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Research paper

The activation of NMDA receptors alters the structural dynamics of the spines of hippocampal interneurons

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ABSTRACT

N-Methyl-D-Aspartate receptors (NMDARs) are present in both pyramidal neurons and interneurons of the hippocampus. These receptors play a key role in the structural plasticity of excitatory neurons, but to date little is known about their influence on the remodeling of interneurons. Among hippocampal interneurons, the somatostatin expressing cells in the CA1 *stratum oriens* are of special interest because of their functional importance and structural characteristics: they display dendritic spines, which change their density in response to different stimuli. In order to understand the role of NMDAR activation on the structural dynamics of the spines of somatostatin expressing interneurons in the CA1 *stratum oriens*, we have studied entorhino-hippocampal organotypic cultures obtained from mice in which this interneuronal subpopulation expresses constitutively EGFP, and have imaged them in real-time. We have acutely infused the cultures with NMDA, a strong NMDAR agonist, and have analyzed the structural dynamics of somatostatin expressing interneurons, prior and after its administration. The appearance and disappearance rates of their dendritic spines increased 24 h after the NMDA infusion and returned to baseline levels 48 h afterwards. By contrast, their stability rate decreased 24 h after the infusion and also returned to control levels 48 h later. The relative density of the dendritic spines remained unaltered throughout the assay. Altogether, our results show that the activation of NMDARs can influence the structural dynamics of interneurons. This is especially important because of the involvement of these receptors in neuronal potentiation/depression and their putative role in the etiopathology of certain neuropsychiatric disorders, such as schizophrenia.

1. Introduction

NMDA receptors (NMDARs) have been extensively studied regarding neural potentiation and synaptic plasticity [1]. These receptors also play crucial roles in different neurodevelopmental events, such as neurogenesis, neuronal migration and synaptogenesis [2]. In recent years, we have learnt that some of these plastic events persist during adulthood and that they are important contributors to cognition and to the response of the brain to aversive experiences. Neurons in the adult brain can modify their morphology and connectivity in response to different stimuli, especially by altering the complexity of their dendritic arbor and the density of their dendritic spines. These postsynaptic structures have been mainly studied in pyramidal neurons, although they are also present in some interneuronal populations [3]. Furthermore, they have been found to be proper markers for neural activity as they increase their density when the neuron is potentiated [4].

However, the study of changes in spine density does not always provide all the information on the structural plasticity of these elements. In fact, events that result in null alterations in this density can also affect the network by changing the synaptic configuration: the appearance of one spine in one site −and its new synapse- can structurally shadow the disappearance of another spine elsewhere, but the new connection still represents novel information. This has been commonly known as structural dynamics, and it appears to be the force underlying adaptation in the nervous system [5,6]. Structural remodeling of excitatory [7] and inhibitory [8] microcircuits have already been observed in real-time after sensory deprivation. However, studies directed to observe this plasticity *in vivo* can only be performed in the neocortex, due to the limitations of the methodology: imaging through a cranial window. This restriction limits the study of other important areas, such as the hippocampus. Entorhino-hippocampal organotypic cultures, with their intrinsic limitations, are an alternative

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to solve this issue. They allow us to follow the same neuron and report structural changes in real-time. Furthermore, they have been broadly used as an *in vitro* model of the rodent hippocampus [9].

Most structural studies have focused on the morphology of excitatory neurons and have left aside inhibitory circuits and interneurons, important players on the central nervous system physiology. Reports on dendrite-targeting interneurons are particularly scarce, specifically on somatostatin (SOM) expressing cells. These interneurons are critical for the maturation of deep cortical circuits [10] and are important players in other stages of neurodevelopment, brain pathology and neuronal plasticity [11]. In addition, recent studies have demonstrated that their structure is altered after different manipulations, such as chronic stress [12], antidepressant treatment [13], streptozotocin-diabetic challenge [14] or the depletion of plasticity related molecules [15]. In the hippocampus, specifically in the *stratum oriens*, two subpopulations of SOM expressing interneurons can be found: those projecting to extrahippocampal areas, such as the HS cells [16] and those projecting locally to the *stratum lacunosum moleculare*. These hippocampal interneurons are essential for the proper functioning of the hippocampus [17] and their dendrites present dynamic dendritic spines [15].

Glutamate NMDARs are widely expressed in both pyramidal neurons and interneurons, including the SOM expressing cells of the *stratum oriens* [18,19]. The pyramidal neurons of knock-out mice lacking these receptors show altered neuromorphological characteristics, such as modifications in the size and density of their dendritic spines and axonal *boutons* [20]. Moreover, the manipulation of these receptors has an important impact on the structure and density of pyramidal spines [21]. A recent study from our laboratory has shown that the blockade of NMDAR has important effects on the structure of these neurons [19]. However, nothing is known about the impact of the activation of these receptors on interneuronal structure and dynamics.

In the present study, we aim to understand the impact of NMDAR manipulation on the structural dynamics of SOM expressing interneurons in the *stratum oriens* of the hippocampal CA1 region. In order to achieve our goal, we have used a transgenic mice strain with these interneurons constitutively labeled with EGFP, which allows us to study their entire morphology [22]. Using organotypic cultures, we have studied their structure under the confocal microscope in real-time and have determined how an acute NMDA administration alters the structural dynamics of these cells.

2. Material and methods

2.1. Animals and treatment

For the real-time *in vitro* analyses we have used the EGFP-expressing inhibitory neurons (GIN) Tg(GadGFP)45704Swn mice (Jackson laboratories, Bar Harbor, Maine, USA) [22]. Six GIN pups (P7) were used to prepare hippocampal-entorhinal organotypic cultures [9]. NMDA (10 μM in culture media, Abcam, Cambridge, UK) was added to the media after the second imaging session and remained there for 1 h (Fig. 1A).

All the animals were maintained in standard conditions of light (12 h cycles) and temperature, without restrictions in food and water. All animal experimentation was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and was approved by the Committee on Bioethics of the Universitat de València. Every effort was made to minimize the number of animals used and their suffering.

2.2. Organotypic culture preparation

The brain was freshly extracted from P7 mice and the hippocampus dissected along with the entorhinal cortex in order to preserve the perforant path. Slices (400 μm-thick) were obtained with a tissue chopper following a previously described protocol [9,15]. Media was changed 3 times per week and the cultures remained 13 days in vitro (DIV) until the confocal imaging started.

2.3. Real-time imaging

For the real-time imaging of organotypic cultures, short imaging sessions (10–15 min) were carried out with a 40X water immersion objective in a laser scanning confocal microscope (Leica TCS SPE, Germany). An additional 10X digital zoom was used to analyze dendritic segments of about 35 μm in length, located between 100 and 150 μm from the soma (Z step size of 0.8 μm). Laser intensity was kept at the minimum allowing observation, and acquisition conditions maintained unchanged over the different days of observation. Control experiments showed that this procedure did not produce any deleterious effect on cell viability, such as cell death or dendritic beadings. One dendrite was analyzed per organotypic culture slice, and 14 slices from 6 different animals were analyzed. All the somata of the interneurons analyzed were located in the *stratum oriens* of CA1 region. The imaging took place at five different time points referenced to the beginning of the treatment: −24 h, 0 h, 5 h, 24 h and 48 h, starting on DIV 13. The treatment was administered after the second imaging session (0 h) for one hour, and then it was again substituted by normal culture media (Fig. 1A).

2.4. Real-time analysis

The same dendrite was studied linearly to calculate the 4 parameters related to the structural dynamics of the spines: appearance, disappearance and stability rates, and the rate of dynamic events. These parameters were calculated relative to the prior number of spines. Appearance rate: number of gained spines/total number of previous spines; disappearance rate: number of disappear spines/total number of previous spines; stability rate: number of stable spine/total number of previous spines. Therefore, both disappearance and stability rates are linked. The rate of dynamic events is the sum of gained and lost spines relative to the previous number of spines.

2.5. Statistics

After checking the normality and homoscedasticity of the data, we used repeated measures ANOVA to compare control and experimental groups in the 24 h-sparse experiment. In order to compare both the linear effects within and between groups, we performed *post hoc* analyses (LSD). To compare the 5-h time point between NMDA and control groups, we used an unpaired *t*-test. In every case α was set to 0.05 and the slice was considered the "n".

3. Results

3.1. No fast effect on spine turnover after acute NMDA administration

Real-time analysis was performed in control conditions and after the acute administration of NMDA (1 h in the culture medium). In order to register fast effects of the NMDAR activation, we imaged the dendrites 4 h after the 1h-treatment (0 h to 5 h). There were no significant changes in the NMDA group on the appearance ($p = 0.568$), disappearance and stability rates (both $p = 0.763$), or the rate of dynamic events ($p = 0.797$; Fig. 1C1, 1D1, 1C2, 1D2 and 1E).

3.2. Increased appearance and disappearance rates of dendritic spines 24 h after NMDA administration

In control conditions, we found no alterations on the appearance, the disappearance, the stability rates, or on the rate of dynamic events.