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Research Report

Disrupted topography of the acquired trace-conditioned eyeblink responses in guinea pigs after suppression of cerebellar cortical inhibition to the interpositus nucleus

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ABSTRACT

Trace conditioning of the eyeblink reflex, a form of associative motor learning in which presentations of the conditioned stimulus (CS) and the unconditioned stimulus (US) are separated in time by a silent trace interval, requires intact forebrain structures such as the hippocampus and medial prefrontal cortex. Recently, increased learning-related activities have also been observed in specific cerebellar cortical area such as the lobule of HVI during this conditioning task. To date, however, it remains controversial how the cerebellar cortex contributes to trace eyeblink conditioning. In the present study, we addressed this issue by reversibly suppressing the cerebellar cortical inhibition via microinjections of the $GABA_A$ receptor antagonist bicuculline methiodide (BICM) into the interpositus nucleus of guinea pigs. We showed that, in the well-trained guinea pigs, the BICM administrations failed to abolish the acquired trace-conditioned eyeblink responses (CRs). Although the acquired trace CRs were mostly retained, their peak latencies were shortened and their peak amplitudes diminished as evidenced by only half of the spared trace CRs preserving the topography of adaptive peak latencies or middle-/high-peak amplitudes. In the same animals, the acquired trace CRs were abolished by microinjections of the $GABA_A$ receptor agonist muscimol and were unaffected by microinjections of the artificial cerebrospinal fluid. Furthermore, we demonstrated that with concurrent BICM-induced suppression of the cerebellar cortical inhibition and presentations of the tone CSs in the guinea pigs receiving unpaired conditioning training, CR-like eyeblink

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Abbreviations: aCSF, artificial cerebrospinal fluid; BICM, bicuculline methiodide; CR, conditioned eyeblink response; CS, conditioned stimulus; GABA, γ-aminobutyric acid; LTD, long-term depression; MSC, muscimol; PC, Purkinje cell; pcd, Purkinje cell degeneration; pf, parallel fiber; SEM, standard error of the mean; SR, startle eyeblink response; UR, unconditioned eyeblink response; US, unconditioned stimulus

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responses were not generated. Altogether, these results support the hypothesis that GABAergic neurotransmission from cerebellar cortex to the interpositus nucleus may participate in regulating the expression of acquired trace CRs.

1. Introduction

Learning to regulate the appropriate occurrence timing and strength of motor operations is one of the most striking properties of mammalian cerebellum (Middleton and Strick, 1998; Spencer et al., 2003; Strata et al., 2009). However, our understanding of the way by which the mammalian cerebellum participates in motor learning is currently a matter of intensive research (for reviews, see Woodruff-Pak and Disterhoft, 2008; Thompson and Steinmetz, 2009).

To date, classical eyeblink conditioning is one of the most widely used model systems for evaluating the mammalian cerebellar involvement in associative motor learning, which involves paired presentations of a behaviorally neutral conditioned stimulus (CS; such as a tone) and an aversive unconditioned stimulus (US; such as a airpuff in the eye). Initially, the mammalian organism produces only a reflexive unconditioned eyeblink response (UR) to the US. After hundred paired presentations, in response to the CS, the organism learns to close the eye before the onset of the US (called conditioned eyeblink response, CR). As a consequence, the appropriate occurrence timing and amplitude is equally important for the expression of CRs (for review, see Thompson and Steinmetz, 2009). It has been well established that, during eyeblink conditioning, signals produced by the CS and the US are carried to the cerebellum via the mossy fibers and the climbing fibers, respectively, where they ultimately converge on Purkinje cells of the cerebellar cortex as well as on neurons of the interpositus nucleus (Mauk et al., 1986; Steinmetz et al., 1989; Hesslow et al., 1999).

Mounting evidence has suggested that the cerebellar cortex is involved in regulating the latency and/or amplitude of CRs during delay conditioning, in which the CS precedes, overlaps and coterminates with the US. Results from the metabolic and functional imaging researches have revealed that specific cerebellar cortical area such as the lobule of HVI is significantly activated during delay eyeblink conditioning (Ramnani et al., 2000; Miller et al., 2003; Plakke et al., 2007; Cheng et al., 2008). Further analysis of single unit activity in the lobule of HVI during delay eyeblink conditioning has shown a specific manner strongly correlated with the expression of CRs (McCormick and Thompson, 1984; Gould and Steinmetz, 1996). In parallel with the findings mentioned above, disrupted CR latency and amplitude of various degrees have been demonstrated after disconnection from cerebellar cortex to the interpositus nucleus (Perrett et al., 1993; Garcia and Mauk, 1998; Bao et al., 2002; Aksenov et al., 2004; Parker et al. 2009) and in patients with cerebellar cortical degeneration disorder (Gerwig et al., 2005; Dimitrova et al., 2008). Several investigators have proposed that long-term depression (LTD) at the parallel fiber (pf)-Purkinje cell (PC) synapse is a key mechanism within the cerebellar cortex subserving for regulating the CR expression during delay eyeblink conditioning (Koekkoek et al, 2003; Wada et al., 2007).

In contrast, contribution of the cerebellar cortex to the expression of CRs during trace conditioning, in which the CS and US are separated by a time gap (called trace interval), remains controversial. It has been reported that the cerebellar cortical pf-PC LTD deficient mice acquire the adaptive trace CRs (Kishimoto et al., 2001a,b,c; Woodruff-Pak et al., 2006), implying that the cerebellar cortical pf-PC LTD is not essential for trace eyeblink conditioning. In agreement with the findings from the gene-manipulation studies, in human patients with cerebellar cortical degeneration, trace eyeblink conditioning is less impaired than the delay paradigm when the trace interval is relatively long, suggesting that the cerebellar cortical function is minimal during this conditioning task (Gerwig et al., 2008). Contrasted with the aforementioned findings, nevertheless, an extending previous study reported that rabbits with aspirations of the lobules of HVI and HVII transiently decreased the acquired trace CRs (Woodruff-Pak et al., 1985). Furthermore, evidence that increased learning-related activities of the lobule of HVI has been observed in rats (Plakke et al., 2007) and humans (Cheng et al., 2008) during trace eyeblink conditioning, indicating a possible involvement of the cerebellar cortex in this conditioning task. Hippocampal function, in the form of theta band oscillations, is shown to predict and modulate trace eyeblink conditioning (Seager et al., 2002; Griffin et al., 2004; Nokia et al., 2009). Strikingly, in two recent researches, theta band oscillations in the lobule of HVI are demonstrated to be synchronized with those in the hippocampus during trace eyeblink conditioning (Wikgren et al., 2010; Hoffmann and Berry, 2009), supporting an assumption that the hippocampal-cerebellar cortical functional integrity may participate in trace conditioning of the eyeblink reflex. A current fundamental issue is that if area like the lobule of HVI contributes to the expression of CRs during delay eyeblink conditioning, should it also play a similar role during the trace paradigm?

It has long been demonstrated that GABAergic projection from PCs to the interpositus nucleus neurons represents the sole output of the cerebellar cortex (Voogd and Glickstein, 1998). Hence, selective suppression of the cerebellar cortical inhibition via microinfusions of the $GABA_A$ receptor antagonist into the interpositus nucleus has been suggested to be an effective approach to investigate the cerebellar cortical involvement in delay eyeblink conditioning (Mamounas et al., 1987; Garcia and Mauk, 1998; Bao et al, 2002; Aksenov et al., 2004; Parker et al., 2009). This approach is characterized as a temporary disruption of the cerebellar cortical inhibition rather than a permanent decortication of the cerebellum. With this approach, this study aims to investigate how the cerebellar cortex contributes to the expression of acquired CRs during trace conditioning using a 500-ms trace interval. We hypothesize that if expression of the acquired trace CRs requires the cerebellar cortical modulation, latencies and/or