A model of cognitive enhancement would be of benefit as a screening tool in the search for new therapies for cognitive disorders such as Alzheimer’s disease. This article provides arguments in favor of neurophysiological assessments during performance in psychometric tests to fulfil such aims. The first part concerns the basic characterization of event-related potentials (ERPs) and, in particular, the generators of the cognitive response called P300, in terms of temporal and spatial properties. Next, we investigate the effects of both noncholinergic and cholinergic drugs and their interaction in healthy young male and elderly subjects using the extracted ERP parameter as readout.

**Temporal and spatial characterization of cognitive responses**

ERPs are transient modifications in electromagnetic brain signals, which are time-locked to cognitive, motor, or sensory processing. They represent activity directly at the level of neuronal networks and hence form a good method for studying the working brain and obtaining neurophysiological indices of attentional mechanisms and cognitive function. In so-called “oddball” paradigms, in which a subject is instructed to count the number of target stimuli, a positive scalp potential with a maximum amplitude of around 300 ms is recorded and is referred to as P300 (Figure 1). Before the emergence of this type of activation, the brain signals display a sequence of components related to consecutive steps of information processing in the central nervous system (CNS), like encoding of stimulus, orienting reaction, etc. These occur in certain time-windows during normal functioning, and the term chronometry is often used. Hence, such electrical responses from the scalp are relevant because they are the result of a coordinated synchronization in distributed neuronal populations. Topographic analysis
reveals patterns in agreement with such a hypothesis (Figure 2, top). The reversal in polarity in the posterior part (oriented toward the right) suggests the presence of parietotemporal sources. Several lines of evidence indicate that the sources must be located in deep structures, such as the hippocampal formation. Multiple generators located in limbic frontal and inferotemporal brain regions are described as neuronal substrates, \(^2,3\) as has been confirmed by positron emission tomography (PET) imaging \(^4\) and, more recently, by hemodynamic responses in functional magnetic resonance imaging (fMRI). \(^5,9\) Speculations are made that the underlying generators are located in medial temporal lobe regions.

A method that is promising to better characterize these phenomena is the recording of the magnetic fields. Regarding auditory P300, contradictory results have been reported, ranging from no response \(^10\) to an effect in the left hemisphere, \(^11\) or more global activations during a lexical test \(^12\) or equivalent oddball tasks in the visual modality. \(^13\) These studies have been carried out either with gradiometers or with a limited number of sensors, covering only a small portion of the cortex. We used a more sensitive method, namely a whole-head magnetometer array (148 channels, Magnes 2500 WH, BTI, San Diego, Calif) to study the equivalent P300 generators. Figure 2 displays a top view (bottom left) and lateral view (bottom right) of the average activation pattern obtained in the P300 paradigm for the same views as for the electrical responses (Figure 2, top). As can be seen, a highly structured pattern with a positive and a negative pole, called the magnetic dipole, is present. A similar but mirror-imaged pattern is present over the right hemisphere. The rotation by about 90° with respect to the orientation of the electrical pattern should be noted.

Many researchers have developed three-dimensional reconstruction or source localization techniques. With my colleague L. Soufflet, we have also implemented such noninvasive imaging procedures. We recently demonstrated that analysis using magnetoencephalography (MEG) can model this type of cognitive response, yielding, as expected, a relatively simple solution of the complex neuronal interactions, by a preliminary process of localizing the sources using a spherical model of the head, topologically adapted for the anatomical substrate, as shown in Figure 3. The locations of current vectors, which explain more than 90% of the observed brain signals, have their origin in the hippocampal formation. Hence, limbic structures contribute to information processing during cognitive discrimination in the auditory paradigm.

**Functional aspects and neuropharmacology**

P300 characteristics such as amplitude and latency are altered during aging. \(^14,15\) The pathophysiological state is reflected in the brain activity. \(^16,18\) Moreover, the deterioration effect has been shown to coincide with the clinical severity of mental illness in demented patients. \(^19-21\) The activation pattern has been shown to be under cholinergic control: scopolamine is able to attenuate or even abolish the P300 response in young healthy volunteers. \(^22-24\) Figure 4 shows the postdosing evolution and statistical comparison of the effect of the cholinergic antagonist scopolamine (0.5 mg, subcutaneous [SC], top). As can be seen, a significant frontocentral attenuation is present in healthy volunteers (upper right...
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Panel). Such effects parallel the deterioration in mnemonic capacities induced by the drug (see below). Acetylcholinesterase (AchE) inhibitors, on the contrary, induce increases in these topographic regions after oral administration. Indeed, oral administration of donepezil, a representative of the class of nootropic compounds, induces the opposite effects (Figure 4, bottom). This type of effect is in full agreement with the existing literature.26-27

The message so far is that the relationship between neuropsycharmacology of the cholinergic system, which is fairly straightforward, on the one hand, and evoked (cognitive) responses reflecting conscious attention, which fits with the functional brain anatomy of limbic circuitry, on the other, form an ideal basis to study drug interaction in research in the field of psychiatric disorders.

Figure 2. Topographic distribution of P300 responses. Top: electrical activity from scalp electrodes top view (left) and lateral view (right) of the left surface with the nose pointing toward the left. Bottom: the same as above, but for the magnetic measurement at several millimeters from the scalp. EEG, electroencephalography.

Figure 3. Limbic sources of P300m dipoles. Three-dimensional reconstruction of the major sources explaining the pattern presented in Figure 2. Note the location in the posterior part of the hippocampal formation.
Proof of concept

The “pseudo–state/trait marker” concept

As mentioned previously, the aging brain provides a natural decline in the properties of the cognitive response, such as the P300 waves (Figure 5, top). Comparison of healthy male subjects aged 55 years and older with young subjects aged between 18 and 35 years yields significant attenuation in large parietal and temporal scalp regions. On basis of this change in CNS state, a proof of concept can be proposed by administration of equivalent drugs presumed to be beneficial and by verifying the presence of improvement in healthy elderly subjects. The idea is that of a selective marker because in the elderly particular nootropic drugs are able to significantly restore P300.28

Due to the lack of clinical efficacy of AchE inhibitors,29 more and more alternative mechanisms of action on central receptors or enzymes are being explored. An example of the effect of a noncholinergic drug is given in Figure 5 (bottom).30 Indeed, a clear-cut indication of recovery can be observed, even though the increase in absolute terms is modest.

The concept of a pharmacological model in young volunteers

The established approach in behavioral neuropsychopharmacology is the use of a pharmacological challenge to reversibly provoke symptoms. As an example, we refer to the model that makes use of the comparison of performance in a battery of psychometric tests (eg, digit vigilance speed) and recording of continuous electrical cerebral activity.31 Both types of examination undergo changes with scopolamine and some of these effects have been shown to be reversed by AchE inhibitors. Hence P300 responses constitute a useful tool in neuropsychopharmacology in exactly the same way as continuous electrical cerebral activity, for the reasons shown in Figure 4. Interestingly, manipulation with benzodiazepines in order to provoke—like scopolamine—symptoms of cognitive impairment at the clinical level in, for example, free word recall,32 induces similar collapses in

Figure 4. Cholinergic control of P300 in young healthy volunteers. Top: Mapping result show the dramatic deterioration under scopolamine (0.5 mg, subcutaneous). P values are issued after multiple nonparametric comparisons (Wilcoxon) between two experimental conditions for each electrode with significance levels coded in the rainbow scale at the lower right-hand side. (Increase in red; decrease in blue, for details the reader is referred Statistical Decision Tree or SDT25.) Bottom: Postdosing mapping of donepezil (5 mg) yields enhancement of P300 responses.
P300 in auditory33-36 (eg, lorazepam, Figure 6) and visually37 evoked cognitive responses. In our experience, the effects on neurophysiological parameters are often much more sensitive than the effects seen in performance changes. Schematically, the procedure can be summarized as follows:

- Drug 1 induces a simulation of the acute state of “non-treated” patient (symptom provocation).
- Drug 2 is used to verify its potency to (partially) reverse the deterioration (validation for pharmacotherapy).

An example of the reversal of the challenge-induced deterioration (drug 1) with an AchE inhibitor (drug 2) is shown in Figure 6 (for study design see reference 38). The interesting aspect of such a model is the possibility of preventing the induction of symptoms by compounds without direct cholinergic effects39 and using a neurophysiological readout as surrogate marker at the same time.

**Perspectives and conclusions**

Using the P300 as a marker of relevant brain function, a pharmacological challenge in young healthy volunteers to provoke symptoms like in aging can be counteracted by treatment with cognition-enhancing drugs. A longer pretreatment with nootropics (such as AchE inhibitors), which simulates more closely the clinical setting, may have more persistent effects on challenge-induced deterioration in P300, but this hypothesis remains to be investigated. A lower dose of symptom-provoking agents associated with P300 changes may also increase the

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**Figure 5.** Age-related modifications in P300. Top left: Average curves for elderly (aged >55 years; blue lines) and young volunteers (gray lines) for several scalp positions. Top right: Statistical comparison between elderly and young subjects. Bottom left: Average curves in untreated (placebo) and treated elderly male volunteers. Bottom right: Statistical comparison showing significant improvement.
Figure 6. Superimposed average P300 responses for baseline conditions (light-blue lines) and after a drug challenge (dark-blue lines). The effect of an acetylcholinesterase (AchE) inhibitor (gray lines) restores the deteriorated signal in the direction of baseline levels. Inset: statistical mapping of treatment drug 2 + drug 1 versus drug 1 alone. Scaling as in Figure 4.

Les marqueurs objectifs des effets médicamenteux sur la fonction cérébrale à partir d’enregistrements de l’activité électrique sur le cuir chevelu de volontaires sains

Afin de souligner l’importance des réponses cognitives appelées P300 dans le développement des molécules, nous décrivons les caractéristiques temporo-espaciales de ces potentiels évoqués objectifs en réponse à une stimulation cible. Ces activations cérébrales reflètent la fonction mnésique dans laquelle les structures limbiques jouent un rôle. Il a été démontré qu’un test de provocation pharmacologique utilisant, par exemple, le système cholinergique chez de jeunes volontaires sains, entraînait des modifications des réponses P300 rappelant celles retrouvées dans le cerveau vieillissant. Nous avons utilisé ce type d’observations pour construire un modèle qui permet de vérifier si cette détérioration peut être contrebalancée par des molécules « procognitives ». Si l’on accepte l’extrapolation des effets pharmacologiques à la symptomatologie, l’analyse des potentiels au niveau du cuir chevelu constitue un outil approprié pour l’étude des interactions médicamenteuses dans des modèles utilisés en phase précoce de démonstration de concept.

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Marcadores objetivos del efecto de fármacos en la función cerebral a partir del registro de la actividad eléctrica del cuero cabelludo en voluntarios sanos

Para enfatizar la importancia de las respuestas de la onda P300 en el desarrollo de fármacos, nosotros describimos las características témporo-espaciales de este potencial evocado objetivo que describe una respuesta a un estímulo. Estas activaciones cerebrales reflejan la función mnémica en la cual juegan un papel las estructuras límbicas. Está demostrado que un desafío farmacológico, que se relacione por ejemplo con el sistema colinérgico en voluntarios sanos jóvenes, induce modificaciones en la onda P300 que hacen pensar en un cerebro envejecido. Nosotros utilizamos este tipo de observación para construir un modelo en el cual se pueda verificar si el deterioro se puede neutralizar mediante el tratamiento con fármacos “aumentadores de la cognición.” De aceptar la extrapolación de los efectos farmacológicos a la sintomatología, el análisis de la actividad eléctrica del cuero cabelludo ofrece una herramienta apropiada para el estudio de las interacciones de fármacos en los modelos iniciales de “proof of concept.”

The first steps toward a validation of a surrogate marker can now be considered as accomplished.

If one accepts the extrapolation of the effects pharmacologicals to the symptomatology, the analyze of the potentials at the level of the scalp constitute an appropriate tool for the study of the interactions medicamentous in the models used in the phase precoce de demonstration of concept.
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