we can reconcile this established genetic data with what we know to underscore the cognitive decline of AD remains a fundamental challenge to a unified theory of AD pathogenesis. An emerging conceptual approach, aimed at delineating the synaptic origins of this disease, is driven from a synaptic point-of-

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Corresponding Author: Prof. Kwangwook Cho,
HW-LINE,
Dorothy Hodgkin Building,
Whitson Street,
Bristol, BS1 3NY
Tel.: +44 (0)117 331 3048
e-mail: kei.cho@bristol.ac.uk

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view.

In this regard, a growing body of evidence now supports the hypothesis that AD results from aberrant synaptic elimination that is similar to and/or associated with mechanisms of long-term depression in synaptic transmission (LTD). Concomitantly, long-term potentiation (LTP; an activity-dependent, sustained increase in synaptic strength) is impaired in both *in vivo* and *ex vivo* models of AD (Walsh et al, 2002; Shankar et al, 2008; Jo et al, 2011). These two processes of synaptic plasticity involve an array of molecular signalling pathways that ultimately lead to changes in the efficacy of synaptic transmission (Malenka and Bear, 2004; Collingridge et al, 2010). Here we present a hypothetical model describing how dysregulation of these pathways, specifically those driving LTD, critically contribute to synaptic deficits associated with AD. Central to this model is the aberrant internalization of synaptic AMPA receptors (AMPARs) and the consequent weakening and elimination of synapses.

Through a better understanding of fundamental synaptic biology we are better able to identify these processes that go awry in pathology. This synaptic approach to understanding AD has already provided a number of startling insights, most notably regarding apoptotic signalling pathways and the protein tau. Here, we provide a timely snapshot of the evidence garnered so far, and begin to tie together different strands of AD research within one conceptual framework.

AMYLOID-β AND WEAKENED SYNAPTIC CONNECTIVITY

Pioneering early work demonstrated that loss of cortical volume occurs progressively in AD and correlates with the severity of dementia (Braak and Braak, 1991). The majority of excitatory neuronal connections occur at protrusions from dendrites, known as spines. Herein lie the compartmentalized regions of postsynaptic signalling, and thus, a significant proportion of the computational power of the brain (Newpher and Ehlers, 2009). Of particular importance is the relationship between structure and function of dendritic spines, whereby changes in spine size and stability are critically linked to learning and memory processes (Kasai et al, 2003). Impaired memory performance may therefore be associated with loss of dendritic spines, which has long been documented in AD patients (DeKosky and Scheff, 1990; Terry et al, 1991) as well as in several transgenic mouse models of AD, such as the Tg2576 (Almeida et al, 2005; Spires et al, 2005), PDAPP (Lanz et al, 2003) and APP/PS1 (Moolman et al, 2004) mice. Understanding how these synaptic connections are lost in brains with elevated amyloid-β and/or other pathogens, and the mechanisms responsible, will give some new insights into fundamental components of AD pathology.

The levels of soluble oligomers of amyloid-β, in particular, correlate with severity of dementia and are believed to be directly responsible for the disruption of synaptic plasticity *in vivo* (Lue et al, 1999; Klyubin et al, 2005). A number of groups have now shown that exposing neurons to these oligomers leads to a decrease in spine size, together with marked spine loss (Shrestha et al, 2006; Lacor et al, 2007; Calabrese et al, 2007; Shankar et al, 2007; Wei et al, 2010; Wu et al, 2010). Interestingly, it is also well established that "physiological" activity-dependent LTD-inducing stimuli are able to promote the shrinkage and retraction of dendritic spines (Nagerl et al, 2004; Zhou et al, 2004). These facets of research together provide the first clues that neurotoxic species of amyloid-β, which drive AD pathology, may be acting at the synapse in a similar manner to physiological LTD inducing stimuli.

Of course, the dendritic phenocopies induced by LTD and amyloid-β only provide us with a limited understanding for the loss of neuronal connectivity in AD. A deeper understanding requires insights into the detailed molecular signalling pathways that underlie these effects. For example, more direct evidence for this LTD homology comes from studies suggesting that spine loss induced by amyloid-β in these models is dependent upon NMDA receptors (NMDARs), followed by the rapid and localized activation of the enzyme calcineurin (Shankar et al, 2007; Wu et al, 2010; Wu et al, 2012). NMDARs and calcineurin are key components of LTD signalling that lead to the endocytosis of AMPARs (Morishita et al, 2005), underpinning the downscaling of synaptic transmission (Carroll et al, 1999). Efforts to understand AD-associated synaptic elimination have therefore been further directed at deciphering the

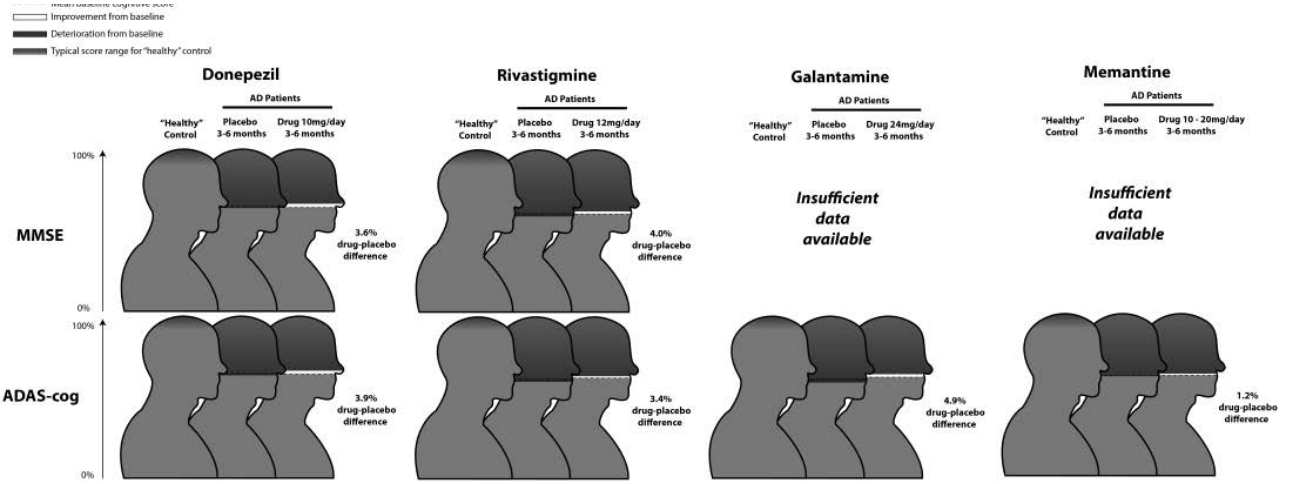

Fig. 1. *Drug efficacy in mild to moderate Alzheimer's disease treatment. Illustrated meta-analyis of 3 - 4 randomized placebo-controlled clinical trials (RCTs) for each drug in patients with mild to moderate AD (as determined by baseline Mini-Mental State Examination (MMSE) score of 14 - 24) with outcome measures calculated 3 - 6 months following beginning of treatment with drug or placebo. The outcome measures were the MMSE (30 point scale) and Alzheimer's Disease Assessment cognitive subscale (ADAS-cog; 70 point scale). Baseline and endpoint means were plotted as a percentage score of their respective cognitive test, e.g. a mean score of 35 in the ADAS-cog test would be plotted as 50%.*

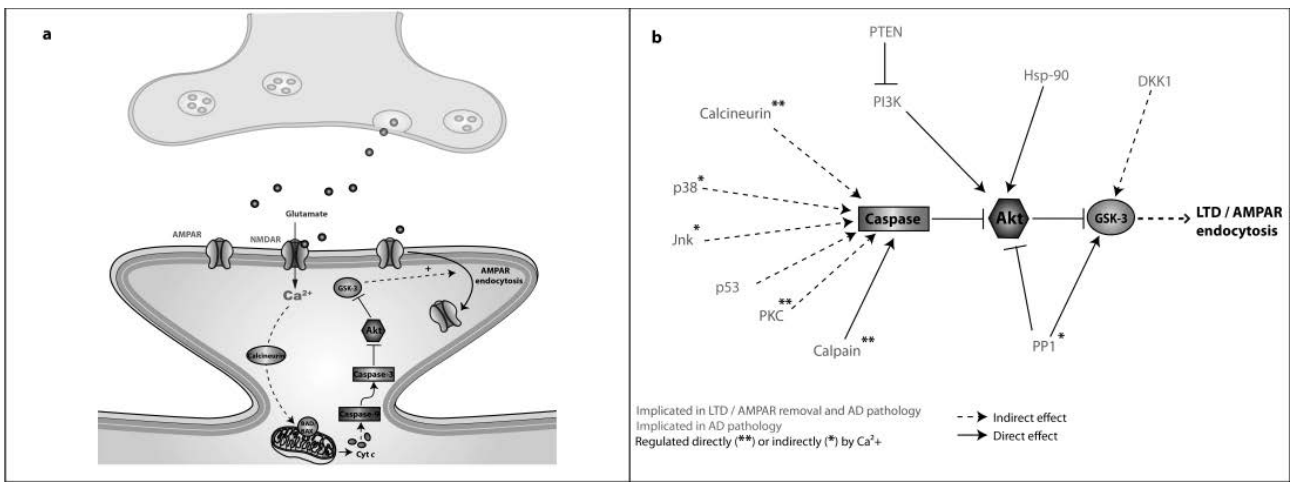

Fig. 2. *The Caspase-3-Akt1-GSK-3b (C-A-G) cascade drives LTD.* ***a)*** *The pro-apoptotic Bcl-2 proteins BAD and BAX are transiently activated by LTD-inducing stimuli, such that BAD translocates to the mitochondria, from which cytochrome c release is stimulated (Li et al, 2010, Jiao and Li, 2011). This leads to the moderate and transient activation of caspase-3 (Li et al, 2010). Caspase-3 causes the proteolysis of Akt1, thereby removing the tonic inhibition of GSK3b (Li et al, 2010). As GSK3b has an essential role in LTD induction (Peineau et al, 2007) , the removal of its tonic inhibition is likely to be a significant contributing event to the induction of LTD and AMPAR internalization.* ***b)*** *A number of other factors associated with LTD and/or AD pathology are known to interact with components of the C-A-G cascade, indicative of a convergence of common signalling pathways. à indicates activating effect, -| indicates inhibitory effect. Abbreviations: DKK1; Dickkopf-related protein 1, Hsp-90; Heat shock protein 90, Jnk; Jun N-terminal kinase, PI3K; Phosphoinositide-3-kinase; PKC; Protein kinase C, PP1; Protein phosphatase 1, PTEN; Phosphatase and tensin homolog.*

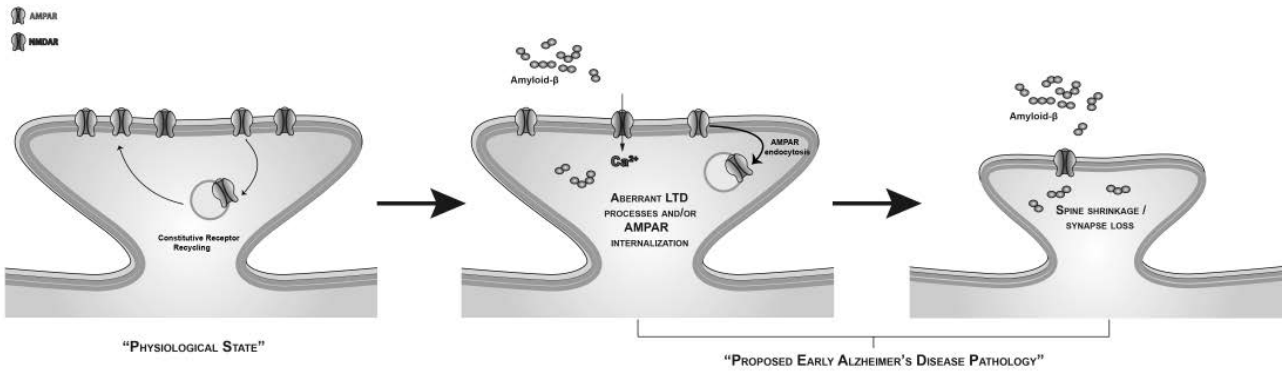

Fig. 3. *Induction of aberrant functional and structural LTD by amyloid-β. Oligomers of amyloid-b act at extra- and intra-neuronal locations to induce aberrant functional and structural LTD. Here, from a physiological state (left) of constitutive receptor recycling from and to the synaptic membrane, amyloid-b induces aberrant LTD processes (centre), leading to eventual spine loss (right).*

regulation and dysregulation of AMPAR endocytosis in more detail.

AMPAR ENDOCYTOSIS: THE DRIVING FORCE

Several lines of evidence have now shown that amyloid-b causes AMPAR endocytosis and can facilitate LTD (Hsieh et al, 2006; Shankar et al, 2008; Gu et al, 2009; Li et al, 2009). Overexpressing amyloid-β results in reduced surface AMPAR immunostaining and a reduced evoked AMPAR-mediated current in organotypic neurons (Hsieh et al, 2006). This is correlated with enhanced phosphorylation of the AMPAR GluA2 subunit at it's serine 880 residue (Malenka and Bear 2004), thereby promoting the internalization of AMPARs via an enhanced GluA2 interaction with the PDZ-containing protein, PICK1 (Kim et al, 2001; Hsieh et al, 2006; Alfonso et al, 2014). Indeed, mimicking phosphorylation at this residue is capable of recapitulating amyloid-β induced spine loss, while a specific AMPAR endocytic-resistant mutant is able to prevent this pathological effect (Hsieh et al, 2006). Together, this supports the idea that amyloid-β aberrantly enhances internalization of AMPARs – a process intrinsic to LTD – and this, in turn, drives synaptic weakening and spine elimination.

Further substantiating the role of amyloid-b in AMPAR endocytosis and LTD, a number of groups have shown a direct facilitatory effect of amyloid-β on post-synaptic electrophysiological responses to sub-threshold LTD inducing stimuli. Soluble amyloid-β from a variety of sources facilitates LTD in acute hippocampal slices, as well as *in vivo* (Kim et al, 2001; Hsieh et al, 2006; Shankar et al, 2008; Li et al, 2009; Cheng et al, 2009). Delving into the molecular mechanics underlying this effect, these studies have also consistently revealed NMDARs as the key mediators of this effect of amyloid-β upon LTD. Specifically, an enhanced NR2B component of NMDAR transmission modulates Ca^{2+} influx and results in the downstream activation of the key LTD enzymes calcineurin and GSK-3β (Hsieh et al, 2006; Li et al, 2009; Li et al, 2011). Of significant note is a recent study demonstrating that inhibition of calcineurin-driven AMPAR endocytosis prevents spine loss induced by oligomeric amyloid-b species (Zhao et al, 2010). This supports the notion that AMPAR endocytosis is a significant driving force for amyloid-b induced synaptic impairment.

LTD AND TAU

Tau has been inextricably linked to the pathology of AD since the initial observations of neurofibrillary tangles containing hyperphosphorylated tau in AD postmortem brains (Goedert et al, 1988;

Braak and Braak, 1991). Of note, the degree of hyperphosphorylated tau strongly correlates with both the degree of cognitive impairment and neuron loss (Braak and Braak, 1991; Gomez-Isla et al, 1997; Santacruz et al, 2005; Nelson et al, 2012). Furthermore, animal and *in vitro* models of Alzheimer's disease have revealed that the presence of tau is critical for the induction of amyloid-β toxicity (Rapoport et al, 2002; Roberson et al, 2007; Shipton et al 2011; Zempel et al, 2013). Yet despite the overt nature of tau pathology in AD, its precise mediatory roles in neuronal and synaptic decline are still unclear.

An axonal segregation is classically attributed to tau (Kosik and Finch, 1987), where it has a well-established role in binding and stabilizing microtubules (Weingarten et al, 1975; Drechsel et al, 1992). Its redistribution to the somatodendritic compartment is associated with the onset of tau hyperphosphorylation and the onset of synaptic loss. However, a number of findings from electron microscopy, subcellular fractionation, immunoprecipitation, and immunocytochemistry studies are revealing that a small percentage of tau resides within the postsynaptic compartment during basal physiological conditions (Hoover et al, 2010; Ittner et al, 2010; Mondragon-Rodriguez et al, 2012; Tai et al, 2012; Liu and Gotz, 2013; Kimura et al, 2014). How such a small amount of tau can come to be located at the synapse remains to be determined, but emerging evidence now suggests that its localization here may have a critical impact upon synaptic function.

In particular, a recent study shows that tau is a fundamental LTD protein (Kimura et al, 2014); LTD mediated by NMDARs (NMDAR-LTD) is unable to be induced in mice lacking endogenous tau, and in rat neurons transfected with tau-shRNA. The involvement of tau in LTD could therefore unravel a further mechanism whereby AD pathogenic contributors converge upon LTD and AMPAR endocytosis. It is not yet clear how tau might be involved in the signalling pathway leading from NMDAR activation to AMPAR endocytosis, although some clues may be taken from a recent study showing that NMDAR activation by NMDA or amyloid-β leads to tau phosphorylation and a reduction in the interaction of a tau/fyn/PSD95/ NMDAR complex (Mondragon-Rodriguez et al, 2012). Furthermore, phosphorylated tau is known to accumulate in post-synaptic compartments of AD brains (Tai et al, 2012) and amyloid-β can directly induce this effect in cell models (Frandemiche et al, 2014). In the context of a recent study documenting the importance of tau phosphorylation events in LTD (Regan et al, 2015), we can now start to see how the hyperphosphorylation and synaptic localization of tau in AD may reflect a facilitation of LTD signalling that ultimately paves the way to a pathological state. In this scenario it will be important to ascertain whether the hyperphosphorylation of tau is simply a by-product of facilitated LTD, or whether it is a direct and critical step in mediating this.

AMYLOID-β, APOPTOTIC PATHWAYS AND LTD

Apoptotic signal cascades have long been implicated in cell death and the pathology of AD (Allen et al, 2001; Cho and Johnson, 2004). A distinct sequence of signalling events are associated with the induction of this intrinsic programmed cell death pathway, beginning with the action of Bcl-2 family members at the mitochondria, the release of cytochrome *c*, the formation of the apoptosome and the subsequent recruitment and activation of various caspases (Li et al, 2010). Amyloid-b has been shown to induce and facilitate apoptosis in AD patients and transgenic mice, through a direct molecular link to the mitochondrial release of cytochrome *c* (Lustbader et al, 2004). Furthermore, AD postmortem brains have shown up-regulated caspase activation (Su et al, 2001;, Zhao et al, 2003), while amyloid-β leads to aberrant activation of caspases and other pro-apoptotic proteins, which is associated with the induction of axonal pruning, loss of synaptic proteins, and cell death (Chong et al, 2006; Nikolaev et al, 2009; Liu et al, 2010; Kudo et al, 2012).

It has recently become apparent that caspases can also participate in non-apoptotic processes, namely synaptic plasticity (Lu et al, 2006; Li and Sheng, 2012). Specifically, caspases have now been shown to have a vital role in NMDAR-LTD and AMPAR internalization via the Caspase-Akt-GSK3b (C-A-G) cascade, as depicted in Fig. 2a. Caspase-3 causes the proteolysis of Akt1, thereby removing the tonic

inhibition of GSK3b (Li et al, 2010). As GSK3b activity has an essential role in LTD induction (Peineau et al, 2007), potentially through its tau kinase activity (Kimura et al, 2014; Regan et al, 2015), the removal of its tonic inhibition is likely to be a significant contributing event to the induction of LTD and AMPAR internalization.

The role of caspases in LTD and proapoptotic signal cascades could pertain to an important mechanism for amyloid-b induced neurotoxicity and/or aberrant synaptic plasticity. A number of recent findings have now begun to shed light on this possibility. Strong evidence indicates that the C-A-G cascade plays a role in the amyloid-b inhibition of LTP, as interrupting the cascade can prevent this effect (Jo et al, 2011). LTP can also be readily induced in hippocampal slices prepared from both caspase-3 and BAX knockout mice, even after exposure to amyloid-b (Jo et al, 2011, Olsen and Sheng, 2012). *In vivo* and *ex vivo* analyses of Tg2576 mice show that local up-regulation of caspase-3 activity in dendritic spines is associated with AMPAR internalization, enhanced LTD and spine loss; all of which are prevented by a caspase-3 inhibitor (D'Amelio et al, 2011). Together, these findings suggest that proapoptotic signalling molecules play a key role in amyloid-b induced synaptic dysfunction, and support the theory that aberrantly regulated LTD mediating molecules are central to this neurotoxic effect. Fig. 2b illustrates the convergence of known LTD and AD-mediating molecules onto the C-A-G pathway, providing further support for this hypothesis.

FATE OF PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL LTD

It is important to consider that synapse elimination and/or transient loss is not an entirely pathological phenomenon; it has an important physiological function during early neurodevelopment, whereby unnecessary synapses are pruned (Bear et al, 1987; Huttenlocher and Dabholkar, 1997; Hua and Smith, 2004). Improper synaptic elimination during these early stages of the immature brain is thought to be responsible for the learning disorder Fragile X syndrome (Irwin et al, 2001; Pfeiffer et al, 2010), and may contribute more broadly to autism spectrum disorders (Penzes et al, 2011), highlighting its fundamental utility as a physiological process. The importance of synaptic elimination here is in stark contrast to the detrimental effects of similar synaptic elimination during late life, which ultimately leads to failure of cognitive functions that characterize dementia (DeKosky and Scheff, 1990).

LTD itself is also vital for encoding physiological phenomena, such as object recognition memory and spatial reversal learning (Cho et al, 2000; Griffiths et al, 2008; Dong et al, 2013; Goh and Manahan-Vaughan, 2013). What is the difference, therefore, between such physiological LTD and the pathological LTD underlying AD pathogenesis (Romberg et al, 2012)? It is likely that the nature of the functional and structural effects on synaptic function exerted by amyloid-b (outlined here and illustrated in Fig. 3) are chiefly responsible. Firstly, the synaptic effects of amyloid-b are persisting. In contrast to physiological LTD, where reductions in synaptic strength occur with physiological relevance in a (relatively) transient nature, elevated amyloid-b is persistent from an early stage of AD onset (Kemppainen et al, 2007). The continuing presence of this LTD-driving entity will lead to functional and structural reductions in synaptic strength that are without physiological relevance and will persist themselves. Eventually, towards the later stages of the disease, prolonged activation of caspases, one of many pathogenes, could trigger a switch from physiological to pathological LTD and thus neurodegeneration.

Further to this, we propose that the crucial differentiating factor between physiological and pathological LTD could lie in the specific mechanisms that bring about synaptic depression. Specifically, an aberrant NMDAR mediated form of LTD is driving AD pathogenesis, rather than 'LTD' *per se.* This is consistent with the view that tonic, rather than phasic, activation of NMDARs is a key excitotoxic factor (Parsons et al, 2007). NMDAR-LTD is considered to be developmentally regulated; it is prominent during early development (Dudek and Bear, 1993) and likely contributes to the synaptic elimination observed during these stages. Following this phase of brain maturation, it appears that NMDAR-LTD is necessarily downregulated, perhaps due to a developmental switch from NR2B- to NR2A-subunit containing NMDARs

(Stocca and Vicini, 1998), and the redistribution of LTD-favouring NR2B-containing NMDARs to extrasynaptic regions (Massey et al, 2004). Thus, only phasic activation of NMDARs may be required in the mature brain for physiological fine-tuning of synaptic connections. A reversion to a more tonic state of NMDAR activation could therefore be causally linked to the onset of aberrant NMDAR-LTD and synapse loss during early AD pathology. Compelling evidence for this change comes from observations of an increased NR2B component of synaptic transmission and a dependence upon NMDARs for synaptic loss following exposure to amyloid-β (Wei et al, 2010; Li et al, 2011). How amyloid-β is itself able to affect NMDAR activation is a matter of intense debate. Direct effects of amyloid-β upon NMDARs (Deshpande et al, 2008), the activation of extrasynaptic NR2B-containing NMDARs following glutamate spillover (Li et al, 2009; Li et al, 2011), and disturbances in local Mg^{2+} concentrations (Glick, 1990; Ozturk and Cillier, 2006), have all been postulated as contributory factors.

Finally, a later consequence of this aberrantly persistent LTD is its irreversibility. Continued spine shrinkage and spine loss induced by amyloid-b will eventually lead to dendrites almost completely lacking in synaptic connections. At this point in adulthood it is unlikely that new synaptic connections are able to grow, and therefore such changes cannot be reversed. This is in contrast to physiological LTD, the synaptic modifications of which can be rapidly reversed by opposing LTP-inducing stimuli (Zhou et al, 2004). Crucially, if irreversible spine loss coincides with the onset of notable cognitive deficits, this has important implications regarding strategies for diagnosing and treating AD effectively.

CONCLUSIONS

Here we have developed a working hypothesis that the fate of synaptic plasticity, determined by the balance between LTP and LTD signalling pathways, forms part of a novel concept of synaptic biology focused towards AD pathological mechanisms. This concept could unveil new levels of understanding of AD-associated synaptic dysfunction and synapse death pathways. Greater understanding of key molecular LTD signalling mechanisms could perhaps ultimately lead to the identification of novel therapeutic strategies for the prevention and/or reversal of AD pathogenesis.

Conflict of interests: The ideas expressed in this review are the personal ideas of the individual authors and do not reflect the views of any agencies or companies with financial incentives.

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NEURONAL ENCODING OF MEMORY IN THE HUMAN AND MOUSE HIPPOCAMPUS

J. CAMPI and R.Q. QUIROGA

Centre for Systems Neuroscience, University of Leicester, Leicester, UK

Despite all the efforts made during the last decades to understand the mechanisms by which we store and retrieve memory, many questions remain unanswered. In this review, we first compare advantages and disadvantages of performing experiments either with humans or animals. Then we describe some results of single cell recording experiments in humans and optogenetic experiments in mice, which unravelled some of the mechanisms of memory formation. Finally, we make a brief comparison between these results and highlight in which way experiments made on humans can complement the ones made on animal models, and vice versa.

In the attempt to understand memory mechanisms, a great number of experiments have been performed in the last decades, both in humans and animal models, but despite impressive progress in the field, much remains unclear [for a review, see (McGaugh, 2000)]. Memory is a fundamental component of our cognitive system. From an evolutionary perspective, fitness-relevant information, such as the location of a nest, or the source of food or water, can be stored and retrieved whenever necessary.

Experiments on animals, as well as on humans, have both advantages and disadvantages. While animal models allow the implementation of invasive techniques and a mechanistic study of the neural basis for memory (e.g. through the use of transgenic lines, ablation or recording techniques), animals are only capable of performing simple tasks and usually need long training periods. In contrast, humans are not only capable of more complex behaviours, but can also give feedback and voluntarily evoke memories. However, despite all the advantages of experiments on humans, studying the human brain has several limitations that are difficult to overcome. On the one hand, while non-invasive techniques, such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG), are broadly used in studies with humans, it is impossible through the use of these techniques to tell apart the firing activity of individual neurons and to isolate the precise neural circuitry involved in different memory functions. Also, they have either low temporal or spatial resolution (Buzsaki et al, 2012). On the other hand, due to obvious ethical reasons, invasive recording techniques cannot be used for studying brain activity at cellular level in humans, which would provide useful information for understanding the mechanisms of memory. A very rare exception is, however, provided by patients with drug-resistant epilepsy which are implanted with intracranial electrodes for clinical reasons. Temporal Lobe Epilepsy is one of the most common cases of intractable epilepsy (Engel et al, 2012) and given the great amount of evidence showing that this area of the brain is involved in declarative memory (Eichenbaum, 2004; Scoville and Milner, 1957; Squire et al, 2004), patients undergoing surgical therapy provide researchers with a unique opportunity of performing experiments to record single cell activity in humans (Rey et al., 2014).

In this review, we describe and compare studies

Key words: memory, hippocampus, medial temporal lobe, concept cells

Corresponding Author: R.Q. Quiroga,
Centre for Systems Neuroscience,
University of Leicester,
9 Salisbury Road
LE1 7QR Leicester UK
Tel.: +44 116 252 5200
e-mail: rqqg1@leicester.ac.uk

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both on humans and animal models aimed at understanding how we store and recall memories.

Concept cells in the human medial temporal lobe

Neurons in the human medial temporal lobe (MTL) were found to respond (i.e. increase their firing rate with respect to baseline) to a specific person, animal or building (Quian Quiroga, Reddy, Kreiman, Koch, and Fried, 2005). For example, a neuron in the left posterior hippocampus of a patient increased its firing rate every time a picture of the actress Jennifer Aniston was presented. For this reason, these neurons were initially known as "Jennifer Aniston neurons" or "concept cells". Regardless of the background of the picture, the position of the actress, the clothes she was wearing, or any other low-level feature, the neuron fired every time the patient was shown a picture of her. Another striking example comes from a neuron found in the right anterior hippocampus of a different patient. This unit responded to pictures of the actress Halle Berry. Furthermore, this same unit also responded to the written name of the actress and to a drawing of her. Interestingly, the neuron fired to pictures of Catwoman, a character interpreted by the actress, where her face was covered with a mask, though the patient knew it was Halle Berry.

On a different patient with electrodes implanted on the left anterior hippocampus, a neuron responded to pictures of the famous television host Oprah Winfrey and to her written and spoken name pronounced by a computer synthetized voice (Quian Quiroga et al, 2009). This was defined as multimodal invariance. Mostly, neurons showing this triple invariance were recorded from the hippocampus and entorhinal cortex. Only a few neurons were found in amygdala, and none in the parahippocampal cortex. Given the fact that multiple sensory modalities converge to the MTL (Lavenex and Amaral, 2000; Squire et al., 2004), it seems reasonable that this area can integrate visual and auditory information. Finally, a unit in the entorhinal cortex responded to pictures of Luke Skywalker, a character from the famous movie Star Wars (Fig. 1). This neuron not only responded to pictures, but also to the written and spoken name of the character. Compared to the ones already described, the novelty of this example resides in the fact that the cell also responded to a picture of Yoda, another related character of the same movie.

Taking all this information together, we can argue that these neurons fire to concepts and not to specific visual or sensory features. The fact that a cell might a) respond with a high degree of invariance to a specific stimulus, b) respond not only to visual stimuli but also to cartoons and to the written and spoken name of the stimulus, and c) respond to two or more associated stimuli, indicates that there is not only a recognition of the stimulus through the integration of information coming from different sensory modalities, but there is also an interpretation of the stimulus, deep enough to associate it to other individuals or objects.

The description offered above resembles the hypothetical "grandmother cells" or "gnostic units" proposed independently by Jerry Lettvin and Jerzy Konorski in the late 60's. This type of neuron is supposed to encode specific concepts, such as one's grandmother. The existence of grandmother cells has been a matter of debate over the last decades (Bowers, 2009; Gross, 2002; Quian Quiroga and Kreiman, 2010; Quian Quiroga, 2013; Quian Quiroga, Fried, and Koch, 2013; Quian Quiroga, Kreiman, Koch, and Fried, 2008). The experiments in epileptic patients described here suggest, however, that these neurons are not grandmother cells. First of all, there cannot be only one single "grandmother cell" per concept (Quian Quiroga et al., 2008; Waydo, Kraskov, Quian Quirogaet al, 2006). If we assume that the number of neurons in the MTL is on the order of 109, it is extremely unlikely that we will find the one and only that responds to a concept.

Clearly, there is not one neuron per concept, but could we have many neurons, each of them responding to one and only one concept? This question is difficult to answer from a methodological point of view. Even if we found that a neuron only responds to one stimulus, we could never be sure that the same cell will not respond to another stimulus that is not being shown. Furthermore, there are many examples of cells that responded to more than one stimulus (Quian Quiroga and Kreiman, 2010; Quian Quiroga et al., 2009; Quian Quiroga, 2012): in a following recording session, the neuron responding to Jennifer Aniston also responded to Lisa Kudrow, another actress from the TV series "Friends"; as mentioned before, the neuron firing to Luke

Skywalker also responded to Yoda; and the neuron responding to the Tower of Pisa also fired to the Eiffel Tower. However, in all the three examples, one could argue that all the stimuli a neuron responded to were related to each other, so the neuron was responding to associated concepts.

Role of concept cells in memory formation

Given the fact that concept cells are located in areas that are known to be involved in memory formation, it has been argued that concept cells constitute the building blocks of memory – i.e. the conceptual representation that allows rapidly forming new associations (Quian Quiroga, 2012). But there is a limit to the amount of information we can store. In everyday life, we are exposed to a massive amount of information that we cannot remember. We tend to remember what is relevant to us, so it is not surprising that concept cells tend to fire to persons, objects or places that were familiar or somehow important to the patients (Viskontas et al, 2009). Concept cells then provide an explicit and abstract representation of a concept, highlighting the most important features of that concept in order to store it in high level memory related areas.

Findings in rodents and non-human primates

As mentioned above, experiments in animals give us the opportunity to dig much deeper into the neural basis of memory. Not only we can use invasive techniques to record specific neural circuits, but we can also control what, when and from where we want to measure.

An important finding in the rodent hippocampus was made by O'Keefe and Dostrovsky in the 1970s (O'Keefe and Dostrovsky, 1971), who found cells in CA1 (one of the subregions of the hippocampus) that increased their firing every time the animal was in a specific location (i.e. the "place field"). These cells share a few characteristics with the concept cells described above (Quian Quiroga, 2012): (1) the so-called place cells, as concept cells, are very selective in their firing, since they only fire when the animal is crossing the place field (Wilson and Mcnaughton, 1993); (2) they are independent of direct visual input: the absence of light or the removal of some visual cues does not change their firing pattern, as long as the location is clear (Quirk et al, 1990; Saveet al, 1998), while concept cells can get active by simply imagining or recalling the stimulus they respond to (G. Kreiman et al, 2000; Gelbard-Sagiv et al, 2008); (3) place cells encode new place fields within minutes, while concept cells can also encode recently acquired concepts (e.g. neurons responded to pictures of the researchers running the experiments, of which the patient had no previous knowledge (Quian Quiroga et al, 2009); (4) place cells' firing patterns can be used to predict the animal's location, just as we can decode what picture a patient is looking at by the activity pattern of single units (Quian Quiroga, Reddy, Koch, and Fried, 2007); (5) place cells have a non-topographic organization (O'Keefe, 1979), which means that neighbouring cells do not necessarily respond to nearby place fields; though this is difficult to prove for concept cells in the human MTL, we have reasons to believe this is the case, since after spike sorting, neurons recorded with the same electrode responded to disparate stimuli (Quian Quiroga et al, 2007; Rey et al, 2014)

Whereas for rodents, it is of vital importance to have a spatial representation of the environment, for humans, being a largely developed social species, it is critical to be able to rapidly recognize each other (Bentin et al, 1996; Navajas et al, 2013). Interestingly, in macaques—another social species—it has been reported the existence of well delimitated areas that respond exclusively to faces (Freiwald and Tsao, 2010; Moeller et al, 2008). These cells are grouped in face patches: regions of cells distributed all over the Infero-temporal Cortex (IT). As concept cells in humans, but to a lower extent, some of them respond to faces in an invariant manner and are individual-specific. It could be argued that, up to now, face cells and place cells are the closest to an animal counterpart of the concept cells in humans.

Neural activity controlled by optogenetics

In the last decade, one of the most exciting developments in Neuroscience has been the introduction of Optogenetics (Lima and Miesenböck, 2005). This technique uses light to excite or inhibit neuronal activity (Deisseroth, 2011). It is based on a series of proteins called opsines, which are gated cation channels sensitive to light. These opsins can either produce optogenetic excitation (Channelrhodopsin)

or inhibition (Halorhodopsin), depending on the ion they are specific for. These proteins are usually activated by light of different wavelengths, which allows their simultaneous use with no interference. Currently, Channelrhodopsine-2 (ChR2) (Nagel et al, 2003) is the most widely used opsine for optogenetics (Cardin et al., 2009; Kohara et al., 2013; Kohl et al., 2011; Sato et al, 2013). When ChR2 is exposed to light of the appropriate wavelength, part of the protein suffers a conformational change which in the end makes the cation channel open, allowing mainly Na+ ions influx (Nagel et al., 2003). Normally, to stimulate with light in vivo, animals are implanted with an optic fibre that delivers light, although for stimulating superficial cells, light can be delivered through the thinned skull (Cardin et al., 2010).

Studying memory mechanisms in animal models

Using a clever design, Liu and colleagues managed to create a false memory in mice (Liu et al., 2012). In order to do this, they genetically marked neurons that were active during a specific behaviour. Following the hypothesis that a sparse population of neurons encode a memory (Hübener and Bonhoeffer, 2010), they asked what would happen if they could re-activate the network encoding a memory (recalling) when it should not be active. However, they first had to deal with a technical problem: if the population of cells encoding a memory is sparse, how would it be possible to only activate those cells that are not necessarily close to each other and not others that are not related to the memory that wants to be recalled?

The solution was to condition the expression of ChR2 to the activation of the c-fos promoter (Fig. 2a and b), a gene normally expressed, among other situations, when a neuron is active (Dragunow and Faull, 1989; Sheng et al, 1993). This gene was proved to be essential for memory formation (He et al, 2001; Tischmeyer and Grimm, 1999). Given that the expression of a gene is subject to the activation of its promoter and that c-fos promoter is activated when the neuron is active, then ChR2 will only be expressed in neurons during activity periods (for a complete description of the system, see (Liu et al., 2012; Mayford, 2013; Reijmers et al, 2007).

A key feature of this design is that it can be turned on and off reversibly. The drug Doxycycline (Dox) inhibits transcription (and thus expression) of the genes downstream the c-fos promoter (ChR2 in this case). When expression wants to be avoided, the animals are fed with Dox, but as soon as the animal goes back to a normal diet (without Dox) the gene is expressed normally.

Taking all this together, only neurons that are active while the animal is not fed with Dox express ChR2.

Creation of a false memory

Knowing that the Dentate Gyrus plays an important role in contextual memory (McHugh et al, 2007), Liu and colleagues injected a virus containing the ChR2 sequence targeting this area of the hippocampus (Liu et al., 2012) along with the implantation of an optic fibre (Fig. 2a). Mice were first exposed to a context (Context A) while receiving food with Dox (Fig. 2c). After this, they were exposed to a second context (Context B) where they received foot shocks, all this while on a Dox free diet, allowing the ChR2 gene to be expressed. Then, while back on Dox, the animals were tested in Context A with light-on and light-off periods. As expected, the freezing levels in Context A were much higher during the light-on epochs, due to the re-activation of the cells encoding the fear memory (Fig. 2d). The authors of this work concluded that a small subpopulation of DG cells is recruited for forming a contextual memory engram, and that the activation of this subpopulation is sufficient to re-activate that memory and also to associate it with a new fear memory.

After these results, a second question arose: Can fear behaviour be induced by the re-activation of an engram encoding a context, while fear conditioning happens in a different context (Ramirez et al, 2013)? For this, mice were exposed to a context (Context A) while off Dox, to allow labelling of the subpopulation of cells encoding that context with ChR2. Next, animals were fear conditioned in a new context (Context B) while cells labelled where being activated by light. Then, the animals were tested either in Context A or in a novel context (Context C). When re-exposed to Context A, mice froze at significant levels, while exposure to Context C had no effect.

Interestingly, the same result was found when

animals were exposed first to Context A (off Dox), then to Context C (on Dox) and finally to Context B, where they were fear conditioned. Memory engrams for contexts A and C are formed before the fear memory. However, one is labelled (and thus can be reactivated with light) and the other one is not. So, when the animals are being fear conditioned, only the subpopulation encoding Context A is being activated. This is why the mice associated Context A, and not Context C, with the aversive stimulus, thus indicating that subpopulations of cells encoding a context memory might be selective for only one context.

These experiments showed that it is possible to generate an association between two different events simply by simultaneously activating the two subpopulations of neurons encoding the memory for each event.

Retrieving a memory is equal to making that memory labile (Nader et al, 2000). In some way, when we evoke a memory and re-activate the neurons encoding that memory, we are making the memory susceptible, and finally that memory can be changed, erased, or reconsolidated (Inda et al, 2011; Sara, 2000). In this case, as we mentioned before, we cannot get any feedback from the animals but the fact that the animals freeze when exposed to a context that was in principle not related to the context where they received the foot-shocks, indicate the possibility that re-activation of the cells encoding Context A while they are in Context B may make them "believe" that they are actually in Context A. The re-activation of these cells makes the memory they encode labile (Nader et al, 2000; Roediger et al, 2007), which allows a "modification" of that memory which now associates it with a high-valence memory: A fear memory.

What can we learn from both experimental models?

As described above, there are neurons in the human MTL that respond to specific stimuli in an invariant manner. It is believed that a group of neurons encode a memory of a concept. Similarly, in the DG of the mouse hippocampus, groups of cells respond selectively to a context, encoding a memory for that context. The experiments on animals we

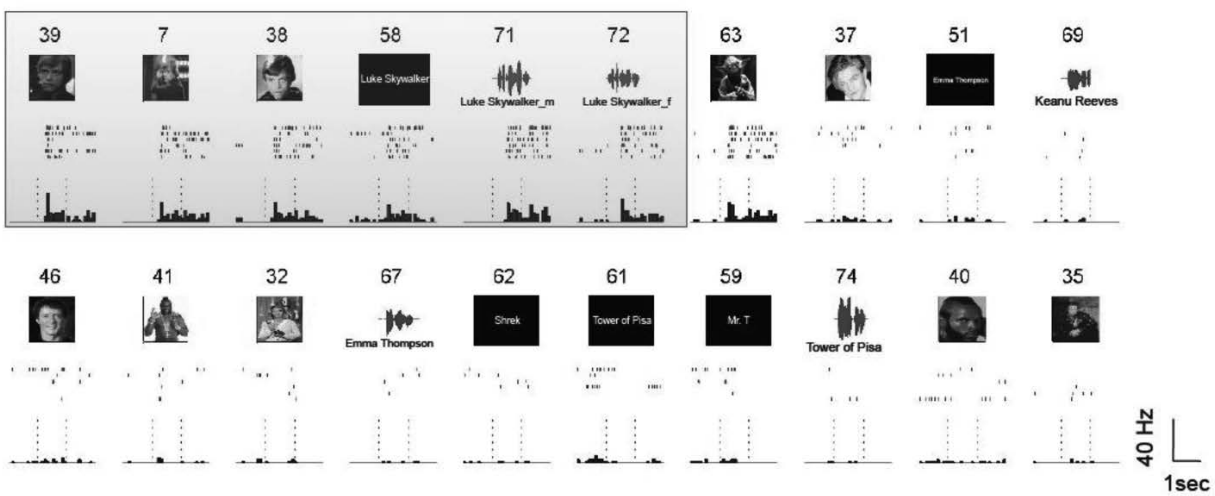

Fig. 1. *Example of a unit recorded from the entorhinal cortex that shows a spiking response to pictures of a Star Wars character, Luke Skywalker. The unit also responded to his written name (stimulus 58) and to his name pronounced by a computer synthetized voice (male voice: stimulus 71; female voice: stimulus 72). There was also a significant response to Yoda (stimulus 63), another character of the movie. For each stimulus, raster plots and post-stimulus time histograms are shown. Dashed lines indicate the onset of the stimulus.*

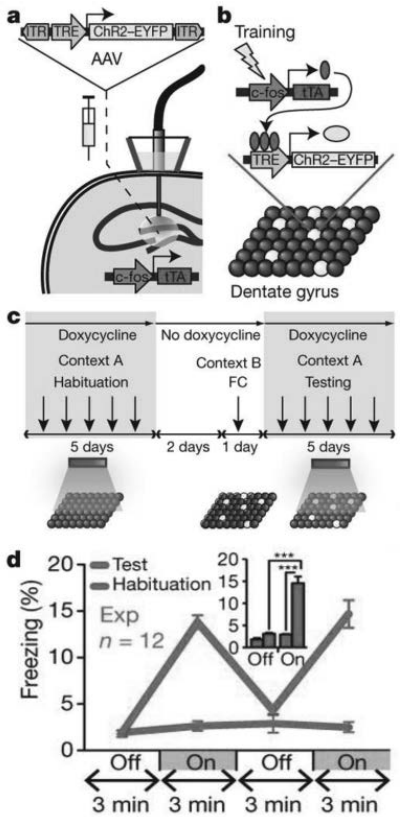

Fig. 2. *a) Injection of AAV9-TRE-ChR2-EYFP and implantation of the optical fibre in the DG of c-fos-tTA mice. b) During training (Off Dox), activity of certain neurons (in yellow) induces the expression of tTA, which through the interaction with TRE, drives CHR2-EYFP expression. c) Mice were habituated during 5 days in Context A with light stimulation and with a Dox-containing diet. Then, after consuming a Dox-free diet for two days, they were fear conditioned in Context B. During the next 5 days, while back on Dox, mice were tested in Context A with light stimulation. d) Mice show increased levels of freezing only during light-on periods after being fear conditioned (red curve), but not during habituation (blue curve). Adapted with permission from (Liu et al., 2012).*

described here show an interesting approach for stimulating a group of neurons that are functionally connected (i.e., encoding the same memory). As already mentioned, experiments with animals allow a series of manipulations that cannot be implemented in humans. Given the great similarities among the mechanisms of memory formation in animals and in humans, and that there is currently no methodology available that could allow these kinds of experiments in humans, the development of an animal model to study concept cells would help to understand how concepts are encoded and how this mechanism serves to declarative memory formation. An animal model would indeed allow the field to further advance in the study of memory formation through abstract concept representation. It would provide data from experiments that could involve lesions, the use of drugs and cutting-edge technology that would lead us to a better understanding of how we represent abstract concepts in our brains. Studying this matter in animals would also allow the use of calcium imaging and optogenetics, which let us study the behaviour of large populations of neurons engaged in a task and manipulate their activity in order to understand the behavioural consequences.

The combination of animal research with state-of-the-art technology would surely help answering many open questions, such as how many neurons encode a concept, how many concepts are encoded by the same neurons, how the engrams are formed, what would happen if a neuron encoding a concept was artificially activated without any cue evoking the memory of the concept, and vice versa (i.e., if a neuron encoding a concept was selectively inhibited when it should be active), etc. With the precedents set by the experiments on mice described above, we think optogenetics and two-photon imaging are very valuable techniques for understanding memory formation. We are confident that in the same way as an engram encoding a context or a fear memory could be artificially re-activated without any natural cue, it should be possible to manipulate the activity of an engram encoding a concept.

CONCLUSION

In this work we have first described the advantages and disadvantages of experimentation on animals and humans, and secondly we reviewed the line of research undertaken with both species in order to understand the cellular mechanisms of memory formation. The experiments on humans reviewed here provide an insight on how we store our memories, through remembering representations of concepts and forming associations. The

experiments on animals show how it is possible to evoke a memory through the activation of solely the cells that are involved in the engram encoding it and how the recalling of the memory can make it labile, susceptible to changes and new associations.

We argue that a combination of studies of the human brain at a single cell level with an animal model of concept representation in the hippocampus will help unravelling the remaining mysteries of memory formation.

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PARSING THE CELLULAR SYNTAX OF MEMORY

KIRIANA K. COWANSAGE

Department of Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, USA

The neural encoding of new episodic memories, or consciously recallable and autobiographical associations, has been studied at length from the vantage point of hippocampal mechanisms and is conclusively established to require structures of the medial temporal lobe. Over time, this information is thought to undergo a process of exchange within anatomically distributed networks of neocortical connections, which theoretically bind individual experiences to more general frameworks of knowledge. Biological models of long-term memory have traditionally cast the cortico-hippocampal relationship as a complementary dynamic, involving two distinct systems, each tasked with a temporally separate phase of the consolidation process. Counter to this idea, however, a growing body of experimental work suggests that both cortical and subcortical systems are concurrently involved in memory across time, and that the machinery of memory systems is less anatomically modular than once thought. This review begins with a brief overview of cellular and molecular mechanisms implicated in associative learning, juxtaposing these ideas with more abstract principles from studies of systems memory consolidation. The discussion focuses on the potential for new technologies to unify conclusions from different levels of analysis, highlighting results from circuit-oriented genetic and optogenetic investigations. These findings draw attention to the need for future work aimed at dissecting small-scale circuit properties that shape large-scale network interactions.

Every day, from infancy until death, we are faced with tasks that require us to recognize and distinguish relevant cues embedded within noisy streams of sensory information. These behaviors are not unbiased reactions to isolated events; rather, they are strongly shaped by our existing internal representations of prior experience (Mishkin 1982; Goldman-Rakic 1988; Froemke et al 2007). The progressive modification or *plasticity* of neural circuits stimulated by external cues is thought to provide a critical mechanism for tuning connections that mediate perceptions and memories involved in behavior. These circuit refinements are not only essential for maintaining a narrative of individual history, but are also necessary for predicting events to come (Morris 2006; Wang et al 2010; Takeuchi et al 2014).

The neurobiological mechanisms implicated in the encoding of *episodic* memories, the subtype of declarative memory encompassing consciously accessible cue-induced associations, have been extensively characterized in both human cases and animal models (Squire 1986; Nadel 1992; Eichenbaum 2000; Moscovitch et al 2005; Smith et al 2006; Shrager et al 2007; Katche et al 2013a). While the formation of new episodic associations has been definitively established to require the hippocampus (HPC), abundant evidence also indicates that over time, neocortical circuits become preferentially engaged in memory storage and progressively less

Key words: associative memory, neocortex, hippocampus, retrosplenial cortex, fear conditioning, contextual learning, sensory representation

Corresponding Author:
Kiriana Cowansage,
Department of Molecular and Cellular Neuroscience
The Scripps Research Institute,
10550 N. Torrey Pines Rd, DNC 202,
La Jolla, CA 92037, USA
e-mail: kiriana@scripps.edu

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dependent on hippocampal structures (Squire 1986; Teng et al 1999; Bayley et al 2003).

Several discrete neocortical divisions, including multiple sub-regions of the prefrontal cortex (PFC) and retrosplenial cortex (RSC), have been consistently implicated in the long-term process of systems memory consolidation, which theoretically integrates or "schematizes" individual traces within a stable framework of knowledge. Although traditionally viewed as a slow time- intensive process, recent experimental studies suggest that cortical circuits are meaningfully activated by learning related activity, and that both areas retain concurrent involvement in memory storage over time (Maviel et al Cowansage et al 2014; Tanaka et al 2014; Goshen et al 2011). These recent insights, in conjunction with more temporally and spatially precise genetic, optogenetic and imaging tools, lay the foundation for more detailed mechanistic analyses of complex anatomical systems.

The goal of this review is to first, provide a brief survey of what is known about cellular and anatomical mechanisms implicated in associative memory, second, to discuss recent strides toward unifying the relationship between circuits and anatomy, and third to highlight certain unanswered questions that will need to be more fully addressed in order to understand how circuits, structures, and systems integrate to support the lifelong persistence of long-term associative memory.

CELLULAR MECHANISMS OF CUE ASSOCIATIONS

The dominant theoretical model for cellular plasticity in hippocampal networks is based on the Hebbian principle that co-activation of two sensory inputs to a common target produces network activity sufficient to strengthen associated connections (He bb 1949; Kandel et al 1968; Kohonen 1972; Kelso et al 1986; Caporale et al 2008). For example, in classic Pavlovian learning, a mouse given a painful shock, while exploring a neutral spatial environment will rapidly learn to associate the aversive cue with the specific space (Martin et al 2000; Maren 2001; Kim et al 2013; Kandel et al, 2004). At the level of synaptic connections, memory encoding is believed to begin when external stimulation drives a spike in voltage, triggering calcium entry via N-methyl-D-aspartate (NMDA) receptors and voltage-gated Ca2+ channels. The resulting activation of kinase signaling cascades, in turn, gate the expression of plasticity-related genes (Martin et al 2000; Silva et al 200; Johansen et al 2011; Redondo et al 2011; Santini et al 2014). The expression of new proteins then leads to shifts in receptor density, synaptic structure, and intrinsic excitability, which are necessary for behavior and may shift the odds of future learning. For example, studies have found that cells overexpressing the active transcription factor, CREB, are more likely to participate in memory (Han et al 2007; Rogerson et al 2014; Yiu et al 2014). Thus, even specific associative events are inevitably shaped by past experiences and biases of the underlying circuit.

SYSTEMS CONSOLIDATION AND THE ANATOMICAL DEBATE

While the physiological and cellular events required for associative learning have been extensively characterized in hippocampal synapses, the concrete molecular basis for long-term memory storage is far less mechanistically defined. Data from behavioral and neurophysiological experiments suggest, however, that this process of *systems memory consolidation (SMC)* depends on the gradual transfer of information from hippocampal to cortical circuits, which are thought to ultimately organize, update, and maintain the content of past association.

Both human cases and animal models have established the importance of the HPC and surrounding medial temporal lobe (MTL) cortices in episodic memory (Squire 1986; Alvarez et al 1994). These areas have unique structural and physiological properties that are well suited to accommodate both pattern completion from partial information and pattern separation from overlapping features (Leutgeb et al 2005a; Leutgeb et al 2005b; Leutgeb et al 2007; McHugh et al 2007).

By all accounts, the most famous longitudinally documented human case of severe episodic memory impairment was the epileptic patient, H.M., whose treatment by surgical resection of the retrograde amnesia, preventing his consolidation of new

associations (Scoville et al 1957). Interestingly, studies of H.M. and related cases revealed that, relative to the time of damage, distant episodic memories remained intact, while recently acquired memories were lost (Teng and Squire 1999). These, along with many subsequent reports, supported the notion that episodic information undergoes a gradual transfer into cortical networks, where it is stored persistently and eventually supplants the hippocampus (McClelland et al 1995; Bayley et al 2003; Maviel et al 2004; Wiltgen et al 2004; Restivo et al 2009; Lopez et al 2012; Tayler et al 2013). Support for the idea of a time-dependent process for SMC was bolstered by primate and rodent studies showing that loss of HPC-function caused memory impairments at recent but not remote post-training time-points (Zola-Morgan and Squire 1990; Kim & Fanselow 1992; Anagnostaras et al 1999). More recently, studies in rodent models have shown that expression of immediate early genes (IEG), robust molecular markers of cellular activity, increase in the HPC after *recent* memory recall (Wiltgen et al 2010) and in the cortex after *remote* recall (Frankland et al 2004; Frankland and Bontempi 2005; Frankland et al 2006; Ding et al 2008).

From a physiological perspective, cortical consolidation is likely to involve a process of "replay," or rapid sequential recapitulations of past stimulus-driven activity. This coherent retrieval- or internally-driven mirroring of past experience has been observed in both hippocampal and cortical networks and occurs during both sleeping and waking states, suggesting that this activity may provide a critical driving mechanism for reinforcing and maintaining the efficacy of relevant connections over time (Hoffman and McNaughton 2002; Kali and Dayan 2004; Karlsson and Frank 2009; Dragoi and Tonegawa 2011; Bermudez Contreras et al 2013; Suh et al 2013). In addition, resulting patterns of coincidental overlap between multiple simultaneously reactivated circuits, could theoretically provide a Hebbian-like mechanism for strengthening inter-association linkages between memories formed at different times. In this way, structural convergences among otherwise unrelated traces could permit new connections to form between existing representations, even long after learning occurs.

Two major cortical areas have been particularly implicated in the cellular storage of episodic memory: 1) anterior cingulate cortex (ACC), as well as, more generally, the PFC, and 2) RSC (Aggleton 2010; Hales and Brewer, 2010; Descalzi et al 2012; Smith et al 2012; Katche et al 2013b; Bucci and Robinson 2014). These regions are critically involved in spatial, sensory and emotional processing, and are connected to numerous sensory and subcortical areas by dense reciprocal connections. (Frankland and Bontempi 2005; Ding et al 2008; Keene and Bucci 2008; Robinson et al 2011).

GENETIC TRACKING AND MANIPULATION OF ACTIVE CIRCUITS

Despite converging lines of evidence supporting the importance of both the HPC and cortex in memory, cortical memory remains poorly understood at the circuit level, likely because of the paucity of available tools for selectively targeting relevant cell assemblies. To begin to begin to investigate the dynamics of cortical plasticity, several novel molecular, genetic and optogenetic tools have been implemented to directly visualize and functionally manipulate behaviorally activated circuits. These technologies make use of IEG expression profiles that are known to correlate with learning-related activity (Milanovic et al 1998; Radulovic et al 1998) and can be used to genetically report natural patterns of neural excitation (Barth et al 2004). Moreover, the cellular expression of IEG mRNA after exposure to a known environment has been observed to selectively increase within cells previously activated by the same context (Guzowski et al 1999; Guzowski et al 2005). Thus, beyond generally correlating with activity, the cellular localization of IEG mRNA expression within stable ensembles can be used to isolate and characterize neural ensemble linked to individual epochs of experience.

More recently, *c-fos* IEG promoter activity has been incorporated into a mouse transgenic system, developed in the laboratory of Mark Mayford, to enable to activity-based "tagging" of active neurons with any target gene of interest. Since this system can be fully suppressed by the drug, doxycycline (dox), the genetic labeling can also be limited

to a specific temporal window, enabling cellular activity triggered by two distinct epochs of activity (i.e. learning versus recall) to be visualized in the same subject (Reijmers et al 2007). Several other inducible IEG-regulated genetic systems have since been validated for use in activity mapping studies (Vousden et al 2014; Guenthner et al 2013; Denny et al 2014). These IEG-based activity tagging systems permit unprecedented brain-wide access to specific ensembles activated at any anatomical site of interest during a given episode of activity. As a result, these tools provide an ideal strategy for investigating the direct relevance of specific anatomical circuits for behavior. This idea has now been tested in several studies, which used *c-fos*-driven optogenetic and pharmacogenetic tagging systems to artificially manipulate and reactivate tagged cell populations that had previously been active during learning (Garner et al 2012; Liu et al 2012; Ramirez et al 2013; Cowansage et al 2014). To test the direct involvement of the dentate gyrus (DG) of the HPC in memory formation, Liu et al (2012) expressed the blue light-sensitive ion channel, Channelrhodopsin (ChR), in *c-fos*-expressing neurons activated by context fear learning. Stimulation of these cells was sufficient to induce contextual memory recall, providing the first proof-of-principle evidence that learned behavior can emerge from activity within a stable and explicitly targetable cell population. To investigate whether learning also activates an early, behaviorally-relevant network in specific neocortical circuits, we, in the Mayford lab, used a *c-fos*-regulated transgenic mouse to express the ChR variant, ChEF, during contextual fear conditioning. Optical stimulation of tagged neurons in RSC revealed that as in the DG, direct optical stimulation of tagged RSC neurons could trigger context-specific behavior. Interestingly, artificially-triggered memory recall could be triggered even when the HPC was pharmacologically inactivated, suggesting that a functional representation memory sufficient to drive behavior was established in RSC within the first day after training (Cowansage et al. 2014). This finding is consistent with previous work, showing that cortical circuits are explicitly activated, and in some circumstances required, for new associative memory formation (Corcoran et al 2011; Einarsson and Nader 2012; Tayler et al 2013). Moreover, a complementary finding from the laboratory of Brian Wiltgen showed that optogenetic *silencing* of learning-tagged HPC neurons during tests of memory retrieval not only impaired behavior recall, but also blocked cellular reactivation of the learning-activated RSC neurons, suggesting that early synaptic potentiation of a cortico-hippocampal trace may coincide with learning, establishing a critical circuit for future reinforcement during replay or retrieval (Tayler et al 2013; Tanaka et al 2014)). These findings are also consistent with an earlier report by Lesburgueres et al (2011) that neocortical ensembles are rapidly recruited during learning and become necessary for behavior at later time-points.

CORTICAL SCHEMAS

While rapid, coincidence-dependent Hebbian plasticity has been clearly implicated in the cellular basis for memories of individual "vignettes," this theory is more challenging to apply to understanding how large multi-memory associations give rise to a unified and consciously accessible "memoire" of all life history. These so-called schemas are thought to develop over time, and represent complex aggregations of many types of acquired knowledge, interconnected as a stable and generalizable network (Eichenbaum 2000; Preston et al 2013). Interestingly, behavioral results from the laboratory of Richard Morris suggest that once established, cortical schemas are rapidly incorporate new episodic elements without the typical need for hippocampal involvement. Thus, rats trained in the principles of a complex HPC-dependent task could more rapidly acquire new associations linked to the same task, even when the HPC was offline (Tse et al 2007), and showed increased levels of neocortical IEG induction (Tse et al 2011; Wang et al 2012). Schemas may therefore be defined as large multi- association networks, which linking temporally separate events together on the basis of conceptual relatedness. Such a system ostensibly permits animals to make predictions and inferences based on more general past experiences, when explicitly recognized cues are unavailable.

CONCLUSION: UNDERSTANDING THE PARTS IS NOT ENOUGH

The concept of associative memory has traditionally been studied from two frames of reference: A) static associations formed when unrelated cues overlap in time—for example, the memory of a smell in a particular place; and B) dynamic associations formed when existing memories are conceptually linked, despite their non- overlap in time—for example, an internally generated memory relating multiple separate places to a commonly associated smell. While the former example is typically viewed as the purview of hippocampal structures, the latter case is thought to depend on neocortical connectivity.

Expanding on this dichotomy, *new* associations are, by definition, dependent on the timing of external events, whereas the variables that shape *existing* association are largely dependent on internal factors that are generally outside the realm of experimental control. Once established, memories are free to strengthen, weaken, change or persist in both the presence and absence of external cues, making these networks comparatively difficult to track. As a result, the same experimental techniques that have been used for decades to control, manipulate, and observe the precise cellular events of memory encoding do not have the same power for addressing questions about memory consolidation.

Given the technical complexity of tracking and studying post-encoding events in specific memory-related ensembles, and given the large body of evidence for MTL involvement in spatial processing and associative learning, memory research has been strongly oriented toward a view of memory centered on the supremacy of hippocampal structures. Only in the last decade, and with the acceleration of technologies aimed at spatially and temporally precise circuit manipulations, have the contributions of cortical structures to associative plasticity mechanisms gained attention in studies of cellular memory mechanisms. These different technical strategies used to study "recent" hippocampal encoding and "remote" cortical consolidation, has created a fuzzy conceptual gap at the interface between these two systems.

Nevertheless, the validation of new technologies that permit long-term multi-system tracking and manipulation of specific circuits has led to renewed efforts to unify models of cortico-hippocampal dynamics (Rosenbaum et al., 2001; McCormick et al., 2010). Intriguingly, as more studies have begun to investigate cortical systems in direct parallel with hippocampal plasticity, the mechanistic involvement of these two anatomical systems has appeared to increasingly converge. Thus, inasmuch as the HPC may retain an extended role in long-term memory maintenance (Debiec et al 2002; Winocur et al 2010; Goshen et al 2011; Lopez et al 2012; Schlesiger et al 2013), the neocortex may carry unexpectedly detailed representations of memory, regardless of post-training timepoint (Bucci et al 2007; Corcoran et al 2007; Keene et al 2008c; Keene et al 2008b; Wang and Morris 2010; Corcoran et al. 2011; Tse et al 2011). These rapidly formed cortico-hippocampal connections therefore seem likely to provide a rapid mechanism for linking, and possibly comparing, new input with previously stored content.

Future brain-wide studies that map discrete epochs of learning- and memory-related activity over long intervals of time may provide greater insights into the true mechanistic distinctions between cortical and hippocampal systems. Other critical questions for future investigations include: 1) How are patterns of cellular activity related to stored information within specific cells? 2) What is the smallest module of useable information, and how are these units organized within circuit and synapse populations? 3) Finally, how are complete associations rendered in circuits as unitary cellular representations of experience? Studies aimed at addressing these questions will ultimately be needed to fully disambiguate the tangible circuit mechanisms that permit "now" experiences to become persistently incorporated into "then" memories.

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POST-TRANSLATIONAL MODIFICATION OF NERVE GROWTH FACTOR BY PEROXYNITRITE: PATHOGENIC SIGNIFICANCE IN NEURODEGENERATIVE DISEASES

M. RICHTER[1], V. VARELA[1], E. TRIAS[1,2] and L. BARBEITO[1]

[1]Institut Pasteur de Montevideo, Uruguay; [2]Insitituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

Previous studies have demonstrated a role for Nerve Growth Factor (NGF) species stimulating p75–neurotrophin receptor (p75NTR) mediated apoptotic pathways in several types of neural cells. The neuroinflammatory response in several neurodegenerative diseases is associated with activation of glial cells, which express increased levels of NGF species that potentially may trigger p75NTR-dependent apoptosis in target cells. At the same time, activated astrocytes and microglia also produce oxidants and nitric oxide-derived species that could react with NGF causing post-translational molecular modifications. Here, we review the rationale supporting NGF nitration by peroxynitrite and the mechanisms explaining its pathogenic role in neurodegenerative diseases. The occurrence of nitrated NGF species in Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's disease (AD) offers an exciting new mechanism by which the canonic neurotrophin signaling could be subverted under inflammatory conditions.

The emerging concept of NGF was developed in 1949 by Rita Levi-Montalcini, as a soluble factor released by tumor tissues that strongly stimulated neurite growth (Levi-Montalcini, 1952; Levi-Montalcini and Hamburger, 1951). Subsequent studies allowed the identification and purification of NGF as a protein, taking advantage of its high concentration in adult male mouse sub-maxillary glands (Levi-Montalcini, 1987). Nowadays, NGF is the best characterized of all neurotrophins. Although originally established as an essential trophic factor for the development of central and peripheral nervous system, NGF also mediates trophic and regenerative signals in the adult nervous system, including an emerging role in pain transduction mechanisms associated to tissue inflammation (McMahon, 1996). NGF has also emerged as a key factor mediating the complex interplay between the nervous and immune systems, being referred as a "neurokine" because of the ability to be produced in inflammatory and modulated immune cells (Levi-Montalcini et al, 1996). Such neuroimmune role of NGF may play a critical role in several pathologies including neurodegenerative diseases, acute brain or spinal cord injury, chronic pain syndromes, and autoimmune diseases.

As observed for other neurotrophins, NGF signaling is complex and regulated at several cellular levels. NGF exerts its actions through two non-homologous transmembrane receptors, the high affinity tyrosine kinase receptor TrkA and the p75NTR (Ebendal, 1992; Meakin and Shooter,

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Corresponding Author:
Dr. Luis Barbeito,
Institut Pasteur de Montevideo,
Mataojo 2020, Montevideo 11400, Uruguay
e-mail: barbeito2006@gmail.com

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1992). p75NTR is a member of the tumor necrosis factor receptor superfamily and can act as a death receptor signaling apoptosis in several neuronal populations (Barrett, 2000; Nykjaer et al, 2005). The mechanism of p75NTR-induced apoptosis can involve downstream production of nitric oxide and peroxynitrite formation (Pehar et al, 2004). In addition, NGF is synthesized as a precursor protein (proNGF) that is then processed extracellularly or by specific proteases in the trans-Golgi compartment, to form mature NGF (Greene et al, 1968; Seidah et al, 1996). Since proNGF and mature NGF interact with different receptor complexes displaying specific biological activities, the site and efficiency of proNGF processing may greatly influence the resulting physiological effects. Finally, both proNGF and NGF might experience post-translational modification by oxidants and nitrating molecules that are produced in inflammatory conditions (Harrington et al, 2004; Pehar et al, 2006b).

While previous reports have been published on the pathological role of the proNGF/NGF signaling in neurodegenerative conditions (Belrose et al, 2014; Capsoni et al, 2011; Fahnestock et al, 2001; Tiveron et al, 2013), the present review summarizes recent evidence on post-translational modifications of NGF that could lead to a gain of function of NGF in neurodegenerative diseases such as ALS and AD.

NGF AS A TARGET OF PEROXYNITRITE DURING INFLAMMATION

Peroxynitrite is a potent nitrating compound that is formed by the spontaneous reaction of nitric oxide and superoxide (Beckman and Koppenol, 1996; Radi et al, 2001). It is typically produced in inflammatory environments where microglia (or macrophages), astrocytes, endothelial cells or infiltrating immune cells have the ability to produce excessive levels of oxygen radicals and nitric oxide (Fig. 1). In turn, peroxynitrite behaves as a potent oxidant and nitrating agent capable of reacting and modifying a wide variety of biomolecules including proteins, lipids, and DNA (Beckman, 1996; Radi, 2004). Tyrosine nitration (i.e., addition of a nitro group to the aromatic ring) is considered as a footprint of oxidative damage mediated by peroxynitrite (Daiber et al, 2013; Viera et al, 2013). Although alternative pathways involving peroxidases using nitrite could also nitrate tyrosine residues, peroxynitrite has been shown to be the predominant nitrating agent in neurodegeneration (Estevez et al, 1998; Pacher et al, 2007). Several proteins such as Mn-SOD, actin, neurofilament-L, cytochrome c, thioredoxin-1, tyrosine hydroxylase, Hsp90, fibrinogen, glutathione Stransferase and α-synuclein are known to be nitrated by peroxynitrite, inducing subsequent changes in protein structure, function or turnover (Ischiropoulos and Beckman, 2003).

The NGF monomer contains 2 well-conserved tyrosine residues at positions 52 and 79 (Bradshaw et al, 1994) that are target for peroxynitrite-induced nitration (Pehar et al, 2006b). Such post-translational modification may have important consequences in NGF biological activity in conditions of inflammation.

NITRATED-NGF AS A MEDIATOR OF MOTOR NEURON APOPTOSIS IN ALS

ALS is a fatal paralytic disorder characterized by selective death of motor neurons. While causes of progressive loss of motor neurons in ALS remain largely unknown, it is now accepted that glial cells and related inflammatory phenotypes play a role in disease pathogenesis (Barbeito et al, 2004; Henkel et al, 2009; Ilieva et al, 2009). In mice carrying the SOD1G93A mutation causing familial ALS, NGF levels are increased 2-fold in the spinal cord, being localized in a subset of reactive astrocytes surrounding degenerating motor neurons. Moreover, up-regulation of astrocytic NGF is coincident with the expression of p75NTR in damaged motor neurons, conditions that could elicit neuronal apoptosis (Pehar et al, 2004).

Since adult motor neurons lack TrkA and p75 receptors they are not responsive to NGF. However, motor neurons can re-express p75NTR after nerve injury and in ALS (Lowry et al, 2001; Rende et al, 1995; Seeburger et al, 1993), thus becoming vulnerable to NGF. Accordingly, p75NTR has been implicated in motor neuron death occurring in transgenic mice models of ALS (Copray et al, 2003; Lowry et al, 2001). Interestingly, studies performed in embryonic motor neuron cultures expressing p75NTR have allowed the characterization of NGF-

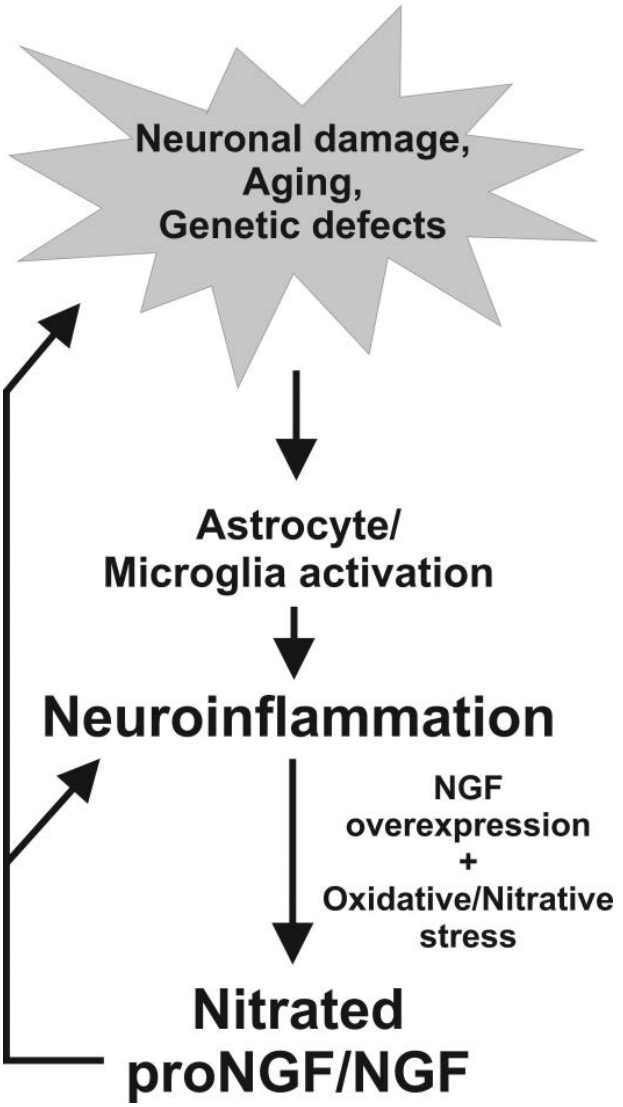

Fig. 1. *Scheme showing the hypothetical mechanism leading to post-translational modification of NGF by peroxynitrite.*

induced apoptosis and its close association with peroxynitrite (Pehar et al, 2004). In this model, NGF-induced motor neuron apoptosis is dependent of a physiological flow of nitric oxide that, in turn, promotes the intracellular formation of peroxynitrite and extensive nitrotyrosine staining in apoptotic motor neurons. Therefore, nitric oxide and related nitrating species as peroxynitrite appear as relevant mediators or co-factors in motor neuron apoptosis, one of the potential targets being NGF.

We previously reported that spinal cord extracts from ALS mice contain sufficient NGF to stimulate p75NTR-dependent apoptosis of cultured motor neurons in the presence of an external source of nitric oxide (Pehar et al, 2004). However, the levels of NGF measured by ELISA in the degenerating spinal cord from mutant hSOD1 mice were in the range of pg/ml, a concentration much lower than necessary for purified NGF to induce apoptosis in motor neuron cultures. These findings lead us to search for specific NGF species having more potent effects on p75NTR-dependent apoptosis. Remarkably, Pehar et al found that oxidation and nitration of NGF by exposure to peroxynitrite increased the NGF potency for inducing apoptosis of motor neurons by 10,000-fold in the presence of nitric oxide. In this model, blocking antibodies to p75NTR or downregulation of p75NTR expression by antisense treatment completely prevented motor neuron death, indicating nitrated NGF activates p75NTR signaling (Pehar et al, 2006a). Thus, peroxynitrite-treated NGF appears as the most potent neurotrophin eliciting p75NTR-dependent cell death in culture at physiologically active concentrations (pg/mL). The same study showed evidence that Tyr52 is more susceptible to be nitrated by peroxynitrite. Thus, the gain of apoptotic activity by nitrated NGF seems to be dependent of tyrosine nitration, leading to conformational changes in the protein and subsequent aggregate formation (from dimers to octamers) (Pehar et al, 2006b). Such oligomeric NGF species may interact with increased number of p75NTR receptors (e.g. inducing trimerization) or facilitate the formation of atypical receptor complexes (e.g. simultaneous p75/TrkA activation). Experimental data showing that only a small fraction of nitrated NGF is necessary to elicit apoptotic signaling in motor neurons strongly supports the hypothetical model of nitrated NGF gain of function. The precursor form of NGF, pro-NGF, is also known to induce motor neuron death via engagement of the p75NTR (Domeniconi et al, 2007). ProNGF binds with lower affinity to p75NTR than NGF, but it forms a high-affinity signaling complex by simultaneously binding to p75NTR and sortilin (Nykjaer et al, 2004). In the spinal cord, proNGF accumulates at higher concentrations than mature NGF, thus also becoming a potential target for peroxynitrite-mediated nitration (Beattie et al,

2002).

Interestingly, Cassina et al have proposed a mechanism by which spinal motor neurons release Fibroblast Growth Factor-1 (FGF-1) in response to damage, inducing NGF expression and nitrative stress in surrounding astrocytes (Cassina et al, 2005). Spinal motor neurons express high levels of FGF-1, which can be released in response to axotomy or other stressful conditions (Cassina et al, 2005; Saito et al, 2007). FGF-1 is known to potently induce NGF expression and secretion in astrocytes (Yoshida and Gage, 1991). In cultured spinal cord astrocytes, FGF-1 treatment is sufficient to induce a sustained up-regulation of NGF expression together with oxidative and nitrative stress (Cassina et al, 2005; Vargas et al, 2004). As expected, FGF1-stimulated astrocytes potently stimulate p75NTR-dependent apoptosis of co-cultured motor neurons (Pehar et al, 2004), further suggesting an autotoxic pathogenic loop.

While current evidence suggests nitrated NGF species could be produced or released by activated glial cells in ALS degenerating spinal cord, there is no direct evidence for its occurrence whether in cell cultures or tissue extracts. Most analytical techniques to detect and quantify modified NGF species with high sensitivity and specificity (e.g. mass spectrometry) cannot be easily applied to this protein because of several limiting factors such as: i) the low concentration of proNGF or NGF in the spinal cord (pg/mg of protein), ii) the small proportion of NGF that would react with endogenous nitrating species, iii) the lack of antibodies that specifically recognize nitrated NGF species and iv) the variety of oxidized and nitrated molecular species likely to be produced by the reaction of NGF and peroxynitrite.

Finally, it is unlikely that nitrated NGF be the only nitrated protein produced during a pathological inflammatory condition. Several other proteins may be simultaneously modified by peroxynitrite (Ischiropoulos and Beckman, 2003; Pacher et al, 2007). In ALS tissue from both patients and animal models, nitrotyrosine can be detected by immunostaining in damaged motor neurons as well as surrounding glial cells (Abe et al, 1997; Peluffo et al, 2004; Trias et al, 2013), suggesting extensive nitration of more abundant proteins. Interestingly, nitration of a single tyrosine residue on Hsp90 turns this pro-survival chaperone into a toxic protein to motor neurons (Franco et al, 2013), suggesting that nitration of NGF and Hsp90 might have a synergistic effect.

NGF NITRATION IN ALZHEIMER'S DISEASE

Besides ALS, another neurodegenerative disease in which NGF nitration is thought to play a crucial pathogenic role is Alzheimer's disease (AD). In 2009, Bruno and Cuello (Bruno et al, 2009) reported the first evidence for the in vivo occurrence of "peroxynitrited" NGF species. This group detected nitrated proNGF in AD brain samples after immunoprecipitation with an anti-NGF antibody and subsequent PAGE/WB analysis using non-specific anti-nitrotyrosine antibodies. This analytical approach provides a solid evidence for a proNGF post-translational modification associated with AD pathology. While oxidative stress and protein nitrotyrosine modification associated to concurrent neuroinflammation has been described in AD (Maccioni et al, 2001), nitrated proNGF appears to be a specific target displaying a pathogenic potential. In turn, administration of peroxynitrited NGF into rat hippocampus was found to induce a significant reduction of the TrkA phosphorylation level (Bruno et al, 2009), suggesting a down-regulation of the normal trophic signaling pathway mediated by NGF.

According to the Rita Levi-Montalcini and Viktor Hamburger´s neurotrophic theory, secreted NGF by cholinoreceptive neurons is captured by specific receptors expressed on cholinergic nerve terminals (TrkA and p75NTR) and then retrogradely transported to the cell body, where it exerts its neurotrophic activity (Aloe et al, 2012; Hamburger and Levi-Montalcini, 1949; Yuen et al, 1996). In AD, dysfunctional production of NGF species (high proNGF/low mature NGF) in cerebral cortex and hippocampus and the decreased NGF retrograde transport by cholinergic basal forebrain neurons may be responsible for the decline in cholinergic innervation and subsequent decline of cognitive functions (Cattaneo and Calissano, 2012). In accordance, the concentration of proNGF, but not of mature NGF, is increased in AD post mortem brains (parietal cortex and middle frontal gyrus) as compared to controls (Bruno et al, 2009; Fahnestock et al,

2001). This anticipates that a simply dysregulation of NGF signaling would be sufficient to trigger an autotoxic loop leading to AD's pathology as suggested by different studies. For example, transgenic mice expressing a recombinant anti-NGF AD11 neutralizing antibody in the adult brain, develop age-dependent neurodegenerative Alzheimer's disease-like pathology including the formation of amyloid plaques, hyper-phosphorylated tau and cholinergic deficits (Capsoni et al, 2000). Capturing NGF by mAbαD11 induces a functional imbalance between NGF and its precursor protein proNGF resulting in an excess of proNGF, which reproduces the alterations found in patients with AD (Capsoni and Cattaneo, 2006). In another study, transgenic mouse lines expressing a furin-resistant form of mouse proNGF that accumulates the proneurotrophin in high levels, also reproduces the AD-like neurodegenerative phenotype. Finally, Aβ oligomers itself also affect NGF metabolism, inducing NGF degradation and decreasing proNGF processing (Tiveron et al, 2013), suggesting a close association between dysfunctional NGF signaling and AD's pathology. In this context, the nitrative modification of NGF species, either proNGF or mature NGF, might greatly influence the NGF signaling through p75NTR, TrkA and sortilin receptors.

In conclusion, available experimental evidence suggests that NGF and proNGF may be produced in aparticular pathophysiological inflammatory environment, where NGF is overexpressed coincident with a cellular status of oxidative and nitrative stress. Nitrated NGF species are likely to be produced in ALS and AD to mediate specific signaling associated to neuronal damage or glial activation. Further studies are needed to determine whether nitrated NGF can be used as a marker of neurodegeneration and/or be a target for neuroprotective neutralizing antibodies.

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