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Classical conditioning of motor responses: What is the learning mechanism?

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ABSTRACT

According to a widely held assumption, the main mechanism underlying motor learning in the cerebellum, such as eyeblink conditioning, is long-term depression (LTD) of parallel fibre to Purkinje cell synapses. Here we review some recent physiological evidence from Purkinje cell recordings during conditioning with implications for models of conditioning. We argue that these data pose four major challenges to the LTD hypothesis of conditioning. (i) LTD cannot account for the pause in Purkinje cell firing that is believed to drive the conditioned blink. (ii) The temporal conditions conducive to LTD do not match those for eyeblink conditioning. (iii) LTD cannot readily account for the adaptive timing of the conditioned response. (iv) The data suggest that parallel fibre to Purkinje cell synapses are not depressed after learning a Purkinje cell CR. Models based on metabotropic glutamate receptors are also discussed and found to be incompatible with the recording data.

1. Introduction

1.1. Eyeblink conditioning in the cerebellum

It has been known for almost three decades that classical or Pavlovian motor conditioning, such as eyeblink conditioning, depends on cerebellar mechanisms (Christian & Thompson, 2003; Hesslow & Yeo, 2002). If a neutral conditioned stimulus (CS), often a tone, a light or a skin stimulus, is repeatedly followed by an unconditioned ocular stimulus (US) that elicits a blink reflex, the CS will acquire the ability to elicit a blink in advance of the US. This conditioned response (CR) is abolished or severely impaired by lesioning or pharmacological inactivation of the cerebellum (McCormick, Clark, Lavond, & Thompson, 1982; Yeo, Hardiman, & Glickstein, 1984). Evidence from Yeo and collaborators show that pharmacological inactivation of the cerebellar cortex prevents consolidation of the learning, suggesting that the cortex is the main locus of memory storage (Attwell, Cooke, & Yeo, 2002; Kellett, Fukunaga, Chen-Kubota, Dean, & Yeo, 2010). However, in spite of intensive research the synaptic mechanisms involved have remained unclear.

Ever since the theoretical ideas of Albus (1971) and Marr (1969) it has been the dominant working assumption in the field that the CS is transmitted to the cerebellar cortex via the mossy fibres (mf) and parallel fibres (pf) whereas information about the US is provided by climbing fibres (cf) originating in the inferior olive. Albus explicitly suggested that the behavioural CR was driven by a pause in the simple spike firing of the Purkinje cells. The US is assumed to induce plastic changes in recently activated synapses in the cerebellar cortex so that the CS, after training with paired CS–US presentations, will elicit a suppression of simple spike firing in the Purkinje cells. Because these cells are inhibitory, such suppression would be expected to cause disinhibition of the deep nuclear cells and an excitatory signal downstream through the red nucleus and the motor neurones (Hesslow & Yeo, 2002). The pf–Purkinje cell synapses are particularly well suited for associative learning because of the extreme degree of convergence at this locus where a couple of hundred thousand parallel fibres may terminate on a single Purkinje cell (Harvey & Napper, 1991). The cerebellar circuit assumed to be involved in the learning is shown in Fig. 1.

1.2. Conditioned Purkinje cell responses

The view of conditioning summarised above was supported by anatomical findings by Yeo, Hardiman, and Glickstein (1985) and has also received strong support by recordings from Purkinje cells during conditioning. It has been shown that, during eyeblink conditioning, Purkinje cells in an area of the C3 zone of the cerebellar cortex, that controls the eyelid, develop a pause response to the CS (Hesslow & Ivarsson, 1994; Jirenhed, Bengtsson, & Hesslow, 2007). This response, henceforth called a Purkinje cell CR, also reliably appears if the CS is direct stimulation of mossy fibres entering the cerebellum and if the US is direct stimulation of climbing fibres or the inferior olive. For an example, see Fig. 3.

The Purkinje cell CR mirrors many aspects of the overt CR (Gormezano & Moore, 1969; Kehoe & Macrae, 2002). For instance, the Purkinje cell CR develops gradually during paired CS–US

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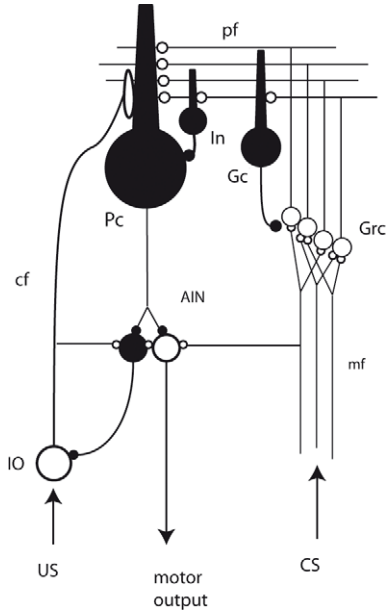

Fig. 1. Synaptic organisation of cerebellar module. Purkinje cell (Pc) controlling blink receives conditioned stimulus (CS) signal via mossy fibres (mf), Granule cells (Grc) and parallel fibres (pf). Different CSs are assumed to activate different mfs and pfs. The unconditioned stimulus (US) is signalled by climbing fibres (cf) from the inferior olive (IO). In, inhibitory interneurons (stellate and basket cells); Gc, Golgi cells. Purkinje cells inhibit the anterior interpositus nucleus (AIN), which sends output to the motor system and also an inhibitory negative feedback signal via the nucleo-olivary pathway to the IO.

presentations and is extinguished during CS-alone presentations. It reappears very fast when paired stimulation is reinstated after extinction (Jirenhed et al., 2007). One of the defining features of classical conditioning is that the CR is adaptively timed. The latency of the conditioned blink tends to be adjusted by the learning process so that the maximum amplitude coincides in time with the onset of the US. If the CS–US interval is increased, additional training will cause the CR latency to adapt to the new interval. The Purkinje cell CR is adaptively timed in the same way and it also changes its temporal properties in response to changes in CS parameters just as the overt CR (Jirenhed & Hesslow, 2011a; Svensson, Jirenhed, Bengtsson, & Hesslow, 2010). Furthermore, it has been demonstrated that the Purkinje cells in which the conditioned pause responses occur control the overt CR. Stimulation in the relevant area of the C3 zone, but not in adjacent areas, completely suppresses the behavioural CR (Hesslow, 1994a, 1994b). We therefore think that it is a reasonable working assumption that the Purkinje cell CR drives the behavioural CR.

1.3. Standard theory: long-term depression

The learning mechanism most often invoked to account for the development of the Purkinje cell CR has been long-term depression (LTD), of the parallel fibre to Purkinje cell synapses. Modulation of these synapses due to simultaneous (or close temporal proximity) of pf and cf input was proposed as a mechanism for motor learning by Albus (1971) and Marr (1969). LTD was demonstrated by Ito and Kano (1982) and has been extensively studied both *in vivo* and *in vitro* since then. Although long term potentiation as well as learning in cerebellar interneurons has been demonstrated (Jörntell & Ekerot, 2002; Linden, 1999), Purkinje cell LTD has remained the critical mechanism in most models of conditioning (Hansel, Linden, & D'Angelo, 2001; Mauk & Buonomano, 2004; Medina & Mauk, 2000; Yamazaki & Tanaka, 2009).

The idea that LTD is the essential mechanism underlying eyeblink conditioning received early support by the finding that the binding ability of AMPA receptors was reduced in rabbits following conditioning (Hauge, Tracy, Baudry, & Thompson, 1998) but has been questioned by other studies. For instance, Welsh et al. (2005) found no impairment in the ability to adapt the timing of conditioned responses to new CS–US intervals in rats in which LTD had been blocked pharmacologically.

The LTD hypothesis has also been addressed in a number of studies of genetically modified mice. Thus, Aiba et al. (1994), and later Kishimoto et al. (2001), showed that mutants which lack the metabotropic glutamate receptor mGluR1 in Purkinje cells have deficient eyeblink conditioning. However, mGluR1 is not specific for LTD and could have affected other learning mechanisms. A different type of knockout that more specifically targeted LTD in Purkinje cells by inhibiting Protein kinase C also produced deficits in eyeblink conditioning (Koekkoek et al., 2003), but PKC probably also affects other learning mechanisms (Schonewille et al., 2011).

A more recent and improved approach has challenged the LTD hypothesis (Schonewille et al., 2011). To avoid non-specific effects, De Zeeuw and colleagues targeted the expression of parallel fibre LTD directly by modifying AMPA receptors downstream of the molecular cytosolic pathway at the level of the membranes (*GluR2D7* and *GluR2K882A* mice). These animals had no LTD, yet they did not show any deficit in eyeblink conditioning suggesting that parallel fibre LTD is not the crucial mechanism.

When LTD was first proposed as the essential synaptic mechanism in conditioning, there was not much data on the behaviour of Purkinje cells during conditioning and it was not really possible to evaluate this LTD hypothesis. Since then, a number of studies reporting on Purkinje cell behaviour in conditioned animals have been published (Berthier & Moore, 1986; Green & Steinmetz, 2005; Hesslow & Ivarsson, 1994; Jirenhed et al., 2007; Kotani, Kawahara, & Kirino, 2006). The purpose of this paper is to discuss the implications of these and other studies for current models of conditioning. We will argue that although the recording data are not conclusive, they throw some doubt on the hypothesis that LTD is the essential mechanism.

There are four major challenges to the LTD hypothesis. The first problem is that removal of pf excitation, as in LTD, does not necessarily entail suppression of simple spike firing, as in the Purkinje cell CR. A second challenge is that the conditions under which LTD can be obtained do not match those for eyeblink conditioning. In particular, conditioning does not occur with short intervals between the CS and the US, although LTD clearly does work at such intervals. The third problem is that LTD cannot by itself account for the adaptive timing of the CR. The fourth problem with LTD as a basic process in conditioning is the recent evidence that there does not seem to be any depression of the parallel fibre excitation of Purkinje cells during learning. We proceed to discuss these four problems in turn. For additional arguments against the standard view of LTD as the main mechanism of cerebellar learning, see de Schutter (1995) and Schonewille et al. (2011).

2. Challenges to the LTD hypothesis

2.1. Challenge I: mechanism of simple spike suppression

During the Purkinje cell CR, the simple spike firing is completely suppressed by the CS-activated mossy and parallel fibres. It is not evident that this can be the result merely of a depression of excitatory input as assumed by the LTD hypothesis. Removing the excitatory input added by the CS can bring the cell firing back to its background level, but cannot by itself inhibit the cell below this level. It is sometimes assumed that the background firing of the Purkinje cell was caused by a background excitatory input from parallel fibres. However, it has been shown by Cerminara and Rawson (2004) that Purkinje cells have an intrinsic spike