Chapter 20
Granule Cells and Parallel Fibers

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Abstract The granule cells (GrC) are the smallest and most numerous neurons of the brain and constitute the main elements of the granular layer of cerebellum, where they are thought to determine a complex spatio-temporal reconfiguration of incoming signals. GrC functioning is based on some specific properties (D'Angelo, Cerebellar granule cell. In: Handbook of the cerebellum and cerebellar disorders (Springer, ed). Springer, Berlin, pp 765–791, 2013):

1. GrCs have a special structure and connectivity pattern allowing fast combinatorial processing
2. GrC are connected to mossy fibers (MFs) and Golgi cells (GoCs) in glomeruli allowing neurotransmitter spillover and crosstalk
3. GrCs are silent at rest and respond with spike bursts to MF activity by exploiting specific ionic channel properties
4. GrCs are at the core of a complex NMDA- and NO-dependent system that regulates long-term synaptic plasticity in MFs and parallel fibers (PFs).
5. GrCs have a peculiar postnatal development determining their connectivity with MFs and Purkinje cells (PCs) (see Chaps. 13, 15, 17, 18).

Keywords Granule cells • Parallel fibers • Cerebellum

20.1 Granule Cell Structure and Electroresponsiveness

GrCs are composed of a small soma emitting four short unbranched dendrites on average and receive excitatory inputs from MFs and inhibitory inputs from GoCs (Eccles et al. 1967). GrCs are excitatory and transmit their output through the ascending axon (AA) that then bifurcates into the parallel fibers (PF). The AA contacts GoCs on their basal dendrites and the PF contacts both GoCs, PCs and

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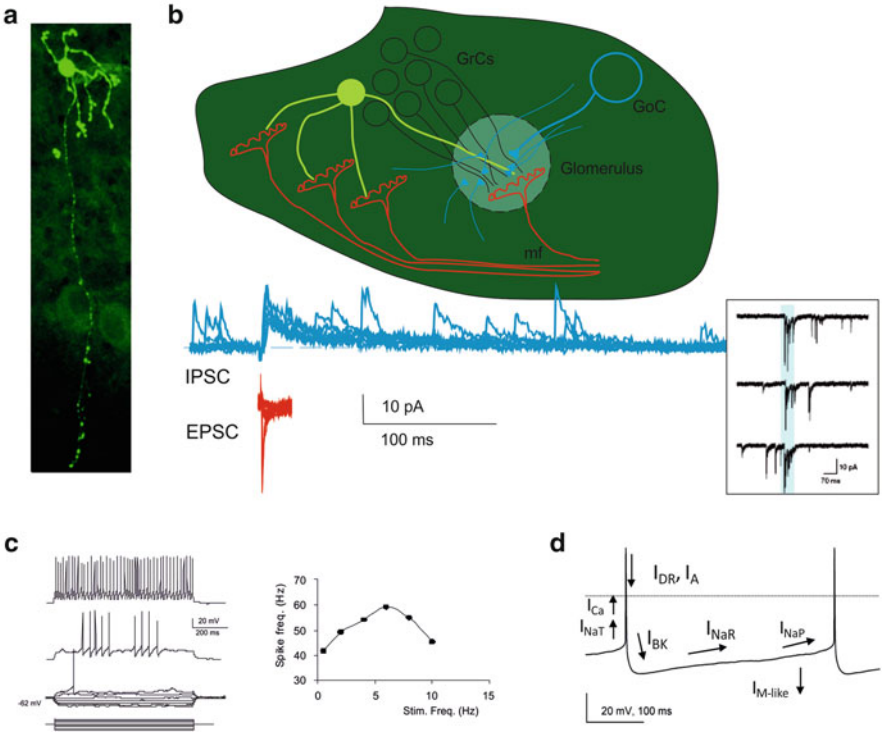

Fig. 20.1 Cerebellar granule cell properties. (**a**) Confocal microscope reconstruction of a GrC injected with neurobiotin. The image shows the dendrites with terminal digitizations and the thin axon ascending through the granular layer into the molecular layer (D Gall and E D'Angelo, unpublished). (**b**) Synaptic transmission at GrC synapses. In this schematic drawing, a GrC receives activation from 4 MFs and 2 GoCs. In the glomerulus, spillover generates slow responses. Several GrC excitatory synaptic currents (EPSC; *red*) and inhibitory synaptic currents (IPSC; *blue*) generated in responses to minimal stimulation are shown superimposed (Redrawn from D'Angelo 2013). Note spontaneous activity generated by GoC discharge. The inset shows the in vivo response of a GrC to air-puff stimulation of the whisker-pad, revealing EPSC bursts with short-term depression (Reprinted from Rancz et al. 2007). (**c**) The GrC is silent at rest and generates repetitive spike discharge during current injection. It can generate bursts and is resonant at theta frequency (Modified from D'Angelo et al. 2001). (**d**) Ionic currents involved in GrC spike electrogenesis (Adapted from D'Angelo et al. 2001). INaT (Transient Na^+ current), INaR (Resurgent Na^+ current), INaP (Persistent Na^+ current), ICa (Ca^{2+} current), IDR (Delayed Rectifier K^+ current), IBK (Ca^{2+}-dependent K^+ current), IM-like (M-like K^+ current)

molecular layer interneurons (MLIs) in the molecular layer (Fig. 20.1; Mapelli et al. 2014).

GrCs express ionic channels conferring specific electroresponsive properties (D'Angelo et al. 2001). Nav1.6 have the highest concentration in the axon initial segment (AIS), where action potentials are initiated, and are located along the axon but are almost absent from soma (Goldfarb et al. 2007). The Nav1.6 sodium channels, in addition to the transient component generating the spike upstroke, produce a persistent current, which amplifies theta-frequency oscillations and resonance.

Moreover, Nav1.6 channels produce a resurgent current reinforcing burst generation. The Ca^{2+} channels are of the high voltage-activated type: in the soma, N-type Ca^{2+} channels are activated during the action potential upstroke and regulate a BK Ca^{2+}-dependent K^+ current, while in the synaptic terminals P/Q and R type Ca^{2+} channels regulate neurotransmitter release (Galliano et al. 2013). A-type K^+ channels regulate spike initiation and M-type K^+ channels determine oscillations and resonance. Finally, GIRK type K^+ channels control GrC resting membrane potential and input conductance. These properties have been incorporated into realistic models (D'Angelo et al. 2001) demonstrating that GrCs are indeed designed to rapidly respond to incoming MF inputs with short spike bursts raising up to about 300 Hz. The spikes are propagated from initial segment backward to GrC dendrites and forward to AA synapses in about 0.1 ms, thus generating a close coincidence between excitation of GrCs and of PCs (Diwakar et al. 2011).

20.2 Glomerular Organization of GrCs Synaptic Inputs

GrC activity is determined by the interplay of excitatory and inhibitory inputs, which impinge onto a specialized structure called *cerebellar glomerulus*. Each glomerulus is made of a glial sheet enwrapping a MF terminal and as many as 50 GrC dendrites, as well as GoC axonal terminals and dendrites. In the glomerulus, in addition to fast synaptic transmission between axonal terminals and GrC dendrites, neurotransmitter diffusion in the glomerulus determines spillover effects and metabotropic activation on all the elements involved, setting up a complex regulatory mechanism (Mapelli et al. 2014).

MFs release glutamate and activate GrC AMPA and NMDA receptors (AMPARs and NMDARs), regulating membrane depolarization and Ca^{2+} influx (D'Angelo et al. 1990; Silver et al. 1992). AMPARs contain GluR2, have fast kinetics and are Ca^{2+} impermeable. NMDARs contain NR2A and NR2C subunit conferring specific voltage-dependence and kinetics (Rossi et al. 2002; Schwartz et al. 2012). GoC terminals release GABA activating GrC GABA-A receptors (Mapelli et al. 2009). Metabotropic receptors on granule cells (mGluR1 and GABA-B), on MF terminals (mGluR2 and GABA-B) and on GoC axon terminals (mGluR2 and GABA-B), regulate neurotransmitter release and GrC ionic channels (Mapelli et al. 2014).

20.3 Synaptic Transmission and Plasticity

The MF-GrC synapse is enriched with synaptic vesicles and can release quanta at high rate for sustained time periods. During bursts, the postsynaptic response (i.e., excitatory postsynaptic current (EPSC)) shows a marked short-term depression due both to vesicle depletion and AMPAR desensitization (Nieus et al. 2014). Glutamate spillover activates NMDARs and contributes to generate a slow of AMPAR-dependent component (Rossi et al. 2002). The GoC-GrC synapse also

shows a marked short-term depression during burst transmission (Mapelli et al. 2009). Both synapses show complex regulatory mechanisms based on metabotropic receptors (Mapelli et al. 2014).

The MF-GrC relay is site of long-term synaptic plasticity, which is manifest as LTP or LTD depending on input bursts patterns: long high-frequency bursts generate LTP, and vice versa (Gall et al. 2005; D'Errico et al. 2009). This LTP and LTD depend on NMDARs and metabotropic glutamate receptors (mGluRs) receptors depending on the input patterns and require Ca^{+2} entry and NO (Gall et al. 2005). MF-GrC plasticity is expressed presynaptically through an increase in release probability and can fine tune the delay to fist spike in GrCs by controlling quantal release and EPSC short-term plasticity (Nieus et al. 2014). Following patterned input bursts, GrCs also show persistent changes in intrinsic excitability (see Chap. 38).

20.4 Spike Coding and Transmission of GrC Output to the Molecular Layer Through AA and PF

By exploiting their ionic channels and synaptic properties, the GrCs efficiently recode input spikes trains into burst with precise timing and number of emitted spikes (Billings et al. 2014; Nieus et al. 2014). GrCs transmit their output spike patterns through the AA and PFs to PCs, GoCs and MLIs (Mapelli et al. 2013). AA and PFs are normally myelinated and conduct spikes at around 0.1 m/s. The PF-PC synapse has normally a low release probability and shows short-term facilitation, so that it responds better to spike doublets or triplets. Moreover, following patterned activity, the PF terminals generate various forms of long-term synaptic plasticity, some of which are presynaptic and involve NMDARs and NO production. Neurotransmission at synapses with MLIs and GoCs also involve forms of short- and long-term plasticity but these are less known (D'Angelo 2014) (see Chaps. 43 and 45).

20.5 Functional Activation of GrCs In Vitro and In Vivo

Experiments in vitro have revealed complex patterns of granule cell activation, which occurs in center surround and can generate combinatorial operations (D'Angelo 2013; Gandolfi et al. 2014). Recordings in vivo have confirmed that the properties observed in vitro actually regulate GrC activity during responses to sensori-motor inputs (Rancz et al. 2007). Most GrCs are normally silent and then respond in short burst or long spike sequences depending on the input MF patterns. Computational modeling has allowed to investigate the implications of GrC properties for the granular layer and cerebellar function (Solinas et al. 2010). The picture that emerges is that of a fast relay neuron, which can regulate timing and intensity of spike transmission to PCs exploiting long-term synaptic plasticity and glomerular interactions.

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