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Classical conditioning of the electrically elicited blink reflex in humans: a new method of data analysis

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Abstract

The eyeblink conditioning paradigm is a well-established model to study learning processes in humans and animals. Especially results from animal studies have supplied new insight into physiological pathways and brain structures involved in associative motor learning and memory. An important role of the cerebellum and its afferent fiber systems could be shown. Recent studies in humans have given evidence that results of animal experiments can be applied directly to the human condition. A high variation of baseline EMG activity and/or spontaneous blinks may influence the analysis of classical conditioning of the electrically elicited blink reflex in humans. To optimize differentiation between real conditioned responses and stimulus-independent EMG activity, we developed an algorithm which is fully automated and independent of a possible bias of an examiner. In a first step the algorithm decides whether a subject fulfills the criteria of a successful learning process or not. The second step quantifies the learning process. For quantification of the learning process, the following parameters were calculated: number of conditioned responses, onset of conditioning, time and amount of maximal conditioning, speed of conditioning and speed of habituation. According to our criteria, 80% of the healthy volunteers acquired conditioned responses. There is an age-related decline in eyeblink classical conditioning. Analysis of patient groups with different types of lesions will further improve our knowledge and understanding of pathways involved in learning processes in humans. The proposed new algorithm of data analysis takes less than 10 s on a standard computer, is more sensitive and more specific in detecting conditioned responses and, therefore, may further improve the value and reliability of the eyeblink conditioning paradigm in clinical research.

Keywords: Basal ganglia; Blink reflex; Cerebellum; Classical conditioning; Habituation; Motor learning

1. Introduction

In neuropsychological research, eyeblink classical conditioning (EBCC) is a frequently used model to study brain substrates of basic associative learning and memory. There is evidence from lesion studies in animals that the cerebellum plays a crucial role in various forms of motor learning. Based on the results of lesion studies, Thompson (1990) developed a diagram of a memory trace circuit for classical conditioning which is in line with the Albus–Marr model of learning (Marr, 1969; Albus, 1971).

However, there is an ongoing controversy as to the neural substrates involved in acquisition, storage and execution of classical conditioning, especially the role of the cerebellum and its deep nuclei (McCormick and Thompson, 1984; Yeo et al., 1985; Bloedel, 1987; Kelly et al., 1990; Welsh and Harvey, 1991; Bracha et al., 1994; Thompson and Kim, 1996; Bracha et al., 1997; Woodruff-Pak, 1997).

Nevertheless, studies in human beings with cerebellar

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lesions, degenerative cerebellar diseases (Lye et al., 1988; Daum et al., 1993; Topka et al., 1993) and olivary dysfunction (Deuschl et al., 1996) have given evidence that results from animal studies can be applied directly to the human condition. An additional role of the basal ganglia, especially the striatum, is assumed on the basis of animal experiments (Kao and Powell, 1986; White et al., 1994) and functional imaging studies (Hugdahl et al., 1995; Logan and Grafton, 1995).

It can be expected that EBCC paradigms will remain of outstanding interest for basic and clinical research. With increasing interest clinical neurophysiologists use conditioning of the *electrically elicited* eyeblink reflex as a functional test to study patients with cerebellar and basal ganglia disorders. Usually an electromyographic (EMG) response that precedes the unconditioned stimulus by less than 200 ms is considered to represent a conditioned response (CR) if the EMG bursts reach a predetermined amplitude, e.g. 2 μV (Topka et al., 1993; Deuschl et al., 1996). Trials of paired stimuli (conditioned/unconditioned stimulus, CS-UCS) are applied blockwise, e.g. eight blocks with each block consisting of eight CS-UCS trials. For analysis of successful learning, the number of CRs is expressed as the percentage of trials containing CRs in proportion to the total number of trials per block.

Stating the problem: (a) definition of the CR by an arbitrarily determined amplitude of the EMG bursts regardless of the variation of baseline activity (BA) may result in under- or overestimation of CRs, depending on the chosen size of the amplitude. Fig. 1 shows the changes in baseline EMG activity during the course of a study in a healthy test person. (b) In subjects with a high rate of spontaneous blinks, there is an increased probability that these EMG events occur in the time window of 200 ms before the UCS and, therefore, may be misinterpreted as CRs (Fig. 2). (c) The width of the time window in which CRs are detected influences the number of registered EMG events. The longer the time period during which CRs are detected (conventionally 200 ms prior to UCS), the more likely spontaneous blinks may be mistaken for CRs. (d) Blockwise analysis of the CS-UCS trials does not allow accurate determination of onset time and maximum of conditioned learning and start of habituation.

The problem how to differentiate between voluntary or spontaneous blinks and conditioned blinks has been discussed for a long time (for review, see Martin and Levey, 1969). The aim of this study was to develop a method of data analysis which improves sensitivity and objectivity in detection of conditioned responses and supplies better resolution to evaluate time of onset and maximum of learning.

2. Methods

Fifty normal volunteers (22 women, 28 men, aged 22–87 years) were studied after giving informed consent.

2.1. Stimulation and recording technique

The blink reflex was conditioned using a classical delay conditioned paradigm (Gormezano, 1966; Martin and Levey, 1969).

2.1.1. Stimulation technique

The stimulus protocol started with five unconditioned stimuli (UCS-alone trials) and five conditioned stimuli (CS-alone trials) to document their independence and the neutrality of the conditioned stimulus (CS). These were followed by 64 CS-UCS trials (conditioning phase) and ten CS-alone trials (extinction phase). The paired stimulation (CS-UCS) consisted of a CS with a duration of 400 ms and an unconditioned stimulus (UCS) which was delivered just at the end of the CS (Fig. 3). The time interval between the trials varied between 8 and 12 s to avoid anticipation. The UCS consisted of an electrical square wave stimulus of 0.5 ms duration delivered to the supraorbital nerve via conventional cutaneous surface electrodes with the

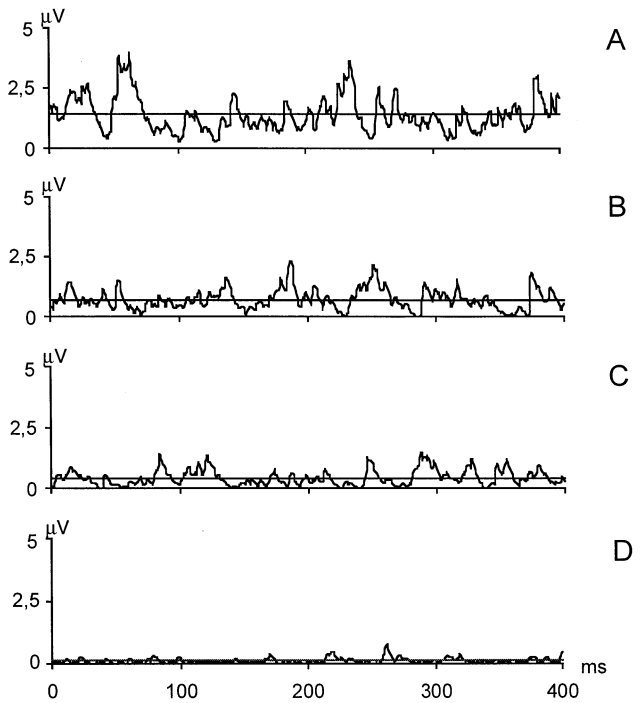

Fig. 1. Changes of baseline EMG activity recorded with surface electrodes from the orbicularis oculi muscle in a test person. Baseline activity in different CS-UCS trials. (A) Trial 4, (B) trial 20, (C) trial 44, (D) trial 60. In some subjects we observed the opposite, that is low baseline EMG activity at the beginning and increasing baseline EMG activity at the end of the study.

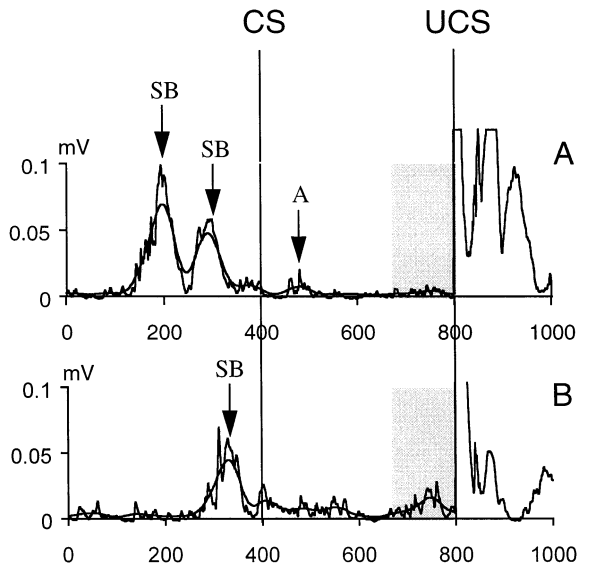

Fig. 2. Spontaneous blinks (SB) may occur at any time and may be mistaken for a CR when preceding the UCS by less than 150 ms. Shortly after the onset of the acoustic stimulus (CS) EMG activity is frequently observed which is smaller than that of a spontaneous blink or a conditioned response and represents an α-blink (A) or orienting response (Martin and Levey, 1969). This response is related to CS and shows an onset shortly after the beginning of the CS and therefore, may not be mistaken for a CR.

cathode placed over the supraorbital foramen. The stimulus intensity was increased gradually until a stable blink reflex was obtained with tolerable discomfort.

The conditioned stimulus (CS) consisted of an acoustic signal of 400 ms duration applied through earphones simultaneously to both ears with an intensity of 70–90 dB (sound-pressure level).

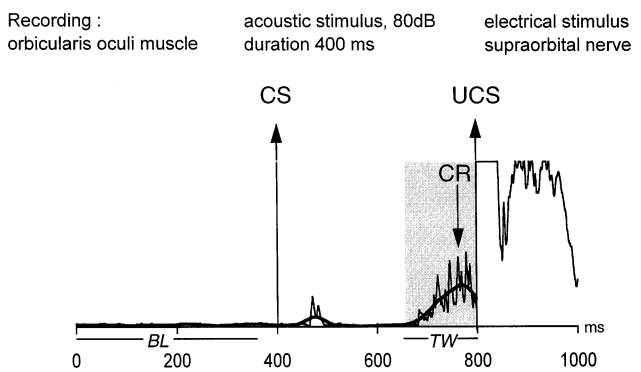

Fig. 3. Stimulation and recording technique. The conditioned stimulus (CS) terminates with the onset of the unconditioned stimulus (UCS). The conditioned response (CR) precedes the onset of the UCS. The bold line represents the filtered EMG activity (sampling frequency 1000 Hz), as described by Eq. (1). TW, time window in which CRs are detected. BL represents the segment of each recorded trial which was chosen for calculation of baseline activity. The frequency of the occurrence of SBs is also determined within the BL.

2.1.2. Recording technique

The EMG responses were recorded from the right orbicularis oculi muscle using surface electrodes. The active electrode was placed over the inferior orbicularis oculi muscle, the reference electrode at the lateral angle of the eyelid. The ground electrode was put around the right arm. The subjects were seated comfortably with their eyes open and were instructed to relax during the session. The EMG signals were rectified and filtered with a time constant of 10 ms and fed into a recording unit which was connected to a standard personal computer. The signals were digitized using a 12-bit analog–digital converter and a sample rate of 1000 Hz starting 400 ms before CS onset and finishing 200 ms after UCS onset (Fig. 3). In this way 1000 data points were obtained for each of the 64 CS-UCS trials.

2.2. Method of data analysis

2.2.1. Smoothing of data

In investigating EMG signals resulting from eye-blinks, one is usually not interested in signals with frequencies higher than about 20 Hz. Therefore, after the data are digitized, rectified and normalized to unit variance, each of the 64 trials was low-pass filtered. We used a triangular smoothing window with a total width of $2m = 70$ ms. Denoting a trial by $d(t)$ and the filtered trial by $\hat{d}(t)$ we get:

$$\hat{d}(\tau) = \sum_{t=-m}^{m} W(t)\, d(\tau + t),$$

where

$$W(t) = \begin{cases} (m+1)^{-1} - \dfrac{|t|}{(m+1)^2} & \text{if } |t| \le m \\ 0 & \text{otherwise.} \end{cases} \quad (1)$$

Fig. 3 (bold line) displays an example of a smoothed CS-UCS trial.

2.2.2. Search for spontaneous blinks (SBs)

The first 365 ms (beginning of the CS minus m) of each trial are taken as baseline (BL, Fig. 3). The standard deviation $\bar{b}$ of the 64 BLs is calculated. Two conditions are now defined to decide whether there are SBs in the BL of a particular trial or not. A detected local maximum has to fulfill two criteria to be regarded as SB: (1) it has to be broader than the smoothing window (in other words, the series of data points monotonically increases and decreases at least 35 ms before and after the maximum, respectively); (2) the maximum is greater than 2.5 times $\bar{b}$.

2.2.3. Estimation of the individual baseline means

For each of the 64 trials, the baseline mean $\overline{BL}$ and the baseline standard deviation BL_{SD} are now calculated. For every trial we get individual values which can be used later for the search for CRs. Therefore, the algorithm takes into account that the baseline may vary between different trials as displayed in Fig. 1.

If the algorithm detects SBs in one or more of the 64 BLs as described above, this or these BLs are excluded from the calculation of the individual $\overline{BL}$ and BL_{SD}. In this case, the average of the BLs of the preceding and following trial is taken.

2.2.4. Probability of the occurrence of spontaneous blinks

The estimated probability of the occurrence of SBs in an individual BL of a record is given by

$$\hat{p} = \frac{n_{spont}}{64}$$

where n_{spont} denotes the number of detected SBs in a randomly chosen time window of the BL. The length of this time window is identical with the length of the time window TW (Fig. 3) where we search for CRs. Confidence intervals for the value $\hat{p}$ are given by the Pearson–Clopper values (Hartung, 1989).

2.2.5. Search for conditioned responses

To optimize the length of the time window TW preceding the UCS in which CRs should be detected, we examined over 900 trials from 39 controls. Of the CRs, 95% preceded the UCS by less than 150 ms. Since a smaller window reduces the probability that SBs occur in the window, we chose the window width $n_w = 150$ ms.

The search for CRs in the selected time window TW takes into account the dynamics of a detected local maximum. We calculated the mean value over a certain area in the window which is determined by the criteria described below. First, the time of the highest point in the window is determined, denoted as t_{peak}. If we denote the interval of the window width by $[t_{low}, t_{high}]$, the following situations may occur:

1. $t_{peak} = t_{high}$: this case corresponds to Fig. 4A. The CR does not reach its maximum before the UCS. The algorithm takes the interval where the data points decrease monotonically to the left side of the window with a maximal width of $n_w = 150$ ms.
2. $t_{peak} = t_{low}$: this case corresponds to Fig. 4C. The maximum value does not usually result from a CR. It results from a stimulus-independent response after the CS. Therefore, the algorithm searches for further peaks as mentioned in the next item and displayed in Fig. 4B.
3. $t_{low} < t_{peak} < t_{high}$: this case corresponds to Fig. 4B, and is most frequently observed. Here, the algorithm takes the interval where the data points decrease monotonically from the highest point.

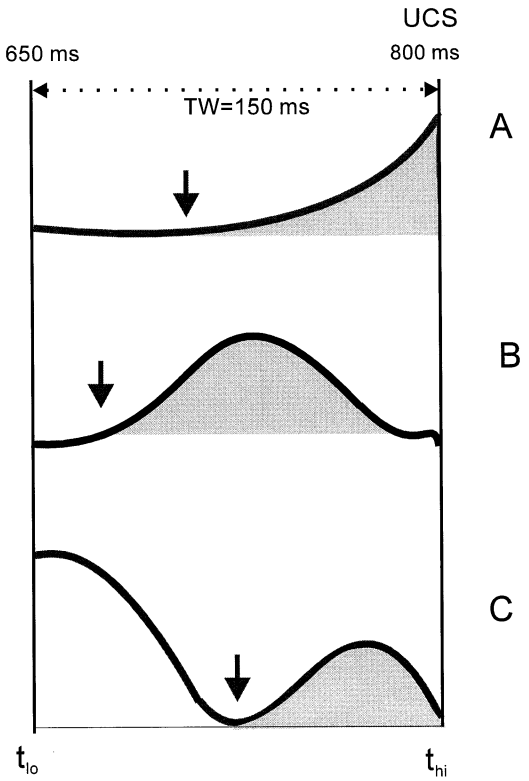

Fig. 4. Time window of 150 ms preceding the UCS in which the CR occur. (A–C) Schematic display of the different possibilities of a local maximum which may occur. For description of the curves see text.

The second item does not exclude the third, if further maxima are found in case 2.

In the next step, the mean value over the determined time interval is estimated, in the following referred as CR_{mean}. We detect a CR in the examined trial, if

$$R = \frac{CR_{mean}}{\overline{BL} + 2.5\ BL_{SD}}$$

is greater than 1, i.e. if the value CR_{mean} is greater than the BL mean plus 2.5 times the BL standard deviation of this particular trial.

If the number of detected CRs is smaller than the probable number of SBs that are expected to occur with respect to $\hat{p}$ and its 95% confidence intervals, the data record must be excluded from further analysis. In this case, one cannot know whether the detected CRs result from a learning process or just represent SBs occurring in the time window under investigation.

2.2.6. Display of results

The values R_i, $i = 1,\ldots,64$ (one for each trial), are taken as values representing CR. As mentioned above, if R_i is greater than 1, there is a CR detected in the ith trial, if it is less than 1, there is no significant response in this trial. It is important to mention that these values are independent of any physical or physiological unit.

We examined several possibilities for the analysis of the 64 values R_i.

2.2.6.1. Trend estimation via a median filter. The advantage of this method is that it easily detects whether there really is a trend to higher values R_i or only a few higher values are present, probably resulting from SBs. This method is useful to basically separate the groups 'not conditioned' and 'conditioned', but can also be used to investigate speed of learning as well as maximum of learning and habituation. The disadvantage of this method is that the amount of learning may be underestimated.

2.2.6.2. Trend estimation via a triangular window (similar to the filter used in Eq. (1)). This method produces smoother curves than trend estimation via a median filter. In opposition to the first method, the disadvantage is that the onset of learning may be estimated too early since a single, very strong response can shift a part of the series upward to values greater than 1. For the same reason this method is not useful in determining if a subject fails to acquire conditioned responses.

The way we calculated speed and maximum of conditioning is schematically displayed in Fig. 5. When present, we also calculated the speed of habituation.

The Kruskal–Wallis ANOVA test was used to test differences between group means.

3. Results

Fig. 6 displays the results of the proposed method of data analysis for a healthy person (Fig. 6A) and a person with a cerebellar lesion (Fig. 6B). We used trend estimation via a median filter (solid line in Fig. 6) to determine whether a person acquired conditioned responses or failed to acquire conditioned responses. We also detected the onset of learning by this method. Trend estimation via a triangular window (dashed line in Fig. 6) was applied for the estimation of the maximum amount of learning in those individuals where trend estimation via a median filter showed a significant amount of learning.

3.1. Blink reflex conditioning in healthy subjects

Table 1 shows the results of 50 healthy subjects. Of all subjects studied, 22% did not acquire any conditioned responses. This remained unchanged even after modification of the stimulation parameters. In the group below the age of 30 years, 17.4% failed to acquire conditioned responses. The number of subjects who did not show any eyeblink conditioning increased with higher age. Differences between the age groups were statistically significant for the number of CRs in the 64 CS-UCS trials and for the maximum amount of conditioning expressed in multiples of the mean baseline EMG activity (Kruskal–Wallis ANOVA, Table 1). Onset of learning was also significantly later with increasing age. However, this was compensated by an increased speed of conditioning.

4. Discussion

The aim of the present study was to develop an algorithmic method of data analysis to improve the value of the electrically elicited EBCC in neuropsychological research and as a functional test in clinical neurophysiology.

Basically there are different methods available to evoke an eyeblink which can be conditioned by an acoustic stimulus. The airpuff technique preferentially

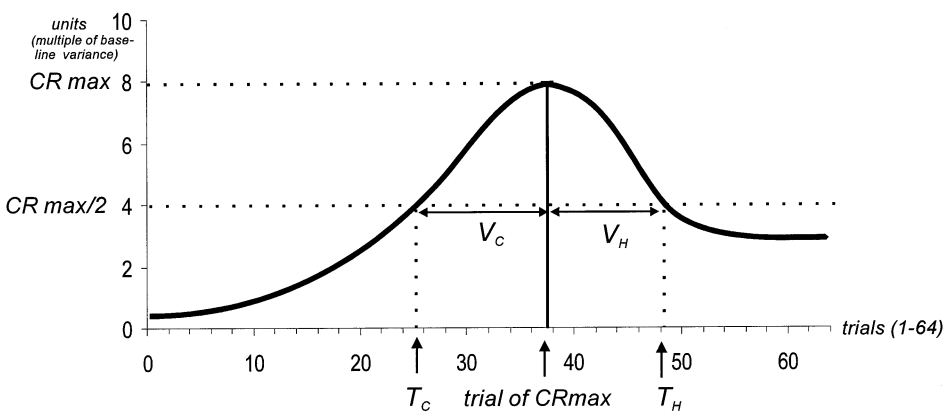

Fig. 5. Schematic display of a smoothed, i.e. trend-estimated, CR curve to explain the extracted values. T_C and T_H are the trial numbers where the curve reached half its maximum value CR_{max} before and after the trial of CR_{max}. V_C is defined as the difference between CR_{max} and T_C and gives an estimation of the 'speed of learning'. V_H is defined as the difference between T_H and CR_{max} and supplies an estimation of the 'speed of habituation'. The higher the velocity, the steeper the slopes. Therefore, a small V_C or V_H stands for a high velocity of conditioning and habituation, respectively.

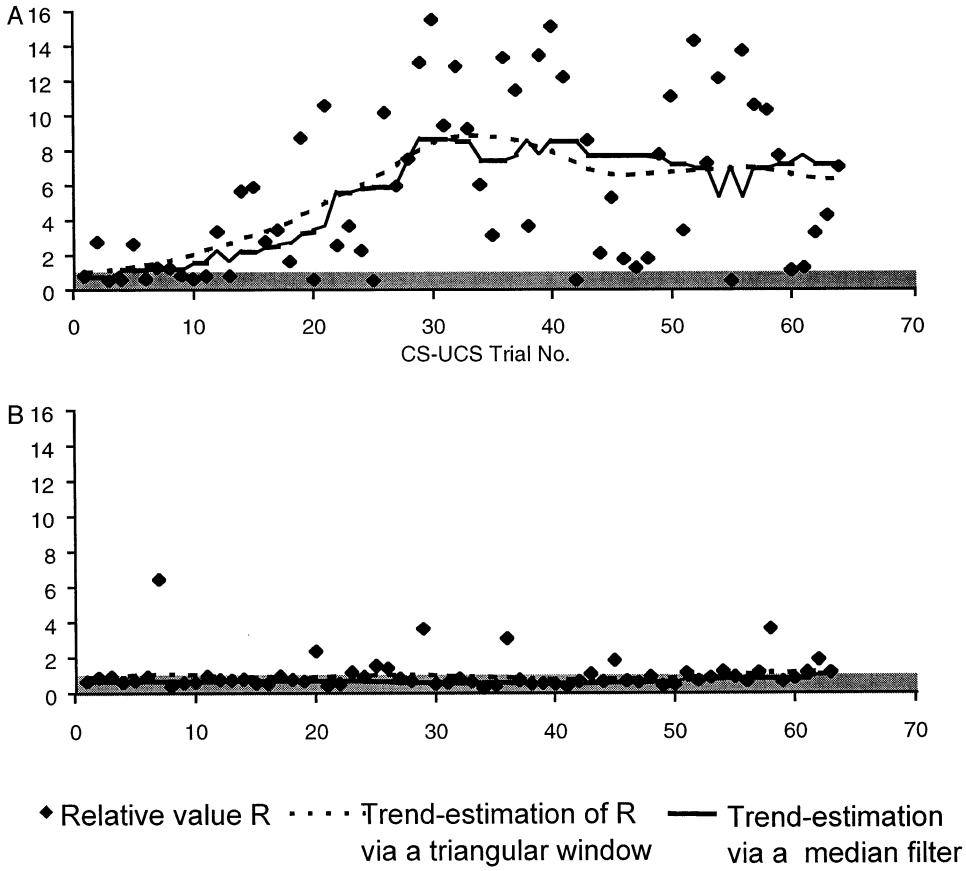

Fig. 6. Analysis of the 64 CS-UCS trials in a normal control subject (A upper trace) and in a patient with cerebellar hemorrhage (B lower trace). If the displayed values R_i are above 1, the algorithm detected a CR. Also in case (B), which was detected as 'not conditioned', there are some values above 1. Nevertheless, the median filtered curve (solid line) is always below 1. This indicates that there is no consistent trend towards higher values of R_i. In case (B), the values R_i above 1 are randomly distributed over all trials. By contrast, for the control subject in (A), the frequency of the occurrence of values R_i above 1 increases consistently beginning with trial 10, reaches its maximum at trial 32 and, finally, decreases again slowly, indicating habituation.

used by neuropsychologists consists of an airpuff applied to the cornea of one eye. A mechanical tap to the forehead may also be used as unconditioned stimulus. The evoked eyelid movement may be recorded in both cases by a photocell system.

We applied the electrically elicited blink reflex which is preferentially used by clinical electrophysiologists and consists of an electrical shock to the supraorbital nerve. The evoked EMG response is recorded with surface electrodes from the orbicularis oculi muscle. To avoid an unacceptably long duration of a session the conditioning phase was restricted to 64 CS-UCS trials, which has proven to be sufficient to evaluate EBCC performance (Papka and Woodruff-Pak, 1996). During this time, habituation of CR was only observed in 28 of 50 control subjects. Therefore, studies on habituation of CR may need a longer conditioning phase.

Baseline EMG activity may change substantially within one session as shown in Fig. 1. High baseline EMG activity may lead to erroneous assumption of a CR if the definition of the CR is independent of baseline EMG activity. To overcome this problem, we defined CR individually with respect to the mean baseline EMG activity of each CS-UCS trial. A similar approach has been used by Thompson (1976) in animal studies when measuring neuronal activity to study the motoneuron substrate of conditioning.

As the duration of the CR showed great variability, it seems more appropriate to consider a mean value over the duration of the CR (CR_{mean}).

With the electrical method, as well as with the airpuff technique or a mechanical tap, spontaneous blinks may influence the results. To estimate the occurrence of spontaneous blinks, we investigated the baseline EMG activity before the onset of the conditioned stimulus over a randomly selected segment with a duration of 150 ms. Thus we were able to estimate the probability of spontaneous blinks appearing in the time window where CRs are detected.

The analysis of time of onset of CRs in 900 trials of 39 control persons revealed that a time window preceding the UCS by 150 ms is sufficient. This is in line with

the close relationship of the conditioned response and the onset of the unconditioned stimulus (Smith, 1968).

Shortening of the time window from 200 to 150 ms further reduces the probability of false-positive CRs. The proposed method increases the specificity in detecting CRs. However, this may lead to a decreased sensitivity by missing CRs, especially at the beginning of the learning process. We assumed lack of conditioning when the number of detected responses in relation to the number of trials and the width of the time window was smaller than the estimated probability of spontaneous blinks in the time window.

Applying these learning criteria, 22% of the healthy subjects studied did not acquire conditioned responses. Failure of conditioning in healthy subjects has not been reported in previous studies. However, the high standard deviations in the reported control groups suggest that lack of conditioning occurred in healthy subjects (Topka et al., 1993; Daum et al., 1996; Deuschl et al., 1996). In those subjects who acquired conditioned responses, conditionability showed very considerable individual differences. This phenomenon has been well known for a long time and has been discussed in detail elsewhere (Martin and Levey, 1969).

The mean inter-trial interval of 10 s seems to be short but has been used for patient studies to limit duration of the examination (Topka et al., 1993; Daum et al., 1996). This may, however, account for the higher number of non-conditioners as the duration of the inter-trial interval influences the learning process. Conditioning occurs most rapidly (in terms of trials required, not total real time) with longer inter-trial intervals (Levinthal and Papsdorf, 1970). Future studies with a longer mean inter-trial interval, e.g. 20–30 s may reveal a lower number of non-conditioners.

EBCC may be a powerful tool in clinical research for group analysis of patients with different neurological disorders to detect deficits in associative learning.

Single trial analysis and trend estimation was used instead of blockwise analysis of the CS-UCS trials to allow more accurate determination of onset and maximum of the learning process and the beginning of habituation.

Table 1
Mean values for conditioning of the electrically elicited blink reflex in 50 healthy subjects

Subjects				<30 years	30–60 years	>60 years	Total
Conditioned responses				15 (88%)	15 (79%)	9 (64%)	39 (78%)
No conditioned responses				2 (12%)	4 (21%)	5 (36%)	11 (22%)
Total				17 (100%)	19 (100%)	14 (100%)	50 (100%)
	Age	Number of CRs (in 64 trials)	Trial of onset	Trial of CR_{max}	V_C[a]	V_H[a]	CR_{max} (units in multiples of baseline mean)
Total							
Mean (*n*)	45 (50)	34 (50)	14 (39)	39 (39)	14 (39)	14 (27)	7.8 (39)
Range	22–87	0–62	3–42	3–64	6–40	5–33	1.2–37.4
SD	19	22	10	14	9	7	7.5
Age <30 years							
Mean (*n*)	26 (17)	41 (17)	10 (15)	43 (15)	18 (15)	16 (10)	9.4 (15)
Range	22–29	0–62	3–32	21–62	7–36	8–28	1.2–37.4
SD	2	19	7	13	9	7	9.5
Age 30–60 years							
Mean (*n*)	45 (19)	34 (19)	14 (15)	39 (15)	13 (15)	16 (11)	7.9 (15)
Range	31–60	0–60	5–31	17–64	6–40	5–33	2.0–24.0
SD	10	23	8	12	9	9	6.2
Age >60 years							
Mean (*n*)	73 (14)	15 (14)	25 (9)	33 (9)	11 (9)	11 (6)	2.3 (9)
Range	61–87	0–53	3–42	3–54	3–18	8–14	1.3–3.4
SD	7	16	15	21	6	2	1.0
Kruskal–Wallis ANOVA		$P \leq 0.05$	$P \leq 0.05$	n.s.	$P \leq 0.05$	n.s.	$P \leq 0.05$

[a] V_C, measure for velocity of conditioning; V_H, measure for velocity of habituation. For explanation of V_C and V_H see Fig. 5. *N*, number of subjects; CR, conditioned response; CR_{max}, amount of conditioning expressed in multiples of baseline mean.

The proposed method is automated, more sensitive and specific in detection of CRs and allows exact determination of different additional parameters, e.g. onset of learning, maximum of learning, velocity of learning, and onset of habituation. The method used is independent of any quantitative physical units, since only relative values for each individual are considered. Furthermore, the single trial analysis compensates intra-individual changes during the experiment. Finally, it is noteworthy that the whole procedure only takes about 10 s for the data of one subject on a standard personal computer. The neurobiological significance of these additional parameters has to be further substantiated in future studies on patients with well-defined cerebellar and basal ganglia lesions. Using this refined technique in patients with selective disorders may lead to better understanding of the underlying mechanisms involved in EBCC in humans.

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