Electrophysiological activity from the eye muscles, cerebellum and cerebrum during reflexive and voluntarily timed blinks

Neil Todd (n.todd@unsw.edu.au)
UNSW Clinical School, Randwick Campus Prince of Wales Hospital
Sendhil Govender
Neuroscience Research Australia
Peter Keller
Western Sydney University Penrith
James Colebatch
UNSW Clinical School, Randwick Campus Prince of Wales Hospital

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Abstract

We report an experiment to investigate the role of the cerebellum and cerebrum in motor learning of timed movements. Eleven healthy human subjects were recruited to perform two experiments, the first a classical eye-blink conditioning procedure with an auditory tone as conditional stimulus (CS) and vestibular unconditional stimulus (US) in the form of a double head-tap. In the second experiment subjects were asked to blink voluntarily in synchrony with the double head-tap US preceded by a CS. Electrophysiological recordings were made of extra-ocular EMG and EOG at infra-ocular sites (IO1/2), EEG from over the frontal eye-fields (C3'/C4') and from over the posterior fossa over the cerebellum for the electro-cerebellogram (ECeG). The behavioural outcomes of the experiments showed weak reflexive conditioning for the first experiment despite the double tap but robust well-synchronised voluntary blinks for the second. Voluntary blinks were larger than the reflex ones. For the voluntary task a contingent negative variation (CNV) was also present in the EEG leads prior to movement, and modulation of the high-frequency EEG occurred during movement. Stimulus-related cerebellar activity was prominent in the high-frequency ECeG for both conditions, while movement-related cerebellar activity was additionally present in the voluntary condition. These results demonstrate a role for the cerebellum in voluntary timed movement analogous to that in classical conditioning.

Introduction

The cerebellum has been considered to be difficult to record from non-invasively (1). Reasons for this include the particular anatomy of the cerebellum causing electrical cancellation. Nevertheless, Andersen et al. (1) felt that recording cerebellar activity should be possible. Our prior observations have suggested that evoked potentials can be recorded from surface electrodes over the cerebellum in response to vestibular stimuli evoked using acoustic and inertial stimuli (2–5) and localised to the posterior cerebellum (6–9). It was also found that cerebellar evoked potentials (CEPs) could be recorded to axial stimuli, which activate muscle afferents (9, 10). These observations suggest that the ability to detect effects may depend upon the type of afferent stimulated whereby vestibular and axial projections can be detected, presumably reflecting the location of their cerebellar targets. A further important development was the realisation that in addition to CEPs, the spontaneous activity from the cerebellum could also be detected using surface recordings (the electro-cerebellogram or ECeG; 11, 12). The ECeG has a characteristic high-frequency power spectral profile, including frequencies well above the high gamma range, extending up to several hundred Hz (11, 12). CEPs of both vestibular and axial origin were associated with powerful short-latency modulation of the ECeG in the form of post-stimulus pausing and bursting (9). These properties led to the suggestion that the CEPs might be of climbing-fibre (CF) origin as post-stimulus pausing of simple spike activity is a known characteristic of CF responses (CFRs) (13). Other properties of CEPs, their polarity, latency and laterality are also consistent with a climbing-fibre origin (5, 14).

In Todd et al. (15) we reported an eye-blink conditioning experiment using a mastoid tap unconditional stimulus (US). This is known to activate vestibular receptors and produce an eye-blink unconditioned

response (UR) and was paired with auditory and visual conditional stimuli (CS). The experiment did produce eye-blink conditioning, albeit weak, which subsequently showed extinction. However, of particular interest were the observations showing ocular EMG activity and both unconditioned and conditioned post-CFR pausing in the ECeG, in turn believed to be the substrate for the eye-blink conditioned response (CR) (16, 17). These observations were confirmed in a subsequent eye-blink conditioning study using a more conventional trigeminal nerve US (18). In particular the presence of conditioned pausing in the high-frequency ECeG was observed to be aligned with the overt CR. Together these support the interpretation of the modulation of the high-frequency ECeG as a manifestation of underlying simple spike activity. However, the results also supported the view that while conditioned cerebellar pausing may be necessary, other structures are likely involved in humans, including the basal ganglia (19). A robust conditioned contingent negative variation (CNV) of likely central origin was also present prior to CR onset in parallel with the changes in the ECeG.

The present experiment is an extension of the Todd et al. (15) design in which the single tap US has been replaced by a double tap US. In addition, the classical eye blink conditioning procedure was followed by an experiment in which subjects were instructed to blink in synchrony with the double head taps, thus allowing direct comparison of cerebellar activity during reflex and voluntary blinks. Prior work has confirmed that human subjects can learn timed voluntary blinks (20). It may be that mechanisms of voluntary and involuntary motor learning overlap (21), and that Purkinje cells participate in learning or predicting sequences of responses in both cases (22). Thus this study could provide evidence for the role of the cerebellum in sensorimotor synchronisation, of interest given the supposed role of the cerebellum in motor timing (23, 24). If sensorimotor synchronisation is conceptualised as a form of classical conditioning, then the cerebellum might play a role in both learning and timing. The hypothesized link between conditioning and timing might in turn be manifest in activity in specific frequency bands. High-frequency bands are of particular interest to the extent that they support sensory-motor integration by allowing both rapid local processing and the synchronization of nodes within functional networks distributed across the cerebellum and cerebrum (25, 26). A further aim was thus to look for evidence for movement related high frequency EEG in parallel with that in the ECeG.

Methods

Participants

A total of 11 healthy adults (five female, six male) without prior history of vestibular, hearing or neurological impairment were recruited from the general community and staff and students at the Prince of Wales Hospital, the University of New South Wales and Western Sydney University. Written informed consent was obtained prior to testing in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee (South Eastern Sydney Local Health District Human Research Ethics Committee).

Unconditional and conditional stimuli

The impulsive (tap) unconditional stimulus consisted of a double presentation, with 600 ms inter-tap interval, of a 3rd order gamma waveform with a 4 ms rise time, applied to the left mastoid. This is an effective means of activating the utricle (27) and also for evoking CEPs (Govender et al., 2020). Customized software was used to generate the gamma waveform using a CED power1401 and fed to a power amplifier (model 2718, Brüel & Kjaer P/L, Denmark). A hand-held mini-shaker (model 4810, Brüel & Kjaer P/L, Denmark) with an attached perspex rod was used by a single experimenter (S.G.) to deliver the stimulus. The intensity used was 20 V peak, equivalent to approximately 14 N peak force level (FL) and delivered using a positive phase polarity (i.e. initial movement of the perspex rod towards the head). This results in an approximately incompressive lateral low-frequency acceleration of the whole head without significant higher frequency bone conduction (2, 3).

The preceding auditory conditional stimulus consisted of a 1200 ms, 2 kHz sinusoidal tone starting 600 ms immediately prior to the first mastoid stimulus. Tones were generated using Signal software (version 6.02, Cambridge Electronic Design, Cambridge, UK) and a CED Power1401 interface, fed to a customised amplifier and delivered bilaterally using insert earphones. The stimulus intensity used was -60 dB re 5 volts peak (equivalent to approximately 80 dB peak sound pressure level (pSPL) or about 77 dBA SPL.

EMG/EOG and EEG/ECeG recordings

FIGURE 1 HERE

Simultaneous measurements were made from beneath the left and right eyes (infra-ocular, consisting of both EMG and EOG: IO1 and IO2 referenced to electrodes 2 cm below), the presumed frontal eye fields (EEG: C3’ and C4’, 2 cm anterior to C3 and C4) and over the posterior fossa (ECeG: CB3 and CB4), referenced to linked ear lobes, using Ag/AgCl electrodes (Figure 1A). The posterior fossa cerebellar electrodes were 6 cm lateral from the CBz location, itself 5% below Iz. The earthing electrode was placed over the sternoclavicular joint. The total length of the recording epoch was 2.1 s with a 300 ms interval preceding the onset of the auditory stimulus and the impulsive mastoid stimuli given 600 ms and 1200 ms later. Signals were amplified (EMG/EOG: x 10 000; EEG and ECeG: x 20,000), filtered (5 Hz – 1 kHz) and sampled (10 kHz) using a CED Power1401 and recorded using Signal software (Cambridge Electronic Devices, Cambridge, U.K.). Subjects were positioned in a recumbent or semi-recumbent position during recordings with their heads resting on a pillow, to minimise any potential contamination from the neck muscles.

Experimental Procedure

For experiment 1 (reflexive conditioning) we used a procedure based upon an adaptation of that of Teo et al. (2009). They used electrical stimulation of the supra-orbital nerve as an eye-blink unconditional stimulus (US), combined with an auditory tone conditional stimulus (CS). The present experiment substituted the double vestibular mastoid tap for the supra-orbital nerve stimulus and like Teo et al. (2009), consisted of six blocks of 11 trials, with an inter-trial interval of 8 –12 seconds. The whole experiment was preceded by a “baseline” block of eleven CS alone trials. In the following 6 learning

blocks, the first nine trials always consisted of an auditory CS plus the US, while the 10th trial was the CS alone and the 11th trial the mastoid tap alone. This was followed by a seventh "extinction" block of eleven CS alone trials.

For experiment 2 (voluntary eye blinks) the stimulus and block procedure were identical to that of experiment 1 except that on the six learning blocks the subjects were given explicit instructions to blink in time with the head taps and this was demonstrated. For the seventh block of CS alone trials, which in the first experiment was the extinction block, the subjects were further instructed to blink at the time when the taps would have occurred. Then on a final block of CS alone trials, subjects were instructed to not blink.

Statistical Analysis

For each subject, automated measurements were made using a MATLAB script on a trial by trial basis of the baseline corrected RMS total power of EMG/EOG in the infra-ocular leads (IO1/IO2), of EEG in the central leads (C3'/C4') and of ECeG in the cerebellar (CB3/CB4) leads. Due to the large individual variability in response amplitudes, analyses were performed on log transformed data. A single trial epoch was segmented into 18 segments of unequal durations, as given in Table 1 & Figure 1B. Segments 5, 6, 10, 11, 15 and 16, at the times of expected responses, were only 20 or 30 ms long and shorter than the remainder. Means across the 8 blocks for the two experiments were made for the five trial types. For experiment 1, CS alone during baseline (B0, T1-T11), CS+US during learning (B1-6, T1-9), CS alone during learning (B1-6, T10), US alone during learning (B1-6, T11) and CS alone during the extinction block (B7, T1-11). For experiment 2, CS+US during learning (B1-6, T1-9), CS alone during learning (B1-6, T10), US alone during learning (B1-6, T11), CS alone during voluntary blinks (B7, T1-11) and CS alone during the voluntary "extinction" block (B8, T1-11). These were further averaged across subjects to make grand means.

TABLE 1 HERE

As there was an unequal number of trials allocated to each of the five types, the analysis of variance (ANOVA) was conducted in two stages. An initial repeated measures ANOVA using "block" (1-6), "trial-type", "side" and "segment" was performed to test for a block effect, then further concatenated across blocks. The responses during the five trial types could then be compared by ANOVA using within-subjects factors of "trial-type" (CS+US, CS alone during learning, US alone during learning, and CS alone during extinction), "side" and "segment". To test for any conditioning effects in the CB leads the ANOVA was repeated for CS alone trials only. To compare across experiments, three common trial types were extracted (CS+US, CS alone during learning, US alone during learning) and combined with a within-subjects factor of "experiment".

The CS + US trial type most clearly exhibited the synchronised blinks and correlated cerebellar activity, features of particular importance for this report. In the description that follows, we have assumed that short latency events following the tap stimuli were reflex or reflexive, while events which preceded (i.e.

anticipated) the stimuli, occurring when the subjects were asked to respond and when they could predict the timing of the stimuli, were considered voluntary.

Spectral Power Analysis

After recording EMG/EEG/ECeG we performed spectral power analyses of the six channels (IO1/2, C3'/C4', CB3/4) using the continuous wavelet transform as implemented in the MATLAB toolbox (R2019b, Mathworks, Natick, CA). In the present analysis a Morlet wavelet was employed at a density of 24 voices per octave over 9 octaves. The CWTs were further transformed to scaleograms (time-frequency images) from the absolute value of the CWT and rescaled to be in dB per Hz re 1 μV^2. Scaleograms were computed for all trials, then further split into eight frequency bands; delta (d: 1.8 Hz – 4 Hz), theta (*q*: 4 – 7.8 Hz), alpha (α: 7.8-12.5 Hz), beta (β: 13-30 Hz), gamma (γ: 30-80 Hz), ultra-gamma (u-γ: 80-160 Hz), very high frequency (VHF: 160-320 Hz) and ultra-high frequency (UHF: 320-640 kHz). These were then further segmented using the same time boundaries as for the RMS analyses and submitted to ANOVA. Wavelet coherence was also computed using the same MATLAB toolbox.

Results

Grand Means and RMS power ANOVA

FIGURE 2 & TABLE 2

ANOVA showed no significant block effect indicating consistent behaviour across the two experiments and justifying averaging over blocks. Figure 2 shows grand means of unrectified signals for three right sided channels (the infra-ocular IO2 for EMG/EOG, the central C4'for EEG, the cerebellar CB4 for ECeG) and five trial types for experiments 1 and 2 respectively. The findings for the equivalent channels on the left were nearly identical and therefore are not illustrated. Equidistant time points (1 – 4) were marked every 600 ms corresponding to onsets of the CS (1), US1 (2), US2 (3) and an implied (silent) US3 (4). The CS alone baseline condition was presented as the fifth trial-type.

Considering first the periocular responses, in experiment 1, a large EMG/EOG signal, corresponding to the blink UR1, was produced in IO channels (Fig 2A) in response to the first tap of the US alone trial type (time point 2). When preceded by a CS, however, the UR to the same tap was much reduced, corresponding to pre-pulse inhibition, as was the UR to the 2^{nd} tap in both CS+US and US only trials (time point 3). For the CS+US trials, weak CRs were present prior to the US taps at IO2. Weak responses can also be seen at the time of the (implied) third stimulus (CR3 and CR, part A). Larger, robust double blink responses were present in experiment 2. Starting with the response to the US only trials (Fig 2B), this began in a manner similar to that of experiment 1, but was followed by a second blink which reached a peak close to the onset of US2 (time point 3). For the CS+US trials, the first voluntary response (VR1) began before US1 and reached a peak close to the onset of US1 and the associated reflexive EMG. The second voluntary blink (VR2) also started in advance of the stimulus, with voluntary EMG occurring ahead of the associated stimulus-evoked EMG. In addition, the timing of the second response was more accurate with

the US than when subjects were asked to blink to the CS alone (CS BLK). A weaker third voluntary blink (VR3) associated with the implied US3 was also evident. A more robust VR3 was also present in CS (BLK) trials. These effects were reflected in the ANOVA of the RMS IO data (Table 2) where a main effect of "trial-type" was obtained for experiment 2 but not experiment 1.

In the central EEG channels, auditory evoked potentials (AEP) were present for trials with a CS for both protocols (C4', Fig 2, parts C and D). For experiment 1, presumed vestibular evoked responses (VsEPs) were present for the US and CS+US trials. For the voluntary task, the earliest response in the US trials resembled the reflexive response, but a large positivity followed. In the CS+US trials a small negative excursion, and presumed contingent negative variation (CNV), started prior to the onset of VR1.

FIGURE 3

For the ECeG in experiment 1 (Fig 2E) each of the mastoid taps was followed by large CEPs with an initial positivity. As we have previously reported (Govender et al., 2020), the latency on the side contralateral the stimulus was shorter than ipsilaterally (overall means; contra: 11.4 ms, vs ipsi: 13.7 ms, $P < 0.001$). The CEP was followed by a pause and small burst in the ECeG (Fig 2E, top 2 traces). For experiment 2 (Fig 2F), similar features were present, with the initial positivity having latencies of 11.6 ms and 13.5 ms for the contralateral and ipsilateral sides respectively. There was no significant effect of movement type on the latencies ($p > 0.05$). The initial CEP amplitudes were nearly identical for both ipsilateral and contralateral responses, for both conditions. There was an additional feature of a following slow negativity, clearest for the CS+US case.

The segmented ECeG RMS power and ANOVA were dominated by the two clear peaks occurring with the US stimuli (Table 2, Figure 3A-D). The CS alone trials show some cyclical reduction in power around the time of the stimuli for both movement types manifest as a "segment" effect ($p < .05$ for the reflexive case), including for the implied US3 (segment 15), and a main effect of "trial-type" in the voluntary case ($p <.01$).

Wavelet power/coherence

FIGURE 4 & 5

Figure 4 shows scaleograms and Figure 5 the associated VHF (160 – 320 Hz) power. The C3'-CB4 coherograms (Fig 4, parts C and D) and VHF mean C3'-CB4/C4'-CB3 coherence (Fig 5, parts C and D) are included. The CS+US trial type is illustrated as this showed the clearest effects. In the IO channel the high frequency EMG and low frequency EOG signals are segregated, most clearly for experiment 2 (Figs 4 and 5, right columns). Whereas the EOG (Fig 4A) cuts off between 10 – 20 Hz, the EMG picks up between 40 to 50 Hz and spreads upwards to several hundred Hz (Fig 4B). The EMG also helps clarify the timing of the blinks for experiment 2, where the first of the pair peaks occurred after the first US tap, but where the second is clearly anticipating the 2nd US tap, given at 1.2s (3). The second EMG burst is also larger by about 2 dB, consistent with more accurate (less variable) timing.

High frequency activity was present in the EEG (Figs 4 and 5 parts E, F), including in the spontaneous EEG, up to several hundred Hz. Of note was the presence of high frequency movement related EEG activity in parallel with but slightly lagging the ocular EMG (part B) for the voluntary case. The CFR peaks in the ECeG were related to the sensory stimuli in both movement cases. However, in the voluntary case some additional movement related modulation in the form of pause-bursting was present within the high frequency component of the ECeG (Figs 4 and 5, parts G and H). ANOVA of high-frequency power across the two tasks with the common three trial types (CS+US, CS learning, US alone) showed a significant three-way interaction of "experiment" by "trial-type" by "segment" for the u-*g* band ($F(34,340)=2.9$, $p<.05$) and a trend to significance for the VHF band ($F(34,340) = 2.4$, $p=.068$). As with the EEG, the ECeG burst appeared to lag the EMG activity. Thus the pause phase occurred during the rising phase of the ocular EMG while the subsequent burst peaked during the falling EMG. There was also evidence of stimulus-related EEG-ECeG coherence for both tasks, manifest as interactions of "trial-type" by "segment" in the *b*, *g* and u-*g* bands (respectively p <.005, p=.001, p = .055 for the reflexive case and p <.005, p=.001, p < .05 for the voluntary case). For the voluntary task there appeared to be possible evidence of movement related high-frequency EEG-ECeG coherence around the time of the two stimuli which seemed to follow the EMG (Figs 4 and 5, parts C and D), but this did not reach statistical significance.

Discussion

We have used electrodes placed over the posterior fossa to record evoked responses and electrical activity (ECeG) that we believe arises from the cerebellum. Samuelsson et al. (29) calculated that ECeG should be detectable in surface recordings but weaker than cerebral EEG. Others have been able to make recordings of cerebellar activity using surface recordings. For example, Bosch et al. (30) confirmed that recordings from over the cerebellum were distinct from those at Oz. Our study compared the electrophysiological features of three types of movement – unconditioned reflexive, in which a response occurs following a stimulus, conditioned reflexive and voluntary both of which may precede predictable stimuli. The nature of the three types of movement was distinct, and the first and the second of the pair of each type differed for both experiments. For the unconditioned reflexive movement, both EMG/EOG responses followed the US, with the second being much smaller than the first. For the voluntary task, the unconditioned reflexive EMG was still present, but the ocular response started before the US and the second movement was as large as the first and appeared less variable. Despite these clear differences, the (cerebellar) ECeG changes were virtually identical around the time of both the US pair, suggesting that they are dominated by the afferent, presumed climbing fibre (CF), input. However, in addition to the stimulus related ECeG changes we also observed superimposed movement-related pause-bursting associated with the voluntary movement, which clearly anticipated the 2nd of the US pair, and may thus be analogous to conditioned pausing in classical conditioning in the circumstance that robust conditioning occurs. The ECeG changes were also accompanied by the presence of a CNV in central leads prior to the onset of voluntary movement and modulation of the high frequency EEG.

While only weak conditioning was evident in the present study, despite the use of dual stimuli, robust voluntary timed blinks were evidence of the presence of sensorimotor synchronisation. Establishing and maintaining synchrony with external pacing sequences are dissociable processes that are likely to rely on distinct mechanisms (31). Much research on sensorimotor synchronization has addressed the mechanisms that enable individuals to maintain coordination (32–34). The process of establishing synchrony at the start of a trial has received less attention (in fact, the first few taps are usually not analyzed in order to focus on the relatively stationary time series that follow). From previous work, we know that establishing synchrony at the start of a trial, or re-establishing it after an unexpected tempo change, typically takes around three cycles (31, 33, 35). This process of temporal adaptation relies on reactive error correction processes that implement compensatory adjustments to movement timing with onset latencies 100–250 ms (depending on tempo) in the movement cycle immediately following a timing perturbation in the pacing sequence (36). Our findings provide detailed evidence of the events occurring both centrally and for the cerebellum at the onset of a sequence performed in two distinct ways.

The lack of a block effect, despite the presence of dual US, indicates that, as a group, no systematic change occurred, i.e. no significant conditioning. We have previously reported that this form of US produced only weak conditioning, which rapidly habituates (15). In contrast, a stronger trigeminal nerve US produced more robust conditioning (18), along with conditioned pausing in the ECeG. We can, therefore, conclude that the two types of movement remained consistent throughout the recordings and thus averaging the responses is representative. To balance the sensory component, an auditory CS was used in both experiments. When subjects determine the timing of their blinks, a readiness potential is present prior to the movement, maximal over the vertex (37). Where movements are contingent upon stimuli instead a CNV may be present, for both conditioned and voluntary cases (38), also observed in Todd at al. (18). Interestingly, the second voluntary movement anticipated the second stimulus more accurately than the first and, unlike the unconditioned reflex movement, was as large as or larger than the first. Responses following a stimulus are more difficult to interpret as reafference generated by the movement may contribute to any potentials present (39). In this context, and noting that little conditioning occurred in the first experiment, the averaged responses to the CS + US stimuli are important as they are the least noisy due to the larger number of trials averaged (Fig. 2). In the reflexive case here, there was no cortical potential preceding the initial response. However, in the voluntary case, there was evidence of a CNV prior to movement, as in Todd et al (18) when learning did occur. Thus we may suggest another parallel between classical conditioning and voluntary learning. No prestimulus potential change was present for the cerebellar recordings for either task, unlike Todd et al (18). The stimulus was associated with a presumed climbing fibre response (CFR) in both cases and a burst of coherence between the cerebellar electrodes and the cortical electrodes.

Given the presence here of pausing in the high-frequency ECeG, an important issue is the possible role of the oculomotor system in voluntary eye blinks, especially since neural pause-bursting is a feature of saccadic eye movements. Reflex eye blinks are largely mediated at the brainstem level (e.g. 40). The ocular-motor areas of the cerebellum include the flocculus/paraflocculus, the nodulus/ventral uvula, two

areas shared with the vestibulo-ocular system, plus the oculomotor vermis (OMV) (41). The OMV, located within lobules V-VII of the vermis, is situated relatively close to the skull surface near Iz and thus plausibly within reach of the window for non-invasive electrophysiology of the posterior cerebellum. This site has been used successfully for TMS influence of the saccadic system (42). The OMV is known to be involved in saccadic eye movement and may participate in reflexive blink-related eye movements as well as voluntary blinks (41). The observed high-frequency pausing behaviour in the ECeG is reminiscent of PC pausing and bursting units in the saccadic system of the OMV. Although they are not saccades it is possible that co-contraction of the extra-ocular eye muscles would be caused by the synchronous bilateral pausing of PCs which would normally have a reciprocal role. The timing of the voluntary movement related ECeG pause-bursting we observed occurred during the rising and falling edges of the EMG, and is thus consistent with a role for the cerebellum in sharpening movement by acceleration after onset and breaking to assist accurate targeting (41).

In addition to the high frequency spectral components in the ECeG we also observed movement related high frequency EEG along with EMG. Movement related sensory-motor high gamma frequency EEG has been described, most commonly in brain-computer interface (BCI) research using the electro-corticogram (ECoG) approach and is believed to be related to somatosensory feedback (e.g. 43). Our data are consistent with the interpretation that the high frequency EEG is related to reafference but with the range extended upwards well beyond the ultra-gamma range (44). Other BCI/ECoG studies have demonstrated the presence of EMG related very high frequencies in motor cortex (45) and high-frequency ECeG-EEG coherence here seemed related to EMG.

Although conditioning and sensory-motor timing are traditionally distinct areas in cerebellar research, they can be seen to be related at multiple levels. At one level, classical conditioning and repetitive, timing-specific movement may rely on common cerebellar substrate for representing temporal information (46), while at another level, they can be viewed as different aspects of the same fundamental process, i.e. conditioning can be viewed as a form of involuntary adaptive motor timing, while adaptive motor timing can be viewed as a form of learning. The latter conceptualization relies on a dual-route framework whereby a neural pathway involving the inferior olive and CFs mediates motor learning based on reflex conditioning while a pathway that projects to cerebellar Purkinje cells via the pontine nuclei and mossy fibres mediates learning based on memory traces (47, 48). On this view, adaptive timing to a vestibular metronome tap can be considered to be a form of learning where the stimulus serves as both an US (via the CF pathway) and a CS (via the mossy fibre pathway). Thus, in a sequence of taps, each stimulus may trigger both an unconditioned response UR to the current and a conditioned response (CR) timed for the following tap. In our present study any conditioning was weak, consistent with the blinks remaining primarily URs to the vestibular US for the reflex task. For the voluntary task, the timing of the second response appeared improved by the US, consistent with it also playing the role of a CS.

Declarations

Ethical Approval. Written informed consent was obtained prior to testing in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee (South Eastern Sydney Local Health District Human Research Ethics Committee).

Competing interests. There are no competing interests.

Authors' contributions. NT, PK & JC contributed to the conception and design of the study; NT & SG conducted the primary acquisition and analysis of the data; NT, PK and JC contributed to the data interpretation; NT wrote the initial draft of the manuscript; JC and PK undertook significant revisions; SG prepared figures; all authors contributed to the final review and draft of the manuscript.

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Availability of data and materials. Data available on reasonable request.

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Tables

TABLE 1: Trial segmentation of RMS power for statistical analysis

Segment	*Interval*	*Name*	*Description*
S1	300 ms	BL	baseline
CS onset (CS – time point 1) – 0 ms			
S2	200 ms	AS	the initial (“alpha”) part of the CS
S3	200 ms	CS1	the interval which followed the CS
S4	200 ms	pre CFR1	immediately prior to the 1st tap
1st tap onset (US1 – time point 2) – 600 ms			
S5	0-20 ms	CFR1	the 1st CFR period post US1
S6	20-50 ms	post-CFRP1	the post-CFR pause post US1
S7	50-200 ms	post-CFRB1	the post-CFR burst post US1
S8	200 ms	CS2	the CS interval which followed the 1st US
S9	200 ms	pre CFR2	immediately prior to the 2nd tap
2nd tap onset (US2 – time point 3) – 1200 ms			
S10	0-20 ms	CFR2	the 2nd CFR period post US2
S11	20-50 ms	post-CFRP2	the post-CFR pause post US2
S12	50-200 ms	post-CFRB2	the post-CFR burst post US2
S13	200 ms	CS3	the CS interval which followed the 2nd US
S14	200 ms	pre CFR3	immediately prior to an implied (silent) 3rd tap
Implied (silent) tap onset (US3 – time point 4) – 1800 ms			

S15	0-20 ms	CFR3	the 3rd implied CFR period post US3
S16	20-50 ms	post-CFRP3	the post-CFR pause post US3
S17	50-200 ms	post-CFRB3	the post-CFR burst post US3
S18	100 ms	return	return to baseline

TABLE 2: ANOVA OF ECeG RMS POWER

	All trial types		***EXP 1***		***EXP 2***	
Electrode	**Factor**	df	*F*	*p*	*F*	*p*
CBs	TT	4,40	2.5	ns	5.8	<.01
	SIDE (Si)	1,10		ns		ns
	SEG (Se)	17,170	11.6	=.001	8.6	<.001
	TT*Se	68,680	12.1	<.001	11.1	<.01
	CS only trial types		***EXP 1***		***EXP 2***	
Electrode	**Factor**	df	*F*	*p*	*F*	*p*
CBs	TT	2,20	0.8	ns	7.5	<.01
	SIDE (Si)	1,10		ns		ns
	SEG (Se)	17,170	2.4	<.05	1.8	ns
	TT*Se	34,340	1.7	ns	1.4	ns

Figures

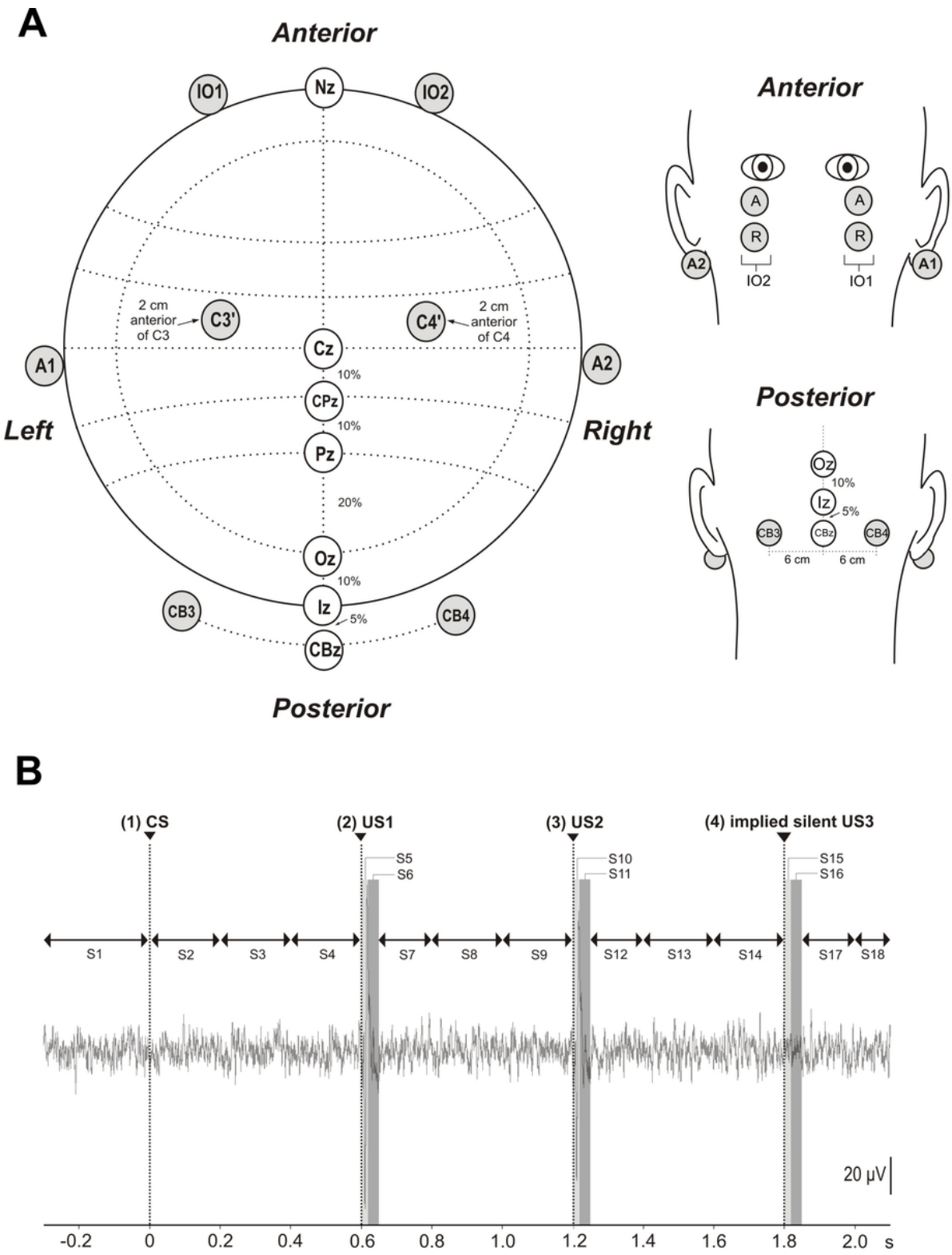

Figure 1

(A) Axial and coronal views of the experimental montage (grey electrodes) consisting of infra-ocular (IO1, IO2), central (C3', C4') and cerebellar (CB3, CB4) electrodes used to record EOG/EMG, EEG and ECeG respectively. The infra-ocular montage consisted of active (A) and reference (R) electrodes positioned slightly lateral and beneath the eyes. EEG recordings from over frontal eye fields were made 2 cm anterior to the conventional C3 and C4 locations. ECeG recordings were made lateral (6 cm) to the midline CBz

location, which is situated 5% below the Iz level. EEG and ECeG recordings were referenced to linked earlobes (A1, A2). (B) The recording epoch demonstrating the stimulus onsets (CS, US1, US2, an implied 3rd stimulus (US3)) and segmentation used for analysis (refer to Table 1 for abbreviations). Single subject data shown is taken from the CB4 electrode.

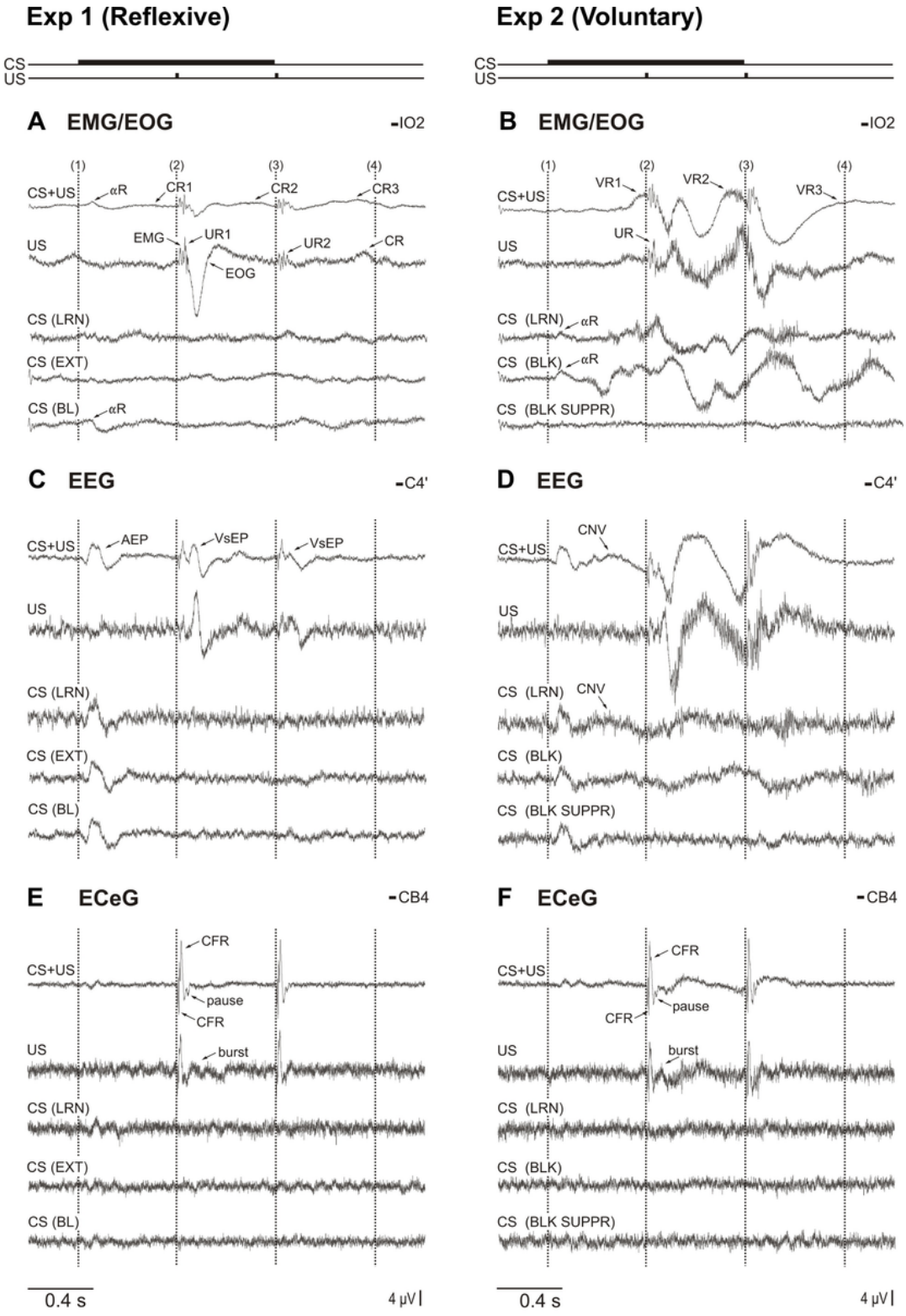

Figure 2

Grand unrectified means for the right sided infra-ocular, (A & B; IO2) central (C & D; C4') and cerebellar electrodes (E & F; CB4) for each of the five trial types for experiment 1 (reflexive conditioning; left column) and experiment 2 (voluntary timed eye blinks; right column). For experiment 1, the infra-ocular electrodes show large EMG/EOG potentials (blink UR) which are diminished upon the second US stimulus in US alone and CS+US trial types. Central electrodes show auditory evoked potentials (AEP) in all CS onset trial types with vestibular evoked potentials (VsEPs) after the US taps. Cerebellar electrodes show a probable climbing-fibre response (CFR) following both US onsets and subsequent pause-burst activity. For experiment 2, the infra-ocular electrodes demonstrate large timed blink responses which often peaked close to the US onsets whereas voluntary blinks during CS alone trials were infrequent and peaked later. For the central electrodes, auditory evoked potentials (AEP) were present in all CS onset trial types, similar to experiment 1. Pre-movement potentials (CNVs) were present in the CS+US and CS alone during learning trial types . Findings for the cerebellar electrodes were similar to that for reflexive responses. LRN = learning, EXT = extinction, BL = baseline, BLK = blink, BLK SUPPR = blink suppression, CNV = contingent negative variation, CS = conditioned stimulus, US = unconditioned stimulus, CR = conditioned response, UR = unconditioned response, VR = voluntary response, positive potentials are up.

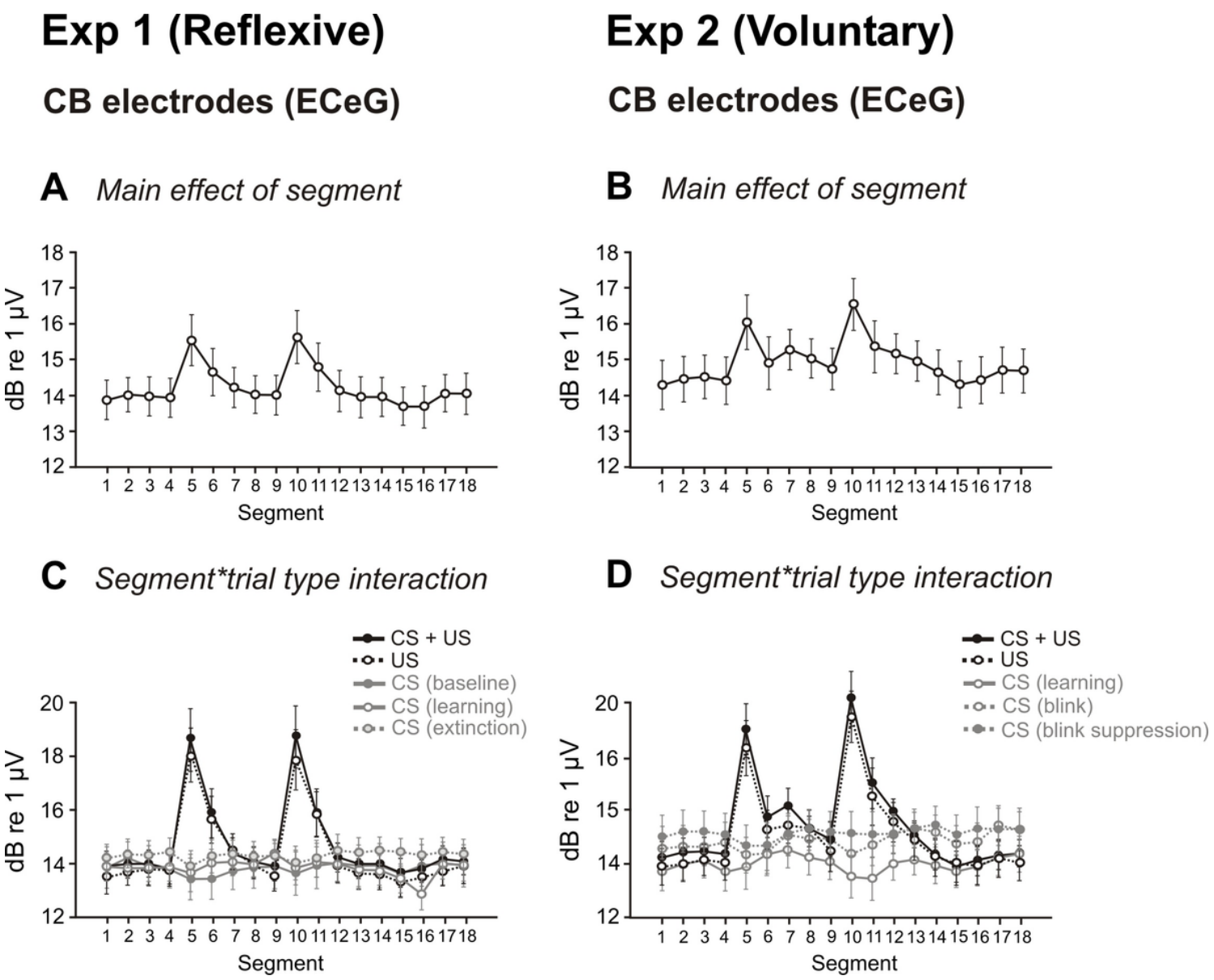

Figure 3

Marginal means using RMS averaging of sequential segments, for the effects of “segment” and the interaction of “segment” and “trial type” cerebellar electrodes for experiment 1 (A & C; left column) and experiment 2 (B & D; right column). Overall, similar patterns were seen for both movement tasks.

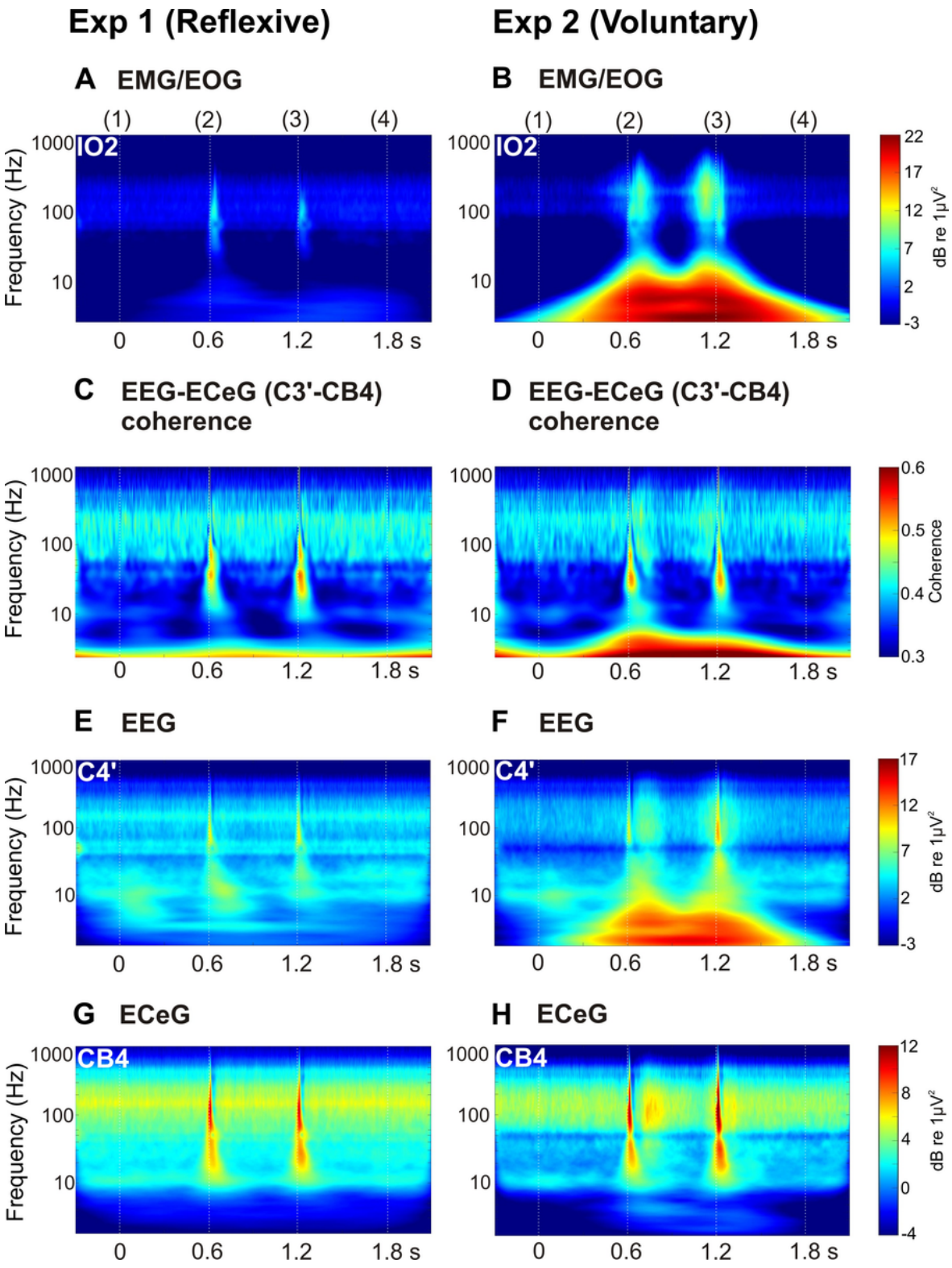

Figure 4

Scalograms for the infra-ocular EMG/EOG (A & B; IO2), central EEG (; E & F; C4') and cerebellar ECeG (; G & H; CB4) for experiment 1 (reflexive conditioning) and experiment 2 (voluntary timed blinks) for the CS+US trial type. C3'-CB4 EEG-ECeG coherograms (C & D) are also illustrated for comparison and was similar to the C4'-CB3 coherogram.

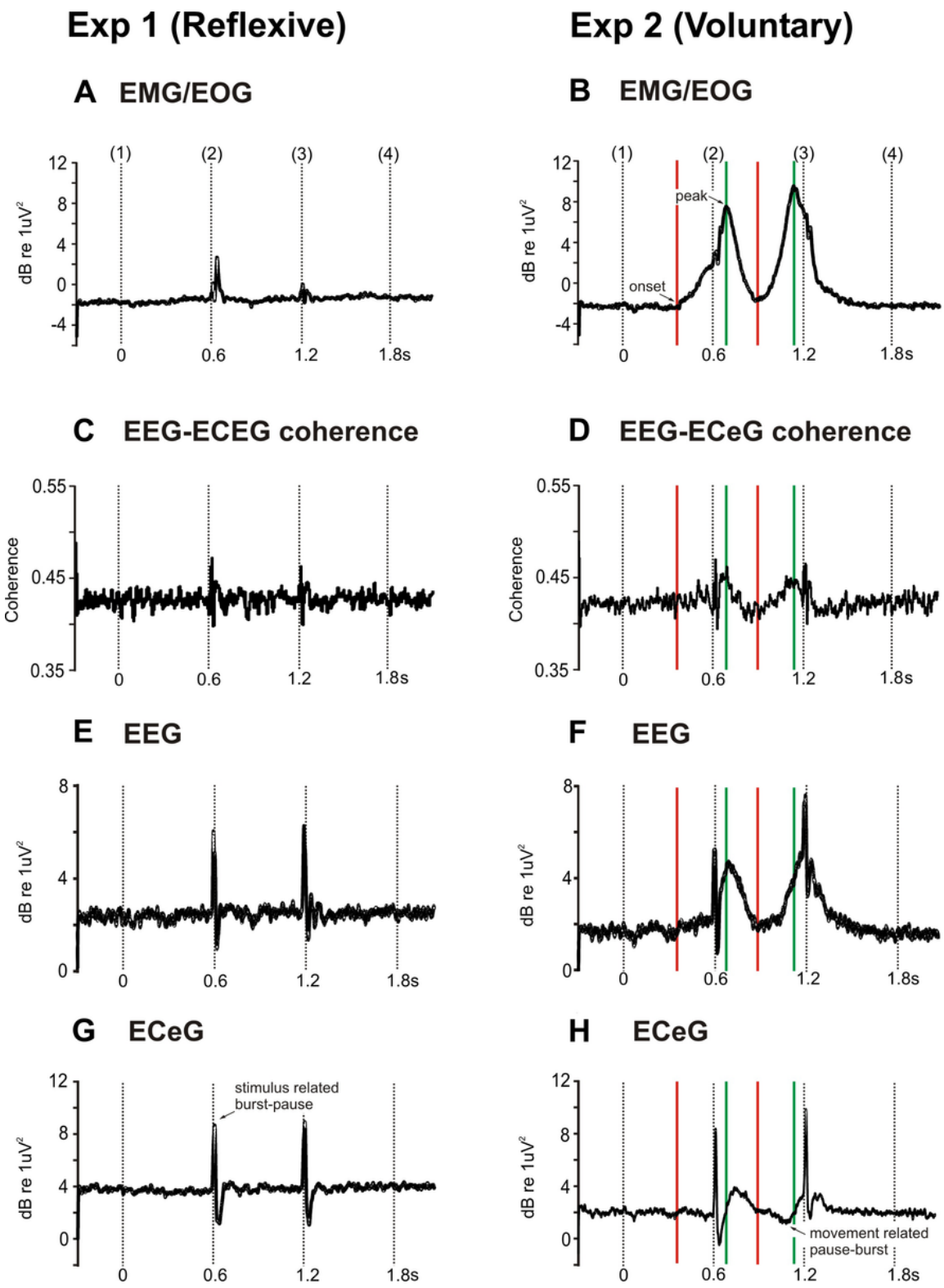

Figure 5

VHF power for the infra-ocular (A & B), central (E & F) and cerebellar electrodes (G & H) and CS+US trial types for experiment 1 (reflexive conditioning) and experiment 2 (voluntary timed eye blinks). VHF power shown reflects the averages between the right and left sides for EMG/EOG, EEG and ECeG graphs. VHF EEG-ECeG coherence bands are also illustrated for comparison (C & D) and are the average coherence of C3'-CB4 and C4'-CB3 comparisons. Red lines reflect the average onset of the voluntary eye blink whereas

green lines reflect the peak EMG/EOG signal. The CFR peaks relate to the stimulus and not the blink response.