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Neurobiology of Learning and Memory 81 (2004) 105–114
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

NMDA receptors and voltage-dependent calcium channels mediate different aspects of acquisition and retention of a spatial memory task

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Received 20 March 2003; revised 24 July 2003; accepted 27 October 2003

Abstract

Activity dependent calcium entry into neurons can initiate a form of synaptic plasticity called long-term potentiation (LTP). This phenomenon is considered by many to be one possible cellular mechanism underlying learning and memory. The calcium entry that induces this phenomenon can occur when *N*-methyl-D-aspartate receptors (NMDARs) and/or voltage-dependent calcium channels (VDCCs) are activated. While much is known about synaptic plasticity and the mechanisms that are triggered by activation of these two Ca^{2+} channels, it is unclear what roles they play in learning. To better understand the role activation of these channels may play in learning we systemically administered pharmacological antagonists to block NMDARs, VDCCs, or both during training trials and retention tests in a radial arm maze task. Wistar rats injected with the NMDAR antagonist MK-801 (0.1 mg/kg) were impaired in the acquisition of this task. In contrast, rats injected with verapamil (10 mg/kg), an antagonist to VDCCs, acquired the task at the same rate as control animals, but were impaired on a 10-day retention test. A group of animals injected with both antagonists were unable to learn the task. The results suggest that each of the calcium channels and the processes they trigger are involved in a different stage of memory formation or expression.

Keywords: LTP; Long-term potentiation; nmdaLTP; vdccLTP; Spatial memory; Spatial learning

1. Introduction

Synaptic plasticity refers to neural connections that change in strength in response to development, experience, and pathology. These synaptic gain changes affect neural communication and may underlie the behavioral changes exhibited in learning (Bliss & Collingridge, 1993; McNaughton & Morris, 1987; Teyler & DiScenna, 1987). The best known increase in synaptic efficacy is long term potentiation (LTP) induced by Ca^{2+} entry into the post-synaptic cell via activation of NMDARs (nmdaLTP; Dunwiddie & Lynch, 1979). A mechanistically different form of LTP is mediated by Ca^{2+} entry through VDCCs (vdccLTP; Grover & Teyler, 1990). While both forms require Ca^{2+} entry and result in a potentiated post-synaptic response, their cellular mechanisms of induction and expression differ.

These two forms of LTP have similar characteristics as well as distinct differences, suggesting that they may work together in the overall process of memory formation, but serve different roles within that process. Both forms of LTP, which can be co-expressed as compoundLTP (Grover & Teyler, 1994), are similar in that they are input specific, induced by increased afferent activity, associative, and are long-lasting (Cavus & Teyler, 1996; Grover & Teyler, 1990, 1995; Levy & Steward, 1979; McNaughton, Douglas, & Goddard, 1978)—all characteristics that support an underlying role in the memory process. Calcium influx through NMDARs triggers a serine–threonine kinase signaling pathway that results in phosphorylation of existing AMPA receptors and inser-

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doi:10.1016/j.nlm.2003.10.003

tion of new AMPA receptors in the post-synaptic membrane (Fukunaga, Muller, & Miyamoto, 1996; Lu et al., 2001; Malinow, Mainen, & Hayashi, 2000), processes that result in a reversible form of LTP. In contrast, calcium influx through VDCCs results in tyrosine kinase activation (Grover & Teyler, 1995) and an irreversible form of LTP (Morgan, Coussens, & Teyler, 2002). Application of tyrosine kinase inhibitors blocks vdccLTP, but has no effect on nmdaLTP (Cavus & Teyler, 1996), whereas nmdaLTP is completely blocked by serine–threonine kinase inhibitors that have no effect on vdccLTP (Cavus & Teyler, 1996; Grover & Teyler, 1995). The neurotrophic factors BDNF and NT-3 are selectively released with the induction of vdccLTP (Patterson, Grover, Schwartzkroin, & Bothwell, 1992) and there is an increase in *trk*B receptor expression (Cavus, Grover, & Teyler, 1993; Teyler et al., 1994). This evidence indicates that Ca^{2+} influx through NMDARs and VDCCs trigger different cellular processes.

A sizeable body of research exists to support the role of Ca^{2+} entry via NMDARs in behavioral learning and memory. Competitive and non-competitive antagonists of the NMDAR which block nmdaLTP in vitro (Grover & Teyler, 1990) and in vivo (Morgan & Teyler, 1999) also can impair acquisition of a number of behavioral tasks including the radial arm maze (Caramanos & Shapiro, 1994) and water maze (Packard & Teather, 1997), fear conditioning (Blair, Schafe, Bauer, Rodrigues, & Le Doux, 2001), conditioned taste aversion (Escobar, Alcocer, & Bermudez-Rattoni, 2002), and simple odor discriminations (Staubli, Thibault, DiLorenzo, & Lynch, 1989). Little research, however, exists examining the role of Ca^{2+} entry via VDCCs in memory formation.

In this experiment we used the 4/8 radial arm maze task, a spatial task known to be hippocampally dependent (Jarrard, 1993), in conjunction with pharmacological antagonists of the NMDA and VDCC Ca^{2+} channels, to examine the distinct role that each may play in the acquisition and storage of spatial information. The radial arm maze task allows for the examination of the time course of memory formation by evaluating performance within a trial, between trials, and after a delay at retention (Olton & Papas, 1979). Animals were trained to obtain a food reward from four consistently baited arms of the 8-arm maze over a period of weeks. To solve this problem, animals must remember from day to day which four arms are baited (reference memory, RM), and within a trial must not re-enter an arm just visited (working memory, WM). Following achievement of criterion by the control group, all animals were left in their home cages for 10 days without training or drugs, and were then tested for retention.

To assess the role of each calcium channel separately, prior to each trial animals were injected (IP) with either the NMDAR antagonist MK-801, at a dose that blocks nmdaLTP in vivo (Abraham & Mason, 1988) but has no effect on vdccLTP, or the VDCC antagonist verapamil, at a dose that blocks vdccLTP in vivo without affecting nmdaLTP (Morgan & Teyler, 1999). In addition, a double drug group received both MK-801 and verapamil at doses shown to block both forms of LTP (Morgan & Teyler, 1999), and a control group received physiological saline. It was hypothesized that blocking calcium influx through NMDARs would impair acquisition, as has been previously demonstrated, while blocking VDCCs would impair long-term memory formation, as suggested by the cellular processes initiated by calcium influx through VDCCs. It was also anticipated that blocking both calcium channels would seriously impair performance in this spatial task.

2. Methods

Fifty-two 70-day-old male Wistar rats, individually housed in clear Plexiglas cages and kept on a 12/12 light/dark cycle, were mildly food deprived to maintain approximately 85% of ad lib weight during the experiment.

The apparatus used was an 8-arm radial arm maze. The maze was elevated 85 cm from the floor and consisted of an octagonal start area (53.5 cm across) with 10 cm by 30 cm arms (with clear plastic sides 20 cm high) radiating outward. Each arm contained a reward cup (3.25 cm high by 4.25 cm in diameter) located 2.5 cm from the distal end of the arm and centered between the side walls. Pneumatically controlled gates separated the start area from the arms. The arms could be removed from the central start area and were periodically interchanged with one another. The maze was located in a 3 m by 3 m room that featured distinct spatial cues on the walls. All animals were initially shaped for 12 days (Table 1) to complete a task in which all 8 arms were rewarded (8/8) with 1/2 of a Kelloggs Froot Loop. Animals received one trial per weekday in all phases of the experiment.

After shaping, animals were randomly assigned to one of four treatment groups: saline ($n = 13$), MK-801 ($n = 13$), verapamil ($n = 13$), or both MK-801 and verapamil ($n = 13$). The 4/8 radial arm maze task was used for this phase of the experiment. The same four arms were rewarded for each animal throughout the experiment. Rewarded arms were varied between animals and balanced across groups. The acquisition phase was run for 40 days. Each animal was injected (IP) approximately 1 h before its acquisition trial with either saline, MK-801 (0.1 mg/kg), verapamil (10 mg/kg), or both MK-801 (0.1 mg/kg) and verapamil (10 mg/kg). At the start of a trial, each animal was placed in the center of the maze with the gates closed. The gates were then opened and the animal was al-