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The plastic neurotransmitter phenotype of the hippocampal granule cells and of the moss in their *messy* fibers

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ABSTRACT

The granule cells (GCs) and their axons, the mossy fibers (MFs), make synapses with interneurons in the hilus and CA3 area of the hippocampus and with pyramidal cells of CA3, each with distinct anatomical and functional characteristics. Many features of synaptic communication observed at the MF synapses are not usually observed in most cortical synapses, and thus have drawn the attention of many groups studying different aspects of the transmission of information. One particular aspect of the GCs, that makes their study unique, is that they express a dual glutamatergic–GABAergic phenotype and several groups have contributed to the understanding of how two neurotransmitters of opposing actions can act on a single target when simultaneously released. Indeed, the GCs somata and their mossy fibers express in a regulated manner glutamate and GABA, GAD, VGlut and VGAT, all markers of both phenotypes. Finally, their activation provokes both glutamate-R-mediated and GABA-R-mediated synaptic responses in the postsynaptic cell targets and even in the MFs themselves. The developmental and activity-dependent expression of these phenotypes seems to follow a "logical" way to maintain an excitation-inhibition balance of the dentate gyrus-to-CA3 communication.

1. Introduction

The granule cells of the dentate gyrus (DG) in the hippocampal formation give origin to the bundle of axons to which Ramón y Cajal gave the adjective of "mossy" because of their appearance, in form of ramified clumps, resembling moss. These mossy fibers (MFs) possess unique anatomical, biochemical, biophysical and information transmitting characteristics, which make their study a complicated task. It turns even more complicated and interesting by the fact that these fibers express different information transmission phenotypes. So I take the liberty to confer them a second adjective: "messy" (which definition by the Merriam-Webster's Dictionary is "marked by confusion"). Indeed, their study has to be technically delicate, thorough and rigorous to avoid obtaining data that can bring confusion when interpreting their physiological meaning.

The mossy fibers make 3 types of synapses along their path through the *hilus* and along the *stratum lucidum* of CA3. One with the pyramidal cells of CA3, which contact is comprised by a giant MF bouton and a dendritic spine of the apical dendrite of the pyramidal cell; second, synapses called "en passant" made by varicosities along the MFs and interneurons and, finally, phylopodial extensions that originate from the MF giant boutons and that also contact interneurons (Acsády et al., 1998).

In the adult rodent, the stimulation of the MF produces depolarizing monosynaptic responses in their target cells: interneurons and mossy cells in the *hilus*, and interneurons and pyramidal cells in the CA3 region. Thus, granule cells have been considered as glutamatergic. Years of research on MF transmission, particularly in *in vitro* preparations, have shown that the characteristics of neurotransmission are highly dependent on the target cells from which recordings are made, and virtually all these studies have been conducted in the presence of $GABA_A$-R antagonists to avoid contamination from the stimulation of interneurons scattered in the *hilus* or *stratum lucidum*. So the typical "experimental assumptions" that the neurophysiologists working on the MF synapse have made are: (1) true MF stimulation would not produce monosynaptic inhibitory responses; however, polysynaptic inhibitory responses can be recorded because interneurons can be driven by MF activation and they, in turn, inhibit pyramidal cells and other interneurons as well. Therefore, blocking glutamate receptors prevents both the monosynaptic

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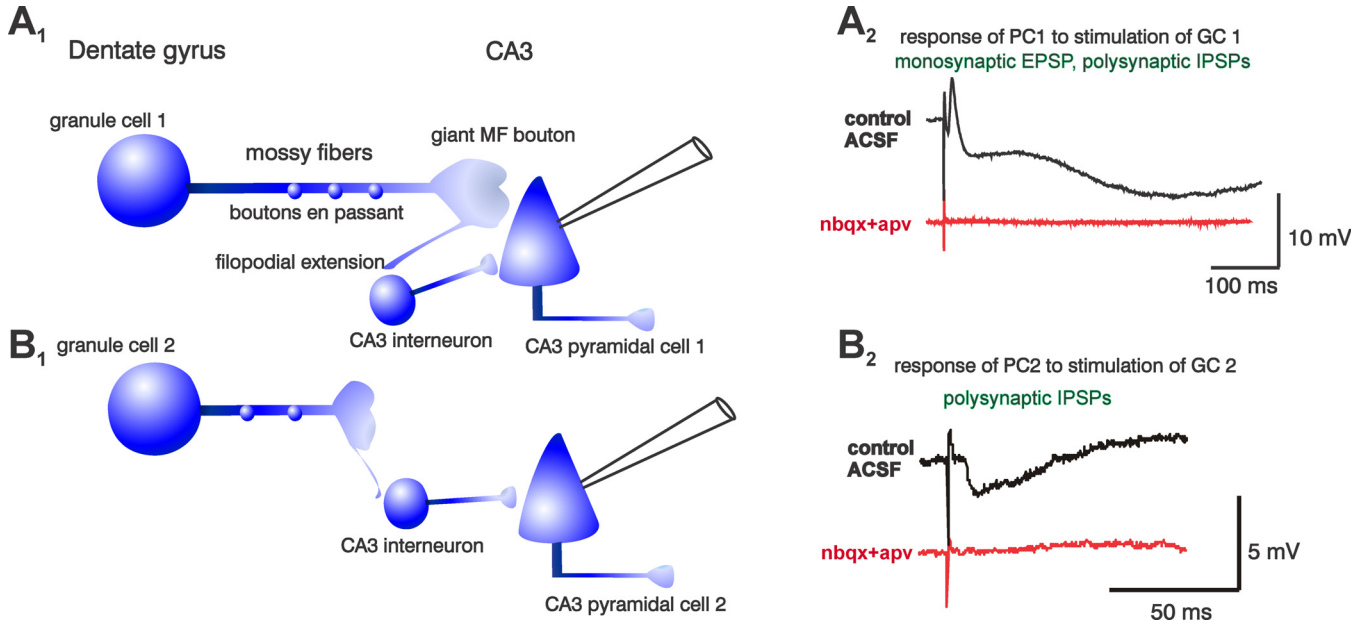

Fig. 1. A_1. The granule cells of the dentate gyrus excite pyramidal cells, through giant boutons, and interneurons, through boutons *an passant* and filopodial extensions. The latter in turn, release GABA to inhibit pyramidal cells and sustain feed-forward inhibition. This scheme depicts glutamatergic-only transmission of the granule cells, after the third week of age. A_2. Stimulation of this arrangement (granule cell 1-to-CA3 pyramidal cell 1) provokes monosynaptic EPSP and polysynaptic $GABA_A$ and $GABA_B$-dependent responses. B_1. The origin of an inhibitory response in a pyramidal cell to MF stimulation is through the activation of an interneuron. B_2. Thus, stimulation of granule cell 2 that does not innervate the pyramidal cell 2, but instead drives an interneuron connected to this pyramidal cell (2), evokes a polysynaptic IPSP.

excitatory response and, thus, the di- or polysynaptic activation of GABAergic interneurons from which GABA-mediated responses originate. (2) On the other hand, the presence of $GABA_A$-R antagonists in the extracellular medium blocks monosynaptic inhibition, which could be elicited by direct activation of interneurons when bulk stimulation is given over the *stratum lucidum*, through which the MFs run (Fig. 1).

Up to this point, all synaptic responses obtained by what is presumed to be selective MF stimulation under the aforementioned conditions seem to be accounted for. Not quite. Glutamatergic responses recorded in CA3 cells can be evoked by the activation of one or more of the anatomically distinct fibers that impinge onto them: the recurrent axons from CA3 pyramidal cells themselves, the perforant path, the commisural and finally the MFs. Therefore, responses to the activation of these sources should be told apart and, for this, some conditions have to be met.

2. Electrophysiological criteria to distinguish glutamatergic transmission of MF origin

For years, consistent results were obtained under the above-mentioned assumptions about glutamate-mediated MF transmission. Therefore, obtaining other type of response (for instance, mono-synaptic GABAergic transmission in the presence of glutamate receptor blockers) would mean that something in these experiments was different from those that everybody conducted and that helped to establish the referred assumptions. Alternatively, their interpretation was wrong because it did not comply with such assumptions. And certainly, there were *ad-hoc* explanations if such a response appeared. For instance, if $GABA_A$R-mediated responses were elicited, that would mean that the electrode supposedly used to selectively stimulate the MFs, directly stimulated interneurons. Thus, a regular practice to avoid "contamination" from interneuronal transmission when MFs are stimulated is the perfusion of a $GABA_A$-R antagonist, either bicuculline, or picrotoxin, or gabazine disolved in the extracellular medium. Even so, selective stimulation of the MF tract over the *stratum lucidum* is "tricky" and it does not always produce pure MF responses because commissural axons, as well as collaterals of other CA3 pyramidal cells can be stimulated too (Henze et al., 1997); so synaptic responses had to "follow certain rules" if they are to be considered of MF origin. Briefly, the physiological and pharmacological characteristics of transmission of MF origin are: (1) strong frequency-dependent potentiation (>300%) in response to modest increases in stimulation frequency; (2) robust NMDA-independent LTP and (3) depression (>80%) of the responses by activation of mGluR, which are present in the MFs. Indeed, proving the presence of these physiological and pharmacological characteristics together defines transmission of MF origin. These and several other physiological characteristics of MF transmission and its plasticity are thoroughly reviewed elsewhere (Henze et al., 2000; Urban et al., 2001; Bischofberger and Jonas, 2002; Lawrence and McBain, 2003; Nicoll and Schmitz, 2005; Jaffe and Gutiérrez, 2007; Galván et al., 2011; Ruiz and Kullmann, 2013).

Despite these experimental manipulations that are routinely used to define transmission of MF origin, the only way to undoubtedly isolate responses to MF activation is by conducting paired recordings, whereby the presynaptic granule cell is depolarized to fire an action potential and a synaptic response to each action potential should be recorded in the postsynaptic target cell. Alternatively, pure MF-mediated synaptic responses can be evoked by the selective stimulation of a MF giant bouton. This has been accomplished through a method that implies recording from a presynaptic giant MF bouton while recording the postsynaptic pyramidal cell in a hippocampal slice (Geiger and Jonas, 2000; Bischofberger et al., 2006). A second method that permits to record synaptic responses to selective stimulation of MF giant boutons implies labeling these boutons, then dissociating the pyramidal cells from their network with the MF boutons attached to their apical dendrites, which can then be identified and directly stimulated by means of a patch pipette while recording from the pyramidal cell (Beltrán et al., 2012; Beltrán and Gutiérrez, 2012). Finally, pure granule cell-mediated synaptic responses can be