Modulation of induced gamma band responses and phase synchrony in a paired associate learning task in the human EEG

Thomas Gruber[a],*, Andreas Keil[b], Matthias M. Müller[a]

[a]Cognitive Neuroscience, Department of Psychology, University of Liverpool, Eleanor Rathbone Building, Liverpool, L69 7ZA, UK
[b]Department of Psychology, University of Konstanz, Konstanz, Germany

Abstract

It has been proposed that associative learning is accomplished by the formation of cell assemblies and synchronous activity among the neurons of such an assembly. Induced gamma band responses (GBRs) and phase synchrony between electrode sites are discussed as a signature of activity within a cell assembly. To examine the activation of this network due to memory recall, a paired associate learning paradigm was used. EEG was analyzed in the frequency domain. Results showed a significant increase of induced GBRs at posterior and anterior electrode sites in the recall sequence of the learning condition. Furthermore, phase synchrony revealed a broad distribution pattern of phase synchrony between posterior and frontal electrode sites. © 2001 Elsevier Science Ltd. All rights reserved.

Associative learning is regarded as an important mechanism in both short-term and long-term memory storage and retrieval of complex episodes or stimulus configurations. In contrast to implicit forms of memory, which are characterized as an unintentional, non-conscious form of memory, associate learning can be regarded as an explicit memory form, described as conscious recognition or recall [14]. In 1949, Hebb [3] proposed that learning is accomplished by the formation of cell assemblies and synchronous neural activity among their neurons. Induced gamma band responses (GBRs) were discussed as a signature of activity within a cell assembly (for recent reviews, see [8,15]), which can be reliably measured in the human EEG [1,7,10,11,16,17]. Furthermore, phase synchrony between pairs of electrodes, independent of power, may provide a better measure for synchronized neural activity forming a cell assembly [9,13].

Imaging studies indicate that object knowledge seems to be stored in a distributed neural network in which information about specific features is stored close to those regions of the cortex that mediate the perception of these features [18]. In addition, neuroimaging studies on explicit retrieval of information from memory reveal an activation of various regions of prefrontal cortex possibly related to intentional retrieval or monitoring processes [14]. In the human EEG, Honda and co-workers [4,5] described a posterior positive evoked potential component approximately 550 ms after the onset of a cue in an associate learning paradigm, possibly related to retrieval of information from memory.

In an attempt to extend Honda's findings to the frequency domain, their paradigm was adopted and high-frequency brain activity was analyzed. We expected activation within a widespread network linking cortical storage sites and recall mechanism. Thus, activation of a network, which represents a stimulus, should result in an increase of induced GBRs and phase synchrony at posterior and anterior electrode sites. To examine this hypothesis, we used a paired associate learning paradigm and measured brain activity by means of a 128-channel EEG-montage. EEG was recorded from 13 right-handed volunteers (seven female, six male; mean age, 26.9 years; SD, 3.7 years). Similar to Honda et al. [4], we used 13 line drawings of simple symbols (for example rectangle, star, circle) as stimuli, which were presented in white on a black background in the center of a computer screen. Each stimulus covered a visual angle of approximately $2.6 \times 2.6°$. The 13 stimuli were allocated randomly to two different experimental tasks. In a blocked design, we presented: (1), a paired associate learning task (PAL); and (2), a choice reaction task (CR), which was used as a control condition to identify brain activity specifically related to memory function in the

---

* Corresponding author. Tel.: +44-151-794-6706; fax: +44-151-794-6937.

E-mail address: tgruber@liverpool.ac.uk (T. Gruber).

learning task. The experiment comprised 360 trials for each task. Before the PAL task, four pairs of stimuli (S1 and S2) had to be memorized for 2 min. The 360 trials for each task were divided into four blocks of 90 trials each, and a new set of pairs for each block was created. One trial consisted of a 500 ms baseline period (black screen). After that, S1 was presented for 100 ms. Subjects were instructed to recall the specific pair belonging to S1 immediately after S1 onset. After another 1900 ms of black screen, S2 was presented as a probe. Subjects had to judge whether or not S1 and S2 formed one of the memorized pairs after S2 presentation. They were instructed to press either a 'yes' key for paired S2 stimuli or a 'no' key for un-paired S2 stimuli. In addition to the presentation of paired stimuli, subjects were confronted with a distractor stimulus, which required always a 'no' response. The time course of one trial in the CR task was equivalent to PAL, but subjects had to decide if S2 was a target stimulus. The target stimulus was defined before each block and different targets were used, respectively. The CR task aimed to have S2 not linked to S1, therefore S1 simply served as a warning stimulus, indicating that S2 would occur in 2000 ms. Furthermore, as in PAL, a distractor stimulus which always required a 'no' response was presented. Altogether, the experimental set-up resulted in six conditions: S1 and paired S2; S1 and un-paired S2 (PAL); S1 and target S2; S1 and non-target S2 (CR); and distractors during PAL and CR. The distractor conditions were introduced to control for general differences of sustained attention or alertness in the two different tasks.

EEG was recorded continuously with an EGI (Electrical Geodesics, 1998) 128-electrode array, referenced to Cz (impedances, <50 kΩ; sampling rate, 500 Hz; 0.1–200 Hz online bandpass). EEG was segmented to obtain epochs starting 500 ms prior and 1000 ms following S1 onset. Artifact correction was performed by means of 'statistical correction of artifacts in dense array studies' (SCADS) [6] based on the average reference. Using this method, three subjects were excluded due to excessive artifacts.

Behavioral data demonstrated that subjects performed well in both tasks. The average percentage of correct answers was 94.33% (SD = 3.17%) in PAL and 98.25% (SD = 1.77%) in CR. Furthermore, a paired t-test revealed that in PAL, reaction times were significantly faster for S2 stimuli forming a memorized pair ($t_{(9)} = -5.65$, $P < 0.001$), indicating that subjects recalled the correct S2 stimulus in the interval between S1 and S2 (paired stimuli: 608.3 ms, SD = 123.7; un-paired stimuli: 687.7 ms, SD = 112.4).

In order to analyze spectral changes in the induced gamma band, a wavelet analysis based on complex Morlet wavelets [16,17] was used, resulting in a time–frequency (TF) representation of the signal. TF energy is averaged across single trials, allowing analysis of non-phase-locked high-frequency components. An epoch from 400 to 100 ms prior to S1 onset was used as a baseline. After wavelet analysis, the mean spectral power averaged across extended 10–20 electrode

sites (indicated in Fig. 2), was represented in TF-plots in the gamma range for distractors, PAL, and CR task. For the purpose of generating these TF-plots in PAL, all trials with a previously learned S1 stimulus were averaged, regardless of whether or not S2 formed a matched pair. For CR, we averaged all trials for which S1 was not the distractor. As depicted in Fig. 1, spectral power in the PAL task showed a maximum in a time window from 400 to 500 ms after S1 onset in a frequency range between 56 and 76 Hz.

Two different ANOVA models were used to analyze the time window and frequency band showing maximal spectral power. (1) An omnibus test with a repeated measurement ANOVA comprising the factors of CONDITION (six experimental conditions) and RECORDING SITE (29 electrode sites corresponding to the extended 10–20 system) was calculated to uncover more general effects of our experimental conditions. (2) To analyze the differences between PAL and CR more specifically, a repeated measurement ANOVA excluding the distractor conditions was calculated with the factors TASK (PAL/CR) × RECORDING SITE (29). The omnibus test resulted in a main effect of CONDITION ($F_{(1,9)} = 3.38$, $P = 0.01$). A post hoc t-test revealed significantly higher gamma power for the paired S2 and un-paired S2 PAL conditions as compared with the distractor (distractor vs. paired S2: $t_{(9)} = -2.84$, $P < 0.05$; distractor vs. un-paired S2: $t_{(9)} = -3.03$; $P = 0.01$). As expected, no significant differences were found between paired/un-paired S2 stimuli. No differences between any of the conditions in the CR task revealed significance. Importantly, no differences were found between the two distractor conditions, suggesting
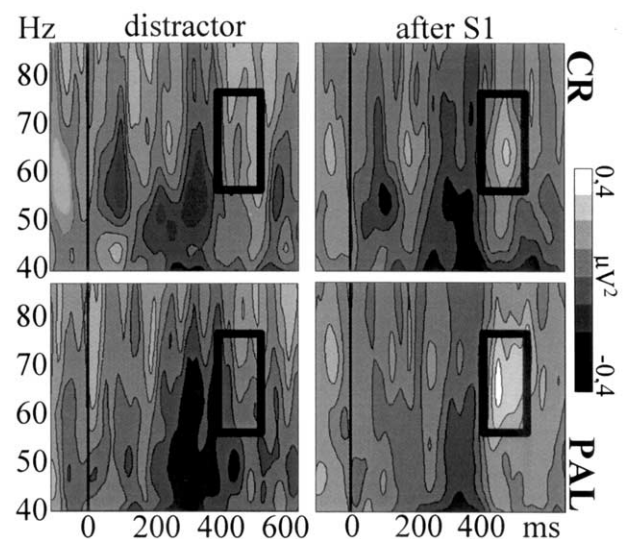

Fig. 1. Grand mean baseline corrected time by frequency plots (*induced* gamma power) for the distractor conditions, PAL and CR task, respectively. Averages across 10–20 electrode sites are presented (see Fig. 2 legend). Note: time and frequency windows used for further analysis are indicated by rectangles. In PAL, an average across paired/un-paired S2 stimuli, and in CR, an average across target/non-target S2 stimuli is shown.

no influence of different levels of alertness or sustained attention in the two tasks. As revealed by a main effect TASK in our second ANOVA, induced gamma band power was higher in PAL as compared with CR ($F_{(1,9)} = 17.64$, $P < 0.01$). The difference was most prominent at centro-parietal and frontal electrode sites (TASK × RECORDING SITE: $F_{(28,252)} = 2.02$; $P = 0.01$). Post hoc paired *t*-tests for six electrode sites showing maximum differences in power between the learning and the CR task revealed significant effects at electrode sites F7 ($t_{(9)} = -3.40$, $P < 0.01$), F8 ($t_{(9)} = -2.49$, $P < 0.05$), Pz ($t_{(9)} = -3.29$, $P < 0.01$), Cz ($t_{(9)} = -3.38$, $P < 0.01$), Cp1 ($t_{(9)} = -2.35$, $P < 0.05$), and Cp2 ($t_{(9)} = -2.67$; $P < 0.05$).

To control for effects in other frequency bands, the same ANOVA models were applied to the alpha band (8–12 Hz), and the gamma range above and below the GBR showing a maximum in spectral power (34–53 and 78–97 Hz). All these analyses revealed no significant effects, indicating: (a), that the reported findings are not due to harmonics of lower frequency bands; and (b), that neural activity in the proposed cell assembly synchronizes in a relatively narrow frequency band in this experiment. Importantly, no significant effects with respect to evoked gamma activity were found.

Phase synchrony analysis was performed elaborating on a procedure suggested by Rodriguez et al. [13]. Synchrony was calculated between pairs of electrodes corresponding to 10–20 electrode sites. In order to find statistically significant phase-locking values between two electrodes, a statistical randomization technique was used [13]. The same time window as for the GBR power peak was chosen. Furthermore, three non-overlapping time windows before, and one after the gamma peak were analyzed. In Fig. 2, significant values of synchrony and desynchrony between pairs of electrodes are depicted ($P < 0.01$). For PAL, most of the significant incidents of synchrony were found in time window 140–240 ms after stimulus onset among distant posterior and anterior electrode sites, which might indicate synchronous neural activity in a broadly distributed network. In the two following time windows, similar overall patterns of synchrony with slightly fewer electrode pairs showing significant synchrony values were observed. In CR, less phase synchrony, as compared with PAL, was observed in all time windows. Practically no significant desynchrony was observed in both conditions.

On the basis of the present findings, the hypothesis that the activation of a widespread Hebbian cell assembly formed by learning processes and related to recall processes from memory seems to be supported. This hypothesis is similar to an account taken by Pulvermüller [12]. He reported an increase of induced GBRs after the presentation of words as compared with pseudo-words, and concluded that this increase is a signature of activity within a cortical representation that exists for words but not for pseudo-words since words elicit a learned representation. The present study extended his results to learned associative links between two line drawings as used in our PAL task.

With regard to the different time course of the increase in gamma power and phase synchrony, it should be mentioned that spectral power and synchrony should not be confounded [13]. Given the long period of the retention interval, it might well be that each subject showed an inter-individual jitter with regard to the time-point of S2 recall. In this case, the latency of the maximum in gamma power should be different for each subject. The consequence in the grand mean spectral power is a significant gamma band increase only in a short time window (see Fig. 1). However, since phase synchrony is a measurement independent of amplitude, phase synchrony may reveal significance before the induced gamma peak, resulting in the long persistence of synchrony depicted in Fig. 2.

The topographical distribution of GBRs in the present study is in line with other studies examining induced GBRs in relation to memory processes. In a previous study using a rapid perceptual learning paradigm, induced GBRs significantly increased at centro-parietal, but not at frontal electrode sites [2]. We assumed this lack of frontal
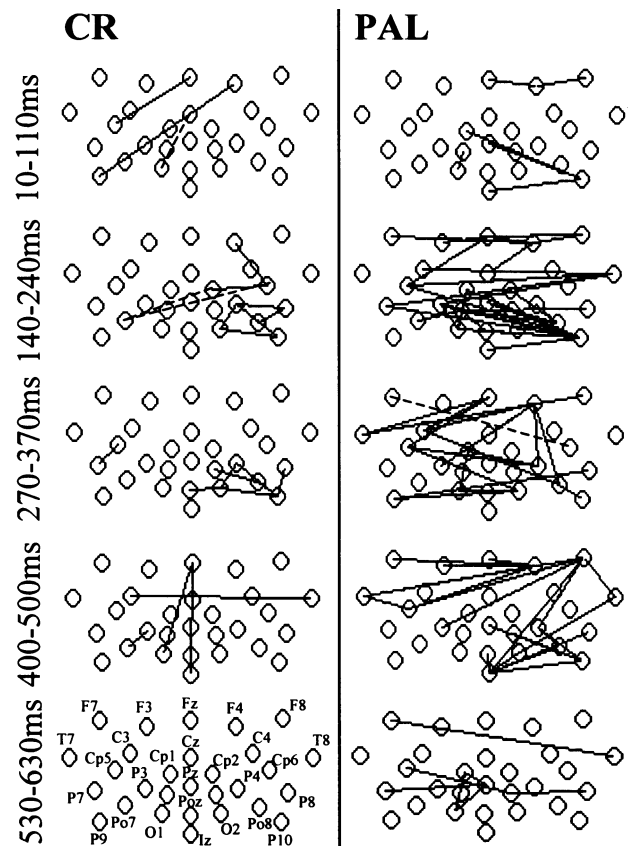

Fig. 2. Synchrony (solid lines) and desynchrony (dashed lines) between 10–20 electrode pairs for the learning and the CR task, respectively. Five non-overlapping time windows are depicted. Lines are drawn only if the phase-locking value is beyond the distribution of shuffled data ($P < 0.01$). Note: extended 10–20 electrode names are given in one electrode layout.

activity was due to the implicit nature of the task in this study. In contrast, the frontal activity in a time window from 400 to 500 ms found in the present PAL task might reflect explicit recall processes, which are necessary to solve the task (see Ref. [17] for similar results). This interpretation is in line with results from imaging studies, which reported activity in prefrontal areas related to intentional retrieval processes [14]. Although the idea of a synchronized cortical network involving prefrontal and posterior areas fits well with the current hypothesis on memory processes, it has to be mentioned that this interpretation is speculative at the moment, because scalp recordings do not allow us to draw direct conclusions on underlying cortical generators.

In summary, the present experiment has an important implication for the understanding of the neural mechanism of associative learning. Induced GBRs and phase synchrony may be a signature of a widespread Hebbian cell assembly covering frontal and posterior areas, which is crucial for the storage and recall of a learned stimulus configuration.

[1] Gruber, T., Müller, M.M., Keil, A. and Elbert, T., Selective visual–spatial attention alters induced gamma band responses in the human EEG, Clin. Neurophysiol., 110 (1999) 2074–2085.

[2] Gruber, T., Keil, A. and Müller, M.M., Modulation of induced gamma band responses in a perceptual learning task in human EEG, J. Cogn. Neurosci. (2001) submitted for publication.

[3] Hebb, D.O., The Organization of Behavior, Wiley, Chichester, UK, 1949.

[4] Honda, M., Barrett, G., Yoshimura, N., Ikeda, A., Nagamine, T. and Shibasaki, H., Event-related potentials during paired associate memory paradigm, Electroenceph. clin. Neurophysiol., 100 (1996) 407–421.

[5] Honda, M., Barrett, G., Yoshimura, N., Sadato, N., Yonekura, Y. and Shibasaki, H., Comparative study of event-related potentials and positron emission tomography activation during a paired-associate memory paradigm, Exp. Brain Res., 119 (1998) 103–115.

[6] Junghöfer, M., Elbert, T., Tucker, D.M. and Rockstroh, B., Statistical control of artifacts in dense array EEG/MEG studies, Psychophysiology, 37 (2000) 523–532.

[7] Keil, A., Müller, M.M., Ray, W.J., Elbert, T. and Gruber, T., Human gamma band activity and perception of a gestalt, J. Neurosci., 19 (1999) 7152–7161.

[8] Keil, A., Gruber, T. and Müller, M.M., Functional correlates of macroscopic high-frequency brain activity in the human visual system, Neurosci. Biobehav. Rev., 25 (2001) 527–534.

[9] Miltner, W.H.R., Braun, C., Arnold, M., Witte, H. and Taub, E., Coherence of gamma-band activity as a basis for associative learning, Nature, 397 (1999) 434–436.

[10] Müller, M.M., Bosch, J., Elbert, T., Kreiter, A., Valdes Sosa, M., Valdes Sosa, P. and Rockstroh, B., Visually induced gamma-band responses in human electroencephalographic activity — a link to animal studies, Exp. Brain Res., 112 (1996) 96–102.

[11] Müller, M.M., Gruber, T. and Keil, A., Modulation of induced gamma band activity in the human EEG by attention and visual information processing, Int. J. Psychophysiol., 38 (2000) 283–299.

[12] Pulvermüller, F., Hebb's concept of cell assemblies and the psychophysiology of word processing, Psychophysiology, 33 (1996) 317–333.

[13] Rodriguez, E., George, N., Lachaux, J.P., Martinerie, J., Renault, B. and Varela, F.J., Perception's shadow: long-distance synchronization of human brain activity, Nature, 397 (1999) 430–433.

[14] Schacter, D.L., Buckner, R.L. and Koutstaal, W., Memory, consciousness and neuroimaging, Philos. Trans. R. Soc. Lond. B Biol. Sci., 353 (1998) 1861–1878.

[15] Tallon-Baudry, C. and Bertrand, O., Oscillatory gamma activity in humans and its role in object representation, Trends Cogn. Sci., 3 (1999) 151–162.

[16] Tallon-Baudry, C., Bertrand, O., Delpuech, C. and Pernier, J., Oscillatory gamma-band (30–70 Hz) activity induced by a visual search task in human, J. Neurosci., 17 (1997) 722–734.

[17] Tallon-Baudry, C., Bertrand, O., Peronnet, F. and Pernier, J., Induced gamma-band activity during the delay of a visual short-term memory task in humans, J. Neurosci., 18 (1998) 4244–4254.

[18] Ungerleider, L.G., Functional brain imaging studies of cortical mechanisms for memory, Science, 270 (1995) 769–775.