TIMING OF CLASSICALLY CONDITIONED PURKINJE CELL PAUSE

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Classical conditioning

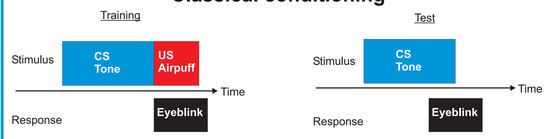


Fig. 1
Schematic illustration of the classical conditioning paradigm. The conditioned stimulus (CS) is presented before the unconditioned stimulus (US) that elicits a reflex response. When testing after learning, presentation of the CS alone will elicit the response.

Experimental set-up

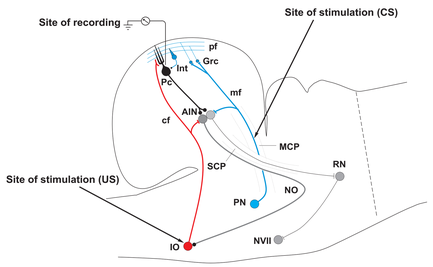


Fig.2
Experimental set-up and wiring diagram of the cerebellar cortex, deep cerebellar nuclei and the inferior olive. Purkinje cell (Pc), Interneuron (Int), Climbing fibre (cf), granule cell (Grc), mossy fibre (mf), parallel fibre (pf), anterior interpositus nucleus (AIN), inferior olive (IO), nucleo-olivary fibres (NO), red nucleus (RN), facial nucleus (NVII), superior cerebellar peduncle (SCP), middle cerebellar peduncle (MCP), pontine nuclei (PN).

Introduction

The cerebellar cortex has been shown to be critically involved in classical conditioning of the eyeblink response (see Fig. 1). Purkinje cells (Pc) and interneurones in the cerebellar cortex receive convergent input from mossy fibres (mf) and climbing fibres (cf), generally assumed to transmit the CS and US-signals respectively. Cerebellar Cortical Conditioning models predict that Pc involved in control of the eyeblink response will learn to pause (since Pc are inhibitory) in response to the CS. Such a pause will disinhibit the anterior interpositus nucleus (AIN) and elicit an eyeblink. We have previously demonstrated that the mf transmit the CS-signal to the cerebellum and that a well-timed pause in Pc activity is present in animals conditioned with a periorbital US. Furthermore, paired mf and inferior olive (IO) stimulation has been shown to induce such a pause in the Pc (poster Neuroscience 2003).

Conditioned eyeblink responses are characteristically well-timed to occur just prior to the onset of the US, thus providing maximum protection from the noxious stimulus. A change in the inter stimulus interval (ISI) between the CS and US will cause a change in eyeblink response timing, such that the response will adapt to the new interval. If Pc are indeed critically involved in the control of conditioned eyeblink responses, then the timing of a conditioned Pc pause should also adapt when the ISI is changed during conditioning with mf and IO stimulation.

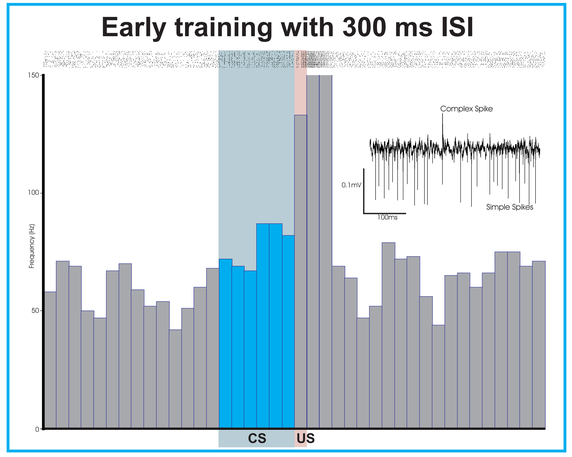
Methods

Two experiments were performed on decerebrate male ferrets (Fig. 2) during standard delay conditioning. In the first experiment, EMG-recordings were made from the *m. orbicularis oculi* and in the second experiment the activity of a single Pc in the c3 zone of the cerebellar cortex was recorded extra cellularly *in vivo*.

- CS: A 50 Hz, 90 μA electrical stimulus train was applied to the mf in the middle cerebellar peduncle.
- US: Two 10 ms electrical stimulus trains (5 pulses at 500 Hz) were applied to the periorbital area (3 mA) in the EMG-recording experiment, or to the IO (150 μA) in the Pc recording experiment.

Initial acquisition training was performed with a 300 ms ISI (and CS duration) until reliable conditioned responses (eyeblinks or Pc pauses) were observed. The ISI and CS duration were then increased to 500 ms. Paired CS-US stimulation with the longer ISI continued for an additional 400 trials.

Early training with 300 ms ISI

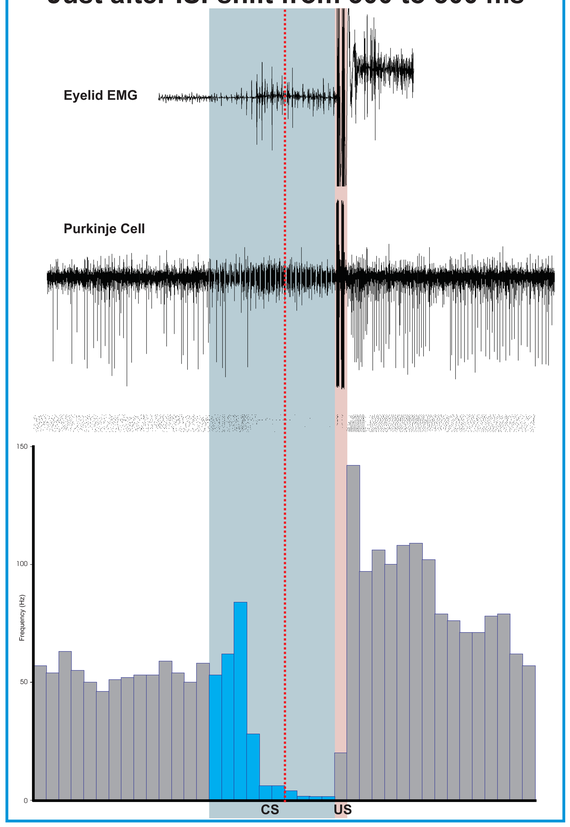


Results

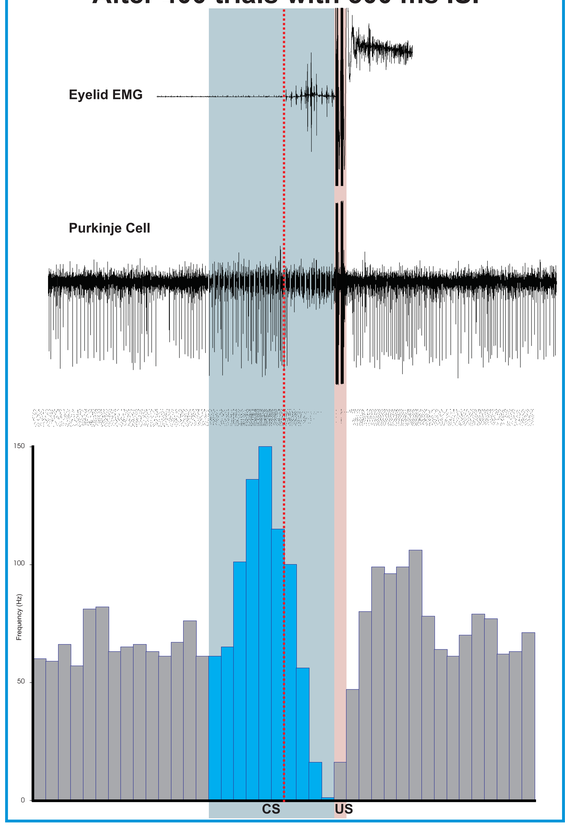
Eyeblink EMG-recordings
After acquisition, eyeblink CRs were timed in accordance with the shorter (300 ms) ISI. The response latency was approximately 200 ms after CS-onset. After the following 400 trials of training with the longer (500 ms) ISI, CRs had adapted to the longer ISI and the latency had increased to ~350 ms.

Purkinje cell recordings
The Pc response to the mossy fibre CS was initially excitatory, i.e. produced increased simple spike activity. However, after 900 trials of paired mf and IO-stimulation, the Pc had developed a reliable pause response to the CS with an onset latency of ~175 ms. After 400 trials of training with the longer (500 ms) ISI, the Pc pause had adapted to the longer ISI and the onset latency had increased to ~350 ms.

Just after ISI-shift from 300 to 500 ms



After 400 trials with 500 ms ISI



Conclusions

- In animals conditioned with a mossy fibre CS and inferior olive US, Purkinje cells acquire a pause response which is timed to the inter stimulus interval.
- An increase of the inter stimulus interval causes the Purkinje cell pause response latency to increase accordingly after 400 trials of paired CS-US stimulation.
- The observed change in timing of the Purkinje cell pause response shows a striking similarity to the observed change in timing of eyelid EMG-activity in animals conditioned with a periorbital US.

These observations are in agreement with previous investigations and strongly support the hypothesis that eyeblink conditioning causes acquisition of a pause in Pc simple spike activity induced by converging mf and cf input. Furthermore, these data suggest a critical role for the cerebellar cortex in the acquisition, expression and the temporal control of conditioned responses.

Aknowledgements

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