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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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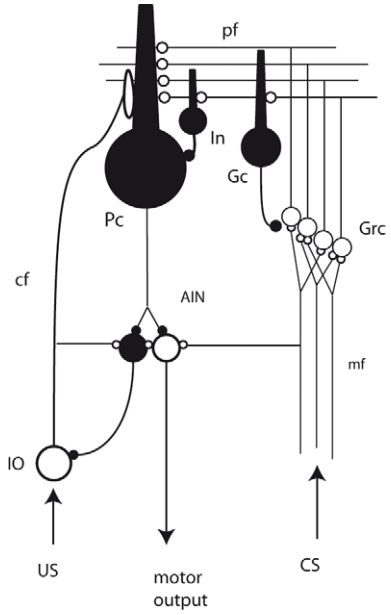

Fig. 1. Synaptic organisation of cerebellar module. Purkinje cell (Pc) controlling blink receives conditioned stimulus (CS) signal via mossy fibres (mf), Granule cells (Grc) and parallel fibres (pf). Different CSs are assumed to activate different mfs and pfs. The unconditioned stimulus (US) is signalled 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

generating mechanism and that they fire at high rates even in the absence of excitatory synaptic input. They found that application of the AMPA receptor antagonist CNQX totally blocked responses to pf input but had little or no effect on the resting level of simple spike firing. To completely silence the cell, as during a Purkinje cell CR, would require an active inhibition; removal of pf excitation is not sufficient.

It could be argued that pf impulses generated by the CS before learning would activate inhibitory interneurons, which would inhibit the Purkinje cell so as to exactly cancel the excitatory input. While this may be possible, it requires some quite strong assumptions. The CS often has no discernible effect on the Purkinje cell before training. This could mean that the pf input has a negligible effect on Purkinje cell synapses, but then LTD would not enable the pf input to depress firing. Alternatively, the absence of a simple spike response to the pf input before training could be due to a perfect balance between excitation and inhibition. This would have to include the time course of the EPSP and the IPSP elicited by the pf input, but this is unlikely. Excitation by pf input to the Purkinje cells will normally be able to elicit a spike before the IPSP elicited by inhibitory interneurons can take effect. Furthermore, the IPSPs normally have a longer duration than the EPSP (Eccles, Ito, & Szentagothai, 1967; Konnerth, Llano, & Armstrong, 1990).

2.2. Challenge II: conditions of LTD induction

It has been well known in the behavioural literature that eyeblink conditioning does not occur when the interval between the CS and the US is shorter than about 100 ms. This is not merely a relative limitation, so that conditioning would be less efficient at short intervals. There is a threshold CS–US interval below which learning does not occur at all (Gormezano & Moore, 1969). We have tested the effect of using short CS–US intervals on Purkinje cell CRs as well. The results are quite clear-cut. Paired CS–US presentations at intervals of 150 ms or more lead to the development of typical pause responses (Purkinje cell CRs). After training with a CS–US interval of 50 ms, the Purkinje cell responds to the CS with an *increase* in simple spike firing (Wetmore, 2009).

LTD, as traditionally conceived, cannot readily account for these results because it would be most effective at short delays between pf and cf input. Most work on LTD since it was first demonstrated has used simultaneous pf–cf input, so-called "conjunctive stimulation", and comparisons of various delays between pf and cf stimulation have indicated that LTD is optimal when the delay is close to zero (Ekerot & Kano, 1989; Ito, 2001; Karachot, Kado, & Ito, 1995).

In contrast, a couple of recent *in vitro* studies have reported that LTD does not, as originally claimed, work best with simultaneous climbing and parallel fibre input. Rather, they suggest that the strongest LTD is obtained when pf input precedes cf input with amounts that resemble the temporal requirements of eyeblink conditioning (Chen & Thompson, 1995; Safo & Regehr, 2008; Wang, Denk, & Häusser, 2000). Such findings have been taken to strengthen the case for LTD as a mechanism for conditioning, but when examining the details of these papers a number of problems with this interpretation become apparent.

Chen and Thompson (1995) investigated the importance of the temporal relationship between pf and cf input for inducing LTD in the rat cerebellar slice preparation. When presenting 100 paired cf and pf stimuli, they found strong LTD when pf input preceded cf input by 250 ms but no LTD when the inputs were simultaneous or when cf stimulation preceded pf stimulation. The authors suggest that this temporal specificity might explain the temporal properties of conditioning.

The observation, that learning may be more effective when pf input is considerably earlier than cf input, is indeed an interesting and suggestive parallel between classical conditioning and LTD. Before concluding that this temporal asymmetry is the explanation for the lack of conditioning at short CS–US intervals, however, we also need to consider some discrepancies between the *in vitro* and *in vivo* work.

Firstly, when these authors applied a training protocol with 600 trials, which is actually much closer to what would be needed to get conditioning in an intact animal, LTD occurred with simultaneous pf–cf activation and even when the cf input *preceded* the pf input by several hundred milliseconds.

A second problem is the short intertrial interval (time between paired stimulus presentations) used. It is generally accepted among students of eyeblink conditioning that learning does not occur if training trials are too closely spaced in time, although the exact minimum interval is not known and may be different in different species. An intertrial interval of 10 s seems to work in mice (Chettih, McDougle, Ruffolo, & Medina, 2011). Kehoe and Macrae (2002) write that 4 s does not result in learning but make no claim about the minimum interval. A study that explicitly tested the possibility of obtaining conditioned blinks in rabbits with an interval of 10 s concluded categorically that this was not possible (Nordholm, Lavond, & Thompson, 1991). Yet, the Chen and Thompson study from the same lab employed an intertrial of only one second, by all accounts much too short to obtain conditioning in whole animals.

A third problem is that the induction of LTD was extremely rapid compared to what is normal in behavioural conditioning in intact animals. In one set of experiments Chen and Thompson used 100 trials at one per second, that is 1.67 min of training, and obtained appreciable LTD within a couple of minutes. In another group of experiments they used 600 trials at one per second. The full LTD effect was present immediately after this ten-minute training period. In contrast, in a typical behavioural experiment, the animal would receive two to three trials per minute and a minimum between one and two hours of such training would be necessary to achieve appreciable conditioning. Reducing the interval between each trial dramatically increases the number of trials needed and cannot reduce the minimum training time by much (Kehoe & Macrae, 2002). This experience of training in intact animals is confirmed by our work with decerebrate ferrets. A minimum of two to three hours is usually necessary for conditioning of both blink CRs and Purkinje cell CRs (Hesslow & Ivarsson, 1996; Jirenhed et al., 2007).

It cannot be excluded that the discrepancies between this study and what is known about the behaviour are due to features of the *in vitro* preparation. A perfect correspondence between results obtained under such different experimental conditions should not be expected. On the other hand, the discrepancies are considerable. For instance, one would not expect learning in a preparation at 22 °C to be very much faster, measured in minutes rather than hours, than that observed in an intact animal at normal body temperature. In our view, therefore, the above considerations suggest that the learning process observed by Chen and Thompson may not be the same as that underlying conditioning.

Very similar considerations apply to the other *in vitro* studies of relative timing of pf and cf inputs. Wang et al. (2000) measured both LTD and the size of the Ca^{2+} signals obtained by paired pf–cf stimulation at different intervals. These "supralinear" Ca^{2+} signals (larger than the sum of those obtained by pf and cf stimulation alone) are believed to induce LTD. They found that both LTD induction and the supralinear Ca^{2+} signals were much more efficient when pf stimulation preceded cf stimulation, but the intervals did not match the behavioural data. The strongest Ca^{2+} signals were obtained when the pf–cf interval was very close to 50 ms and quite strong signals were obtained with a zero and even with negative CS–US intervals.

This study also used very short intertrial intervals. LTD was induced by delivering 50 pairings at 2 s intervals. Each pairing was composed of 5 pf stimuli (100 Hz) and 1 cf stimulus. In spite of this, LTD induction was extremely rapid. After less than two minutes of training had been completed, LTD was appreciable within five minutes and reached its maximal level after just over ten minutes.

The study by Safo and Regehr (2008) is the only one using an intertrial interval that resembles those normally used in behavioural experiments, 10 s. LTD was strongest when the pf input preceded the cf input by about 80 ms, but LTD was almost as strong at zero and even at slightly negative pf–cf intervals, clearly at variance with data from *in vivo* conditioning experiments. Furthermore, as in the previously discussed *in vitro* studies, learning was very much faster than that observed *in vivo*. Training consisted of 30 paired trials with an interval of 10 s. Strong LTD was present immediately after this five minute training period.

It might be argued that the fact that all the above studies observed LTD at short CS–US intervals is not a decisive objection, because LTD was still less efficient at these intervals. Perhaps animals would learn at short intervals if training had been continued for longer periods. This is unlikely, however, because training Purkinje cells with a 50 ms CS–US interval is not only less efficient; it results in an increase in the simple spike response to the CS Wetmore (2009).

It should be noted that the usual way of formulating the temporal requirement in conditioning could be very misleading. The CS must precede the US by a certain minimum amount of time (in the neighbourhood of 75–100 ms), but this refers to the *onset* of the CS. In the standard delay protocol the CS continues throughout the pf–cf interval and terminates together with the cf input. This means that most of the pf input will actually occur at intervals much shorter than the CS-US interval. Some of them will actually coincide with the cf input. It is well known that the delay protocol is more efficient, i.e. leads to faster conditioning, than trace conditioning in which the CS is shorter and leaves a gap between CS and US. It follows that the pf inputs that occur late during the CS and therefore close to or coinciding with the US also contribute to the learning.

The argument above should not be taken as a critique of the *in vitro* work on cerebellar learning. Our point is merely that there are several discrepancies between the properties of LTD, as revealed by *in vitro* experiments, and what is known about conditioning. Furthermore, no single discrepancy by itself can be decisive because of necessary methodological constraints on the experiments. Nor do we want to give the impression that LTD is irrelevant to all forms of cerebellar learning. It could be important for other forms of motor learning and it might play a role in eyeblink conditioning, but if so only in conjunction with other mechanisms, in particular to account for the adaptive timing of CRs.

2.3. Challenge III: adaptive timing and LTD

The third major challenge to the LTD hypothesis is the adaptive timing of the overt blink CR as well as the Purkinje cell CR.

Even if a conditioning protocol could result in LTD, it is not easy to see how LTD alone could make the parallel fibres elicit responses with right temporal properties. But the Purkinje cell CR has a specific learned time course with a long delay, a maximum just before the US and terminates just after the US even if the CS continues several hundred milliseconds after the US.

Models of conditioning based on LTD have therefore included various additional mechanisms (Yamazaki & Tanaka, 2009). Essentially, these assume that there is a temporal pattern in the granule cell responses to the mossy fibre input carrying the CS signal (cf. Fig. 2). If different granule cells respond at different times after the CS onset, they will have different temporal relations to the cf input and the corresponding synapses will be affected differently. The synapses affected most strongly will be those that were activated at a time relative to the cf input that is most conducive to LTD. After learning, only these parallel fibres will take part in generating the Purkinje cell CR, which will then be appropriately timed.

Different models postulate different mechanisms by which such delays and temporal patterns in the granule cell responses could arise (Yamazaki & Tanaka, 2009). We will not be concerned by these here, because we question the existence of such temporal patterns in the granule cells.

As far as we know, granule cells continue to respond to the mossy fibre inputs throughout the CS. This is shown by results in Jirenhed and Hesslow (2011b) and is also supported by the observation in our lab that the excitatory response (in contrast to the inhibitory Purkinje cell CR) that develops with short CS–US intervals continues during the CS and terminates shortly after it (Wetmore, 2009).

In an experiment reported in Jirenhed and Hesslow (2011b) we used a 50 Hz train of electrical stimuli to the mossy fibres as the CS and followed a Purkinje cell for several hours of training with paired CS–US presentations. (A simplified illustration of this experiment is shown in Fig. 3.) The duration of the CS was 800 ms. Before learning, a mossy fibre stimulus in the CS would sometimes evoke a simple spike response in the Purkinje cell. Peri-stimulus time histograms from four selected time-points (only three shown in Fig. 3) during the CS showed two things.

First, the response to a single mossy fibre stimulus was very brief. There was no discernible effect on simple spike firing after 5–10 ms, perfectly consistent with the finding of Jörntell and Ekerot (2006) that granule cells respond to mossy fibre input with a brief and short latency burst of action potentials.

Second, the responses were rather similar throughout the 800 ms CS. An important implication of these observations is that there does not seem to be anything in the parallel fibre input to the cortical neurones that could provide a temporal code. In the same paper it was shown that a very brief CS, in some cases a single mossy fibre stimulus was sufficient to elicit a normally timed Purkinje cell CR with the same onset and offset latencies as a Purkinje cell elicited by a 50 Hz, 400 ms train of stimuli. However, if the time course of the Purkinje cell CR depends on the temporal pattern of the parallel fibre input, it follows that a single mossy fibre impulse can result in the same temporal pattern of parallel fibre input as a 400 ms 50 Hz stimulus train. In view of the data just summarised, this does not seem likely.

2.4. Challenge IV: no LTD was observed in conditioned Purkinje cells

In the experiment just described, we used direct 50 Hz stimulation of the mossy fibres as the CS and followed a Purkinje cell for several hours of training with paired CS–US presentations (cf. Fig. 3). The CS–US interval was 200 ms and the CS duration 800 ms. The CS thus outlasted this interval by 600 ms. As expected, training caused the development of a typical Purkinje cell CR. As illustrated in Fig. 3, A and B of (Jirenhed & Hesslow, 2011b) the spontaneous simple spike firing was completely suppressed during the later two-thirds of the CS–US interval and a few tens of milliseconds after the US. During this period, there was also a depression of the spikes elicited by the mossy fibre stimuli that constituted the CS.

The mossy fibre pulses before and after the CR elicited very similar responses in the Purkinje cell. Now, a very significant observation about these responses was that they were also very similar to the responses elicited before training. That is, although there was some variability, the average Purkinje cell response

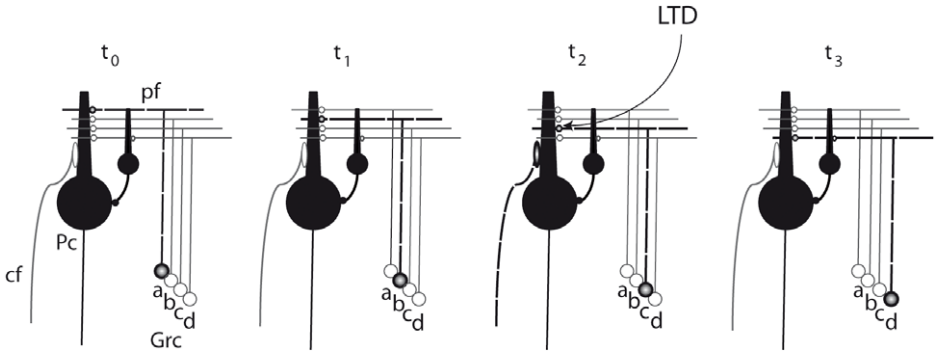

Fig. 2. Delay line theory of CR timing. The CS will activate different granule cells with different delays. At t_0 granule cell a will fire, at t_1 b will fire etc. In this case only cell c fires in close temporal proximity to the climbing at t_2, so only the synapse between a and the Purkinje cell will undergo LTD. In the future, only c will elicit a Purkinje cell response, which, because of the delay, will be correctly timed.

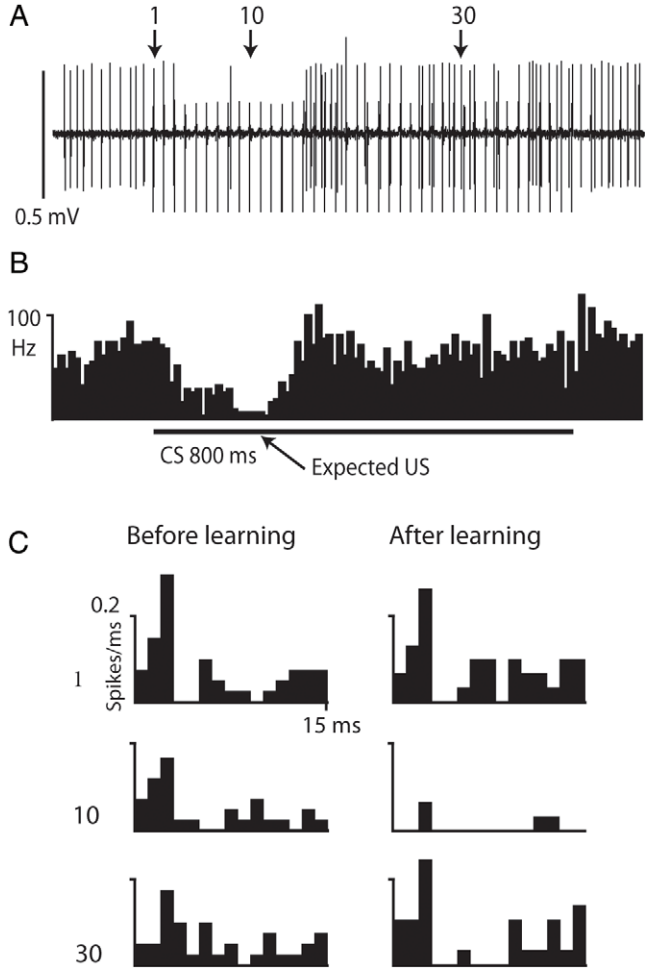

Fig. 3. Experiment showing absence of LTD during eyeblink conditioning (modified after Jirenhed & Hesslow, 2011b). The CS was an 800 ms 50 Hz train of direct mossy fibre stimuli and the US direct climbing fibre stimulation 200 ms after the CS onset. A: Sample record of a Purkinje cell CR. Shock artefacts generated by the CS can be seen as vertical lines extending below the simple spikes. B: Peri-stimulus time histogram over 40 trials. Notice that the suppression of simple spike firing (Purkinje cell CR) ends just after the expected US even though the CS continues for six hundred milliseconds. C: Poststimulus time histograms of responses to the mossy fibre stimuli 1, 10, 30 indicated by arrows in A, before (left) and after (right) training. Each bin is 1 ms and the *y*-axis indicates probability of firing. The response to stimulus 10 is depressed after training. The responses to stimuli 1 and 30 are not depressed, as they would have been if the pf to Purkinje cell synapses had been depressed.

to a single mossy fibre stimulus before and after training was quite similar. It was only the responses during the CR that were depressed after learning. Before and after the CR, the cell responded with roughly equal probability. This would seem to suggest that there was no depression of parallel fibre to Purkinje cell synapses, that is, no LTD.

It could be objected here that the mossy fibre stimuli might activate different sets of granule cells at different times during the CS. This is possible but unlikely. Identical electrical stimuli were presented to the mossy fibres throughout the CS and it has previously been shown that granule cells reliably follow much higher frequencies and with no apparent temporal patterning (Jörntell & Ekerot, 2006).

3. Models not relying on granule cell delays

Although most theories of eyeblink conditioning depend on an assumption of temporal patterns in granule cell firing, other ideas about how timing of CRs can be learned have also been proposed. Fiala and coworkers have proposed a model based on the metabotropic glutamate receptor (mGluR) (Fiala, Grossberg, & Bullock, 1996). These mGluRs elicit slow Ca^{2+} signals and Ca^{2+}-activated K^+ currents, which could hyperpolarise the cell and cause a Purkinje cell CR. The authors suggest that the latency of the Ca^{2+} response depends on the number of available mGluRs. Purkinje cells were assumed to have different densities of mGluRs, producing a corresponding variation across cells in the rate of intracellular Ca^{2+} increases. Depending on the mGluR density, a Purkinje would be "specialised" for a specific CS–US interval.

This model is contradicted by the findings of adaptive timing Purkinje cell CRs (Jirenhed & Hesslow, 2011a). Rather than different Purkinje cells learning different CS–US intervals, it appears that any cell can learn to respond with any latency. All Purkinje cells acquired CRs that were adaptively timed to the CS–US intervals and it was even observed that a cell could change the CR latency when the CS–US interval was changed.

An alternative theory based on mGluRs has been suggested by Steuber and Willshaw (2004). They assumed that the number of mGluRs changes with learning. Training with a particular pf–cf interval would change the number of mGluRs until the appropriate CR latency was achieved. This theory is contradicted by our data on three counts.

Firstly, the model predicts that the CR latency decreases as learning proceeds. In contrast, Jirenhed et al. (2007) found that although size of the Purkinje cell CR increased, the peak latency remained the same throughout learning.

Secondly, changing the time of the US after learning would be expected to cause a gradual change in the CR latency. But, both behavioural studies and our recording data suggest that changing the CS–US interval will cause two simultaneously occurring but independent processes: extinction of the first CR and acquisition of a second CR at the new US time (Jirenhed & Hesslow, 2011a; Kehoe & Macrae, 2002).

Thirdly, as the authors themselves point out, the model would be difficult to reconcile with double peaked CRs. It has long been known in the behavioural literature, that training with two different CS–US intervals at alternating trials will result in a behavioural CR with two adaptively timed peaks (Millenson, Kehoe, & Gormezano, 1977). This seems to be due to two peaks in simple spike depression in the Purkinje cell. When Jirenhed et al. changed the CS–US interval after a Purkinje cell CR had developed, a second Purkinje cell CR appeared before the first one had extinguished, giving rise to a double peaked simple spike suppression (Jirenhed & Hesslow, 2011a).

4. Conclusions

The data from Purkinje cell recordings are difficult to reconcile with any of the published models of conditioning, in particular the LTD hypothesis. We have called the objections "challenges to the LTD hypothesis" to emphasise that no argument by itself can be taken as conclusive. Nevertheless, we believe that, taken together, they considerably weaken the case for LTD. Indeed, they undermine the idea that strengthening or weakening of synaptic connections can account for all forms of learning and memory. The suggestion that different Purkinje cells learn different temporal relationships is also contradicted by the data. The model proposed by Steuber and Willshaw fares somewhat better but also runs into serious difficulties.

We do not want to give the impression that we think that LTD is irrelevant to all forms of cerebellar learning. It could well be important for other forms of motor learning and it might play a role in eyeblink conditioning, but if so, only in conjunction with other mechanisms, in particular to account for the adaptive timing of CRs. There are several forms of synaptic plasticity in the cerebellum and a combination that includes LTD may achieve what this mechanism on its own cannot do (Gao, van Beugen, & de Zeeuw, 2012).

What other kind of mechanism could explain classical conditioning? We do not wish to propose an alternative model and we do not want to imply that there can only be one mechanism or that it has to be the same for all CS–US intervals, but we would like to end this paper by pointing out some features that a plausible mechanism for conditioning must have.

The data suggest that the delay of the Purkinje cell CR does not depend on delays in pf inputs but rather on a mechanism that resides in the Purkinje cell itself (or possibly in inhibitory interneurons). After training with an appropriate protocol, the pf input seems to activate a molecular mechanism with a particular constant delay, after which a hyperpolarising response with a specific duration is turned on. The delay might seem to be adjustable, like in a kitchen timer, except that the CR latency after a shift in the CS–US interval does not change gradually. As pointed out above, the response is extinguished while a new response with a new latency is learned. This suggests that there is a family of "timer units" (perhaps metabotropic receptors or channels or molecular mechanisms that can delay channel openings) and that the learning process selects the appropriate units. Such a mechanism would also be compatible with the fact that the Purkinje cell seems to be able to harbour (at least) two different CR latencies at the same time. Furthermore, once a timer unit has been started, it runs its course with the specific delay and on and offset of the response, regardless of further input. Thus, a very brief pf input or an input outlasting the CS–US interval would elicit similarly timed responses. We do not want to speculate more on the nature of these timer units, but suggest that it might be a fruitful project to try to identify them.

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