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# Behavioral Rehabilitation of the Eye Closure Reflex in Senescent Rats using a Real-Time Biosignal Acquisition System

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*Abstract*—**In this paper the replacement of a lost learning function of rats through a computer-based real-time recording and feedback system is shown. In an experiment two recording electrodes and one stimulation electrode were implanted in an anesthetized rat. During a classical-conditioning paradigm, which includes tone and airpuff stimulation, biosignals were recorded and the stimulation events detected. A computational model of the cerebellum acquired the association between the stimuli and gave feedback to the brain of the rat using deep brain stimulation in order to close the eyelid of the rat. The study shows that replacement of a lost brain function using a direct bidirectional interface to the brain is realizable and can inspire future research for brain rehabilitation.**

## I. Introduction

Brain diseases like stroke or amyotrophic lateral sclerosis lead to damage in specific brain functions. Up to now patients suffering the symptoms of these diseases have to live with them and are not able to use the functions anymore. As a consequence the idea of cerebral prostheses came up. Such prostheses should be able to replace lost functions synthetically by interacting directly with the brain to allow behavioral rehabilitation. Cochlea implants are an existing step in that direction as they receive auditory signals, translate them and stimulate the auditory nervous system with electrodes placed within the cochlea [1]. These devices represent a one-way communication with brain neural systems. Another example is a device which controls a prosthetic limb based on signals taken from electrodes implanted in the patient's motor cortex [2]. In this system visual feedback informs the motor cortex about movement quality. Instead of visual feedback, deep brain stimulation (DBS) can be used to give feedback to the brain directly. This kind of stimulation, although not serving as feedback, is used for instance in Parkinson patients [3]. Knowledge about how neurons code information is needed at least to a certain level of detail in order to construct adequate prostheses.

A well-studied brain circuit which becomes dysfunctional due to aging is the eye closure conditioned reflex. A significant decrease in the acquisition of eyeblink responses in human subjects of about 40 years of age was first observed by Woodruff-Pak et al. [4]. Subjects of an age of 70 years show less than half the performance of 20 year old subjects. A similar effect is observable in rats [5,6].

Experiments dealing with the eyeblink reflex are usually conducted using a classical conditioning paradigm [7,8]. Subjects are exposed to repeated trials of paired conditioned and unconditioned stimuli (CS-US). A tone (CS) is presented followed after a short time interval by an aversive airpuff (US) directed at the cornea of the subject. In naïve subjects, where the learning function is working, the tone-CS does not elicit any response, while the airpuff-US elicits an unconditioned eyeblink response (UR). After the association between the tone-CS and the airpuff-US is established, the subject blinks after the CS onset and before the expected US, i.e., it performs a conditioned eyeblink response (CR). In a situation where the learning function is not working, the conditioned response will not be observable.

The objective of the present study was to test the feasibility of replacing this lost learning function in an anesthetized rat by a computer-based recording and feedback system which acquires the incoming biosignal data, processes them using a computational model and provides feedback to the brain in order to establish behavioral rehabilitation.

## II. Methods

### A. Experimental Setup

Figure 1 depicts the hardware setup for the experiment. Electrodes were implanted into the brain of an anesthetized rat placed in a stereotaxic apparatus for deriving neuronal data. It is known that the pathway of the CS to the cerebellum runs through the pontine nucleus (PN) and the pathway of the US runs through the inferior olive (IO) [9,10]. Therefore the animal was implanted with a titanium-nitride micro electrode array (Faculty of Engineering, Tel Aviv University) with ten channels in the PN to detect the CS and with a tungsten needle electrode (A-M Systems, USA) in the IO to

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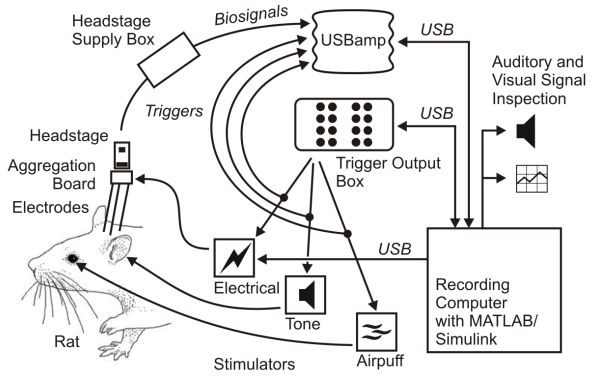


Fig. 1. The hardware setup of the experiment which includes the recording computer with audiovisual inspection facilities and the g.USBamp biosignal amplifier connected through a headstage to the electrodes implanted in the brain. The trigger output box is connected to the electrical-, tone-, and airpuff-stimulators.

detect the US. It is also known that the efferent pathway for the eyelid closing mechanism goes through the facial nucleus (FN) [11]. Therefore a twisted-wire electrode was implanted in the FN to produce the eyeblink using an electrical stimulator. The recording electrodes were connected through aggregation boards to headstages, which pre-amplify the signals. These were connected to the main amplifier, the g.USBamp (g.tec medical engineering GmbH, Graz, Austria) which transmitted the digitized data to the recording computer. Each channel was sampled with a frequency of 19.2 kHz with 24 bits precision. The processing of the recorded biosignals for the generation of the feedback was done within the MATLAB/Simulink environment (The MathWorks, Natick, USA) which allows in combination with the g.USBamp Highspeed Online Processing Toolbox (g.tec medical engineering GmbH, Graz, Austria) the real-time functionality of the system. Moreover the software includes facilities for audiovisual inspection of the recorded biosignal data.

To control CS and US delivery as well as the blink-inducing stimulation, a programmable trigger output box was used. The CS and US timing was done by the box autonomously. The trigger pulse of the cerebellar model was routed via USB to the output box. In order to have all these triggers precisely aligned with the biosignal data they were fed back into the recording system by using the digital inputs of the g.USBamp.

The tone-generator is configured to deliver a white-noise stimulus at 67-70 dB in intensity for 450 ms to the right ear through a hollow ear-bar of the stereotaxic head holder. The intensity of the airpuff was 1.5 bars at the source and its duration was 150 ms. It was delivered through a hose positioned ~2.5 cm from the cornea of the right eye. The inter-stimulus-interval (ISI), the time between CS and US onset, was 300 ms. The electrical stimulator delivered 200 mA 0.1 ms constant-current pulses with a frequency of 80 Hz for 150 ms.

### B. Signal Processing

To extract the onsets of the stimuli based on the signals recorded from the PN and IO an event detection algorithm was developed.

Within the experiment two instances of this algorithm were used in order to detect the events of PN and IO separately. The algorithm consists of two parts: a feature trace generator and a threshold detection stage. First we compute the instantaneous variance of the MUA and, after smoothing this signal, we down sample it to 500 Hz [12]. For the multi-channel data coming from PN we do this computation for every channel. As the last step of the trace generation, the feature traces from PN were averaged. The second part of the algorithm is simple threshold detection which outputs 1 at the point in time that the trace crosses the threshold with a rising flank, and 0 otherwise.

### C. Cerebellar Model

The cerebellar model, which is the central part of the system, was inspired by a previously published model [13] which abstracts in a top-down fashion the actual cerebellar anatomy. For each component of the model a particular underlying biological realization can be identified. Figure 2 shows the structure of the model.

(1) Trace generation: This block receives the detected events of the PN channel and expands them to linearly decaying traces of fixed length. (2) Scaling: Here the decaying trace is multiplied by the factor w. (3) Thresholding: This block triggers the CRs on existence of an active decaying trace that goes below a given threshold. The parameter *w* has to decrease below 0.4 in order to trigger CRs and below 0.28 in order to trigger well-timed CRs. (4) Delay: This block acts as a delay buffer and outputs a delayed copy of the trace produced by component 1. (5) Inhibitory pulse: Here a pulse of fixed duration is generated on the occurrence of a CR. (6/7) Delay/Gating: Again a delay buffer which generates a delayed copy of the inhibitory pulse; the information regarding the IO detections is blocked in cases where an inhibitory pulse is active. (8) Coincidence detection: This block updates the value *w* and represents plasticity. Its operation is determined by the relative arrangement of the PN trace and

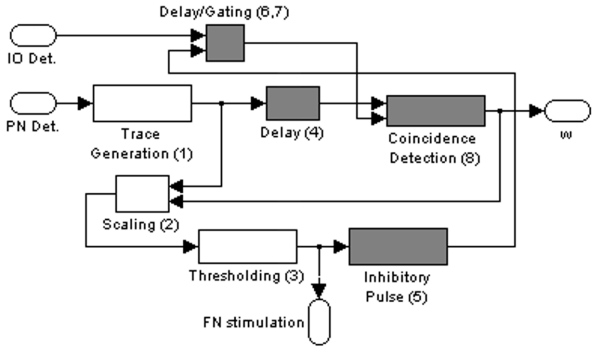


Fig. 2. Simulink blockset of the cerebellar model. The white blocks map PN activity into action, in the case of eyeblink conditioning, they map tone detections into eyeblinks. Such mapping is controlled only by the association weight *w*. The shaded blocks control the mapping, namely they are involved in the adjustment of *w*.

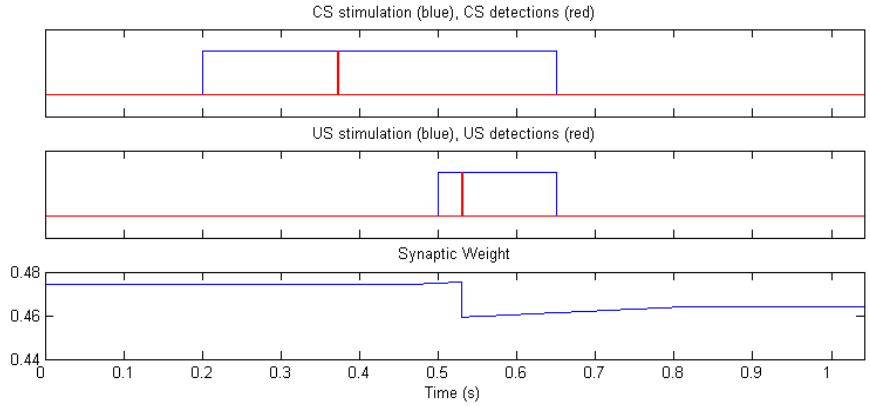


Fig. 3. The evolution of the value *w* as it changes during an acquisition trial. Top: The blue curve shows the CS stimulation period and the red trigger the time of the detected onset. Middle: The blue curve shows the US stimulation period and the red curve the time of the detected onset. Bottom: The output of the cerebellar model which processes the coincidence of the two detections resulting in a depression step in the period of potentiation. In sum the weight *w* decreases on a correctly detected coincidence.

the IO detections. In each time step with an active trace of block 1, *w* is incremented by a potentiation step. In each time step in which an IO detection coincides with the trace *w* is decreased by a depression step. To avoid the stimulation artifacts being detected as reactivity, a mechanism was included into the system which disregarded any detections coming from signal processing during the period of the electrical stimulation.

### D. Experimental Workflow

To allow a correct identification of stimulus onsets in the real-time experiment, training data recorded during conditioning were used offline to calculate parameters for the detection algorithms. This was done according to a receiver operating characteristic (ROC) resulting in a percentage of true positive detections (TP) and a rate of false alarms (FAR) in Hz for each threshold. Subsequently, the parameters for the cerebellar model were optimized to perform according to a predefined specification; i.e., to learn within 60 trials, at a given performance of the signal processing algorithms. This means that one pair of points on the ROC curves for PN and IO was selected out of the vector provided by the signal processing stages. The outcomes are the depression and potentiation factors which influence the value *w* of the model. After these steps and the validation of the calculated parameters the online system reacquired biosignals, detected the onsets of the stimulation and fed these time-points into the cerebellar model which used them to simulate cerebellar plasticity and emit the required behavior to avoid the airpuff.

## III. Results

Training data was acquired during the presentation of 30 combined CS-US trials. The inter-trial interval (ITI) was 10 s. For the PN, four channels which provided the highest signal-to-noise ratio were chosen to serve as input to the PN event detection algorithm. Then the ROC curves were calculated for the PN and IO event detection and were forwarded to the optimization procedure for the parameters of the cerebellar model. The model was configured for an acquisition time of 60 trials and an extinction time of 60 trials. Value *w* of the model was initialized at 0.5. The operating points selected by the optimization procedure were a FAR of 1.14 Hz for IO leading to 48.6% TP and a FAR of 0.11 Hz for PN leading to 91.4% TP. The optimization procedure determined the factor for depressing the weight *w* of the cerebellar model as 0.0161 and the factor for potentiating *w* as 3.36e-5.

The real-time experiment, which lasted for 1h 20min, started with the presentation of 190 paired CS-US trials for acquisition. The ITI was randomized between 10 and 15 s. After that, 180 CS alone trials were presented for extinction. The performance of the signal processing algorithms was 75.1 % TP at a FAR of 0.06 Hz for PN and 32.1 % TP at a FAR of 1.1 Hz for IO. In Figure 3 the evolution of the value *w* of the cerebellar model during one acquisition trial can be seen. After about 25 of these trials (about 800 s after the beginning of the experiment) *w* undercut the value 0.4 and the first CRs occurred. The model was stable during the 130 subsequent paired CS-US presentations (from 800 to 2500 s) and *w* remained around 0.3. Then extinction (CS only presentation) began and *w* started to increase again. The rate of delivered CRs was then decreasing. From 4000 s onwards (trials 310 to 370) *w* remained stable in a range of about 0.5 for the rest of the experiment without delivery of CRs.

Between the specified time for acquisition and the beginning of extinction (from trial 60 to 190) 39.7% of the elicited CRs were well-timed. Well-timed CR's are defined in this analysis such, that they have to cover 80% of the time of the airpuff-US. At trial 190 the extinction period started. As mentioned previously the model was configured to unlearn within 60 trials from trial 190 to 250. After trial 250 no more well-timed CRs occurred and shortly after that (approx. at trial 280) the model stopped delivering CRs completely.

## IV. Discussion

The results of the real-time experiment demonstrate that the system containing the cerebellar model has successfully

been conditioned by paired CS-US presentations resulting in the presentation of a CR. After acquisition, CRs were successfully extinguished by CS-alone stimulation. The value *w*, which represents the synaptic weight of the Purkinje cells in the cerebellum, behaved as predicted after the training, resulting in well-timed CRs after about the amount of trials expected. Also extinction worked correctly within the defined bound of trials. The model managed to stabilize the value *w*, depending on the stimulation, around the expected values. In this experiment the length of the CR-electrical stimulation was exactly as long as the length of the US. This was a very strict setting, as a slightly longer stimulation, i.e. 200 ms, would lead to a higher percentage of well-timed CRs.

For future advances of this concept several issues have to be taken into account. An important step would be to detect the length of the sensory events instead of only the onsets, in order to adapt both the behavior of the cerebellar model and the length of the electrical train that triggers the behavioral CRs. Therefore the granularity of the information extracted from the biosignals is very low.

In the signal processing algorithms the manual choice of channels as it was done for the PN could be replaced by automatic weighting, which could increase the signal to noise ratio compared to the simple averaging which was used in this experiment. Another important point is that several false alarms in the PN occurred due to detections of the reactivity of the PN as a cause of the US which could be avoided by a more advanced signal processing algorithm.

An obvious problem is that during the electrical stimulation no US can be detected due to the stimulation artifact. This has effects on the behavior of the cerebellar model. Methods could be incorporated which can detect and clean this artifact from the biosignals. Another important issue is signal stability. It can be seen that the detection performances of the signal processing algorithms changed slightly from the training recording to the test recording. This is a normal effect and can be traced back to changes in the locations of the electrode relative to the brain, anesthesia state of the animal and other variables. The FAR from signal processing should ideally be stabilized during operation as variation affects the behavior of the cerebellar model and can lead to unstable results.

The next step would be to replicate the experiment in behaving animals. This seems to be a very demanding task because of the decrease of signal quality due to movement artifacts or the time the electrode is implanted, which directly affects the performance of the event detection.

## V. Conclusion

In this study we demonstrate the integration of a real-time biosignal acquisition system combined with signal processing algorithms and a model of cerebellar plasticity. In the experiment reported, the combined bio-synthetic system produced adaptively timed CRs after CS-US stimulation and abolished such responses following CS-alone extinction training. Such results are congruent with a successful replacement of the function the cerebellar microcircuit, even though replication of the results and unpaired CS-US controls are still required in order to validate the correct functionality of the system. Therefore, with this work we illustrate that our approach can support a biologically-inspired system for the replacement of the learning capability of the dysfunctional cerebellum, and is a valid demonstration of the rehabilitating potency of the model.

## Acknowledgment

Within this study g.tec developed the real-time recording environment including the electrical stimulation. The Psychobiology Research Unit, TAU, was responsible for the clinical integration experiments. SPECS, UPF, developed the cerebellar model and the signal processing algorithms. The Faculty of Engineering, TAU, developed the micro electrode arrays.

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