Repeated Measurements of the Auditory Oddball Paradigm Is Related to Recovery From the Vegetative State

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Summary: The auditory oddball response has been found to be of predictive value for neurologic outcome at the early stages of coma. In the present study, the auditory oddball response was examined longitudinally during the recovery from the vegetative state to consciousness. This response was repeatedly examined every 2 weeks for an average period of 3.5 months in severely brain-injured patients. Results showed that amplitude of the auditory oddball response was unrelated to the behavioral changes during the patients' recovery from the vegetative state to consciousness. However, the presence and size of a negative potential at about 350 milliseconds predicted behavioral outcome, both for the short and long term (2 to 3 years after injury). Practical and theoretical implications of these findings are discussed.

Key Words: Brain injury, Vegetative state, Unresponsive wakefulness syndrome, Minimally conscious state, Consciousness, Auditory oddball, P300, N3501.

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At present, more and more studies are focusing on detecting consciousness in unresponsive, awake patients who suffered severe brain injury. It is also increasingly evident that clinical assessment of unresponsive patients using behavioral observation methods alone can be prey to misdiagnoses (Andrews, 1996; Childs et al., 1993; Schnakers et al., 2009), precisely because these methods quantify the (absence of) behavioral reactions to the environmental input. Fortunately, the need for the use of brain imaging or neurophysiological measures is therefore recognized and is becoming more common in these patients (e.g., Coleman et al., 2009; Cruse et al., 2011).

Additional to the question whether consciousness is absent or present in brain damaged vegetative patients, it is important to study recovery processes of the brain and to investigate treatment possibilities to enhance these recovery processes. We investigated the question of how recovery to consciousness occurs and whether recovery can be predicted by brain responses: what happens in the brain in the recovery period from coma to consciousness and are we able to predict outcome? Our approach to tackle the difficult problem of the assessment of consciousness is to assess the subtle changes during the recovery process by using both observation methods and neurophysiological measures.

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Recovery Process From Coma to Consciousness

Many individuals who sustain severe acquired brain injury experience prolonged or permanent disorders of consciousness. Acute severe brain injury inevitably results in coma, a state of loss of consciousness with the eyes closed, with no sleep–wake cycle (Multi-Society Task Force on Persistent Vegetative State, 1994a). Patients respond minimally or not at all to external stimulation, and initiate no voluntary activities. If not resulting in death within a period of 4 to 6 weeks, this coma will develop into a vegetative state (VS; Jennett and Plum, 1972) or Unresponsive Wakefulness Syndrome. (In 2010, the European task force on Disorders of Consciousness presented a new name to describe "vegetative state": Unresponsive Wakefulness Syndrome [UWS; Laureys et al., 2010]. From here we shall both use the old and new term.)

In the VS/UWS the patient seems awake but not aware, uncommunicative and unresponsive to the environment. The vegetative or autonomic functions, such as breathing, maintaining a normal blood pressure, digesting and eliminating foods are sufficiently preserved to permit survival with medical and nursing care. A vegetative/UWS patient may present verbal sounds (e.g., grunting, moaning, or screaming), motor agitation (e.g., grinding their teeth, grimacing, moving arms and legs), and emotional expression (e.g., shedding tears). However, this behavior is not purposeful or voluntary, and it is not reproducible by commands. There is no evidence of language comprehension or expression (Multi-Society Task Force on Persistent Vegetative State, 1994*a*). In some patients, VS/UWS is the final outcome. According to the latest numbers of the Multi-Society Task Force on Persistent Vegetative State (1994*b*), the prognosis is influenced by age, the underlying cause, and its current duration. A little over half of those in a VS 1 month after trauma will regain awareness. With other causes, after a month in a VS, fewer than 20% will recover. The chances of regaining awareness fall as time passes. Beyond 1 year after trauma, and beyond 6 months in nontraumatic cases, the chances of regaining consciousness are extremely low. In the very few of well-documented cases, recovery has usually been to a state of exceptionally severe disability.

If recovery continues, patients regain minimal responsiveness to external stimuli; the minimally conscious state (MCS; Giacino et al., 2002). In MCS, patients have sleep–wake cycles, they are awake for a major part of the day, and they show minimal but definite behavioral evidence of awareness of the self or of the environment (Giacino et al., 2002).

Minimally conscious state was recently subcategorized based on the complexity of patients' behaviors: MCS+ describes high-level behavioral responses (i.e., command following, intelligible verbalizations, or nonfunctional communication) and MCS− describes low-level behavioral responses (i.e., visual pursuit, localization of noxious stimulation, or contingent behavior, such as appropriate smiling or crying to emotional stimuli) (Bruno et al.,

2011). Prognosis of MCS has not been systematically investigated so far. A first study focusing on differences between VS and MCS concerning recovery and prognosis has been performed by Giacino and Kalmar (1997). Minimally conscious state patients with a traumatic brain injury had the best functional outcome (Giacino and Kalmar, 1997). More recent studies were performed, yet on very small samples (Lammi et al., 2005; Luaute et al., 2010). Luaute et al. (2010) showed that one-third of MCS patients still improved after 1 year.

Object manipulation and functional, accurate communication indicate the emergence from the MCS (Giacino et al., 2002, 2004), that is, the recovery of consciousness. Patients have to be able to consistently express goal-directed behavior (Giacino et al., 2004). However, many physical disorders, such as paralysis and motor disorders, may be the result of severe acquired brain damage. Additionally, disorders in intellect, cognition, emotion, and behavior may lead to limitations in the daily life of the patients. Only a small percentage of patients who suffered severe acquired brain injury are eventually able to live a completely independent life, in which an education or a job can be resumed (Frost et al., 2012).

Recovery and Treatment Possibilities

The traditional view on the brain plasticity is that brain cells do not regenerate, and that those that are destroyed are not replaced. However, it has been demonstrated that our brain is able to change and adapt constantly as a result of environmental demands. The functions of the parts of the brain that are lost after brain injury can be taken over by other parts of the brain (functional recovery; Luria, 1973). In addition, new brain cells originate on a daily basis (neurogenesis; Gross, 2000), and new connections can be made between brain cells (sprouting; Merzenich, 2000; Merzenich et al., 1987). Healthy neurons are making new connections constantly, by means of collateral innervations or sprouting, and these processes are being accelerated after damage to the nervous system (Bach-y-Rita and Bach-y-Rita, 1990). The brain has an enormous capacity to respond and adapt to the functional need from the internal and external world (Buonomano and Merzenich, 1998).

The recovery functions of the brain can be influenced by environmental information (external stimulation and input) (Bach-y-Rita and Bach-y-Rita, 1990; Robertson and Murre, 1999). Therefore, the recovery processes after brain damage may be enhanced by "rehabilitation-induced plastic reorganisation" (Robertson and Murre, 1999). The role of neurogenesis after brain injury and its relation with rehabilitation has been reviewed by Garcia et al. (2011). This review presents recent evidence on how therapy-induced plasticity after brain jury, contributes to neurobehavioral consequences. For instance, it has been found that environmental enrichment promotes neurogenesis, angiogenesis, and survival of hippocampal neurons in rodents (van Praag et al., 2000).

In addition, Hebbian cell assemblies may form after frequently repeating particular stimulations (Cruikshank and Weinberger, 1996; Robertson and Murre, 1999). Hebbian learning describes a mechanism for plasticity wherein an increase in the efficacy of cell connections arises from the frequent communication between cells (Hebb, 1949). When practicing a particular skill, the cells involved will form stronger connections, which cause the skill to become easier to perform.

Continuous improvement in health care might have changed expectancies of recovery in VS/UWS (Estraneo et al., 2010), making the number of the MSTF on PVS outdated. The Rehabilitation Centre Leijpark (Tilburg, The Netherlands) uses an early Intensive Neurorehabilitation Program to children and young adults in a vegetative or MCS as a result of severe acquired brain damage. The aim of this treatment is to maximize a patient's ability to process and respond to stimuli and information of increasing variety and complexity. The rationale of the program is based on theories as described above: providing structured sensory input and preventing deprivation to trigger the recovery processes as described above. Eilander et al. (2005) showed that patients who participated in this program had a more favorable outcome than predicted by The Multi-Society Task Force on Permanent Vegetative State (1994*b*). Patients involved in our study were all participating in this early Intensive Neurorehabilitation Program.

A more recent study also indicated the possibility of achieving behavioral improvements in VS/UWS and MCS patients by the use of long-term treatment (Lotze et al., 2011).

Electrophysiological Correlates of Recovery to Consciousness

As we mentioned earlier, research on recovery patterns using neurophysiological indices in these patients is relatively scarce. In 2006, we found a linear relation between parameters of the autonomic nervous system and recovery from VS/UWS to consciousness: parasympathetic activity decreased and sympathetic activity increased with recovery, leading up to improved sympathovagal balance (Wijnen et al., 2006). Probably, these findings were because of the recovery of cortical structures regulating autonomic nervous system balance (Critchley et al., 2003; Matthews et al., 2004). Also we found an almost perfect correlation between mismatch negativity (MMN) amplitudes and the recovery from VS/UWS to consciousness (Wijnen et al., 2007). The MMN (Näätänen et al., 1978) is generated by the brain's automatic response to physical stimulus deviation from the preceding stimulus in repetitive auditory input. In our study, a sudden enhance in MMN amplitude preceded overt communication with the environment, which might be indicative for consolidation of the underlying neural networks. In any case, our findings support plasticity of the nervous system after severe acquired brain damage.

In the field of electrophysiological measurements, mostly event-related potentials (ERPs) like MMN and P300 are regarded as markers of key stages in the information processing hierarchy leading up to conscious perception. Therefore, it was interesting for us to compare our earlier findings on MMN with a more cognitive ERP, for example, P300, and how it would relate to the recovery of consciousness.

Therefore, the present study reports on the auditory ERP in a standard oddball task, which elicits a P300 in awake and healthy people. The P300 is a large, broad, positive potential with typical peak latency between 300 and 400 milliseconds after stimuli in any modality (Sutton et al., 1965). The most common task for eliciting the P300 is the oddball task, in which low-frequency target stimuli (oddballs) are embedded in a series of nontarget stimuli (standards). The subjects are either required to actively respond to each target, or to count the target stimuli (active condition), or to passively attend to the train of stimuli (this is often used in animal studies or in non-responsive human patients). The P300 usually has a central–parietal scalp distribution, although this depends much on the exact nature of the task. When novel or highly deviant stimuli are used as oddballs, the scalp distribution is more frontal than central–parietal, and the potential peaks a little earlier. It is then labeled P3a, or novelty-P3 (Courchesne et al., 1975), as opposed to the more classic P300, or P3b, which is the focus of the current study.

Recently, the use of ERPs in coma, VS/UWS, and MCS has been described extensively (e.g., Chennu and Bekinschtein, 2012; Daltrozzo et al., 2007; Vanhaudenhuyse et al., 2008). Main conclusions are summarized below. The presence of a P300 has been found to be of some predictive value for neurologic outcome at the early stages of coma. For instance, 80% to 100% of comatose patients, traumatic and nontraumatic, who exhibited a P300, regained consciousness (e.g., Gott et al., 1991; Guérit et al., 1999; Kane et al., 2000). No conclusions on the prognosis can be drawn for the absence of a P300, however, because patients without a P300 have been found to have good or bad outcomes alike. In other words, using the presence of the P300 in the early stages of coma as a predictive tool for final outcome is a test with high sensitivity but low specificity.

Studies also revealed that ERPs can be evoked sometimes in VS/UWS and MCS (Guérit, 2005; Kotchoubey et al., 2005), especially when salient stimuli were used, such as the patients' name (Laureys et al., 2004; Perrin et al., 2006), speech, and musical notes (Kotchoubey et al., 2001). No large differences were found between VS/UWS and MCS. Later after the injury, the P300 has been found to occur in patients in VS/UWS (Guérit, 2005; Guérit et al., 1999; Kotchoubey et al., 2001, 2005), and in MCS (Kotchoubey et al., 2005; Laureys et al., 2004). Fischer et al. (2010) found a relation between the occurrence of ERPs and the etiology of VS/UWS and MCS: mainly P300 responses were found when the patients' state was not because of anoxia.

We thus attempted to correlate characteristics of the auditory oddball task with level of consciousness during the recovery from VS/UWS and to assess its predictive value of outcome. Predictive value of the auditory oddball was measured for both outcome of recovery to consciousness and outcome 2 to 3 years after injury of recovery of function. So far, only 1 case study describes the improvement of ERPs from the sixth month after injury, yet emerged from VS/UWS after 20 months (Faran et al., 2006).

Whether these ERPs are also markers of consciousness has been the topic of very recent research in VS/UWS and MCS patients (Bekinschtein et al., 2009; Boly et al., 2011; Faugeras et al., 2012; Schnakers et al., 2008). Bekinschtein et al. (2009) and Faugeras et al. (2012) used a derived function of the oddball paradigm, calling it the ERP Local-Global Paradigm. Only conscious individuals presented a global effect, which is more related to the P3b. In addition, by using extensive mathematical models on EEG data derived from the MMN paradigm, Boly et al. (2011) observed that the only significant difference between patients in VS/UWS and controls was an impairment of backward connectivity from frontal to temporal cortices, indicating that top-down communication from frontal to parietal networks is necessary to be conscious.

Because nonresponsive patients are thought not to be able to follow the instructions to count or respond to the oddball stimuli, usually passive oddball tasks are used in which the patients are not given any instruction to pay attention. In healthy participants, the P300 is usually present under a passive condition to the same extent as in an active condition (Polich, 1989; Rappaport et al., 1991). In a study by Schnakers et al. (2008), only P300 differences between active and passive conditions were observed in MCS, and no differences were found in VS/UWS patients. We therefore included both active and passive conditions in our measurements.

METHODS

Participants

Ten patients with severe brain injury, who participated in an "Early Intensive Neurorehabilitation Program" (Eilander et al., 2005) between November 2002 and January 2004, were included in the study (7 male participants). Age at the time of injury ranged from 8 to 25 years (mean = 17.3 years; standard deviation [SD] = 4.4). Time since injury at admission ranged from 6.2 to 19.4 weeks (mean = 11.6 weeks; SD = 3.6). All but 2 patients suffered from traumatic brain injury caused by traffic accidents. Patients participated in the program between 1.5 and 5.2 months (mean = 3.5 months; SD = 1.03). See Table 1 for a detailed description of the patients participating in this study.

In the healthy control group, 2 participants were excluded because their data contained too many artifacts. Thus, the remaining control group consisted of 15 persons (7 male participants); the groups were matched for mean age ($t(21) = 0.16$, $P = 0.876$). All patients and the healthy control group participated in this study after informed consent was given by one of the parents, a legal representation or partner (all the patients and the healthy control group aged <16 years), or by themselves (healthy control group aged ≥ 16). The study has been approved by a Medical-Ethical Test Commitee for Research in Patients and Test Subjects.

Observation Scales

To assess the level of consciousness (LoC) a categorization was used based on the definitions described by the "International Working Party Report on the Vegetative State" (Andrews, 1996) and the Aspen Neurobehavioural Conference (Giacino et al., 1997). The categorization system describes a comatose state, 3 vegetative sub-states, 3 nonvegetative sub-states, and a conscious state. See Table 2 for the classification scheme in detail.

Recently, the Coma Recovery Scale-Revised (Giacino et al., 2004) has been suggested to be the best assessment scale for assessing disorders of consciousness (Seel et al., 2010). However, at the time of our measurements, this knowledge was not yet available. Based on the literature available, we searched for the best measure to diagnose consciousness, based on the definitions described by the "International Working Party Report on the Vegetative State" (Andrews, 1996) and the Aspen Neurobehavioural Conference (Giacino et al., 1997).

This classification scale showed high reliability and validity (Eilander et al., 2009). The interrater reliability (Spearman rho) varies between 0.85 and 0.94. The interrater agreement (Cohen weighted Kappa) varies between 0.90 and 0.95. The intrarater reliability is 0.96 and the intrarater agreement is 0.94. Correlation of the scores of the rated scores with the Western Neuro Sensory Stimulation Profile (Ansell et al., 1989) varies between 0.85 and 0.90, and with the Disability Rating Scale (DRS; Rappaport et al., 1982) between 0.88 and 0.94 (Eilander et al., 2009).

Overall LoC at the end of the program ($LoC_{discharge}$) was determined by the rehabilitation physician, after a discussion with the multidisciplinary treatment team about each patient. Note that the level of consciousness at discharge was measured independently of the ERP measurements, often more than a week thereafter. Thus, the $LoC_{discharge}$ did not necessarily correspond to a particular ERP measurement for a given patient.

To determine the long-term functional outcome, the DRS (Rappaport et al., 1982) was administered. The DRS consists of 8 items, which can be summed up to values from 0 to 29. A high score on an item indicates a low level of functioning on that aspect. To make the 2 scales more comparable, the DRS was reduced to 8 categories according to Rappaport et al. (1982): 1 = dead (score 30), 2 = VS (score 22 to 29), 3 = extremely severe disabled (score 17 to 21), 4 = severely disabled (score 12 to 16), 5 = moderately severe disabled (score 7 to 11), 6 = moderately disabled (score 4 to 6),

TABLE 1. Summary of Patients' Details

Patient	Gender	Age	Cause	GCS	T1	T2	T3	T4	CT Scan Features*	CT Category	LoC 1	LoC 2	DRS Cat	GOSE	T5
1	M	17.6	Traffic accident	2t	72	80	28	139	Epidural hematoma (right), punctual hemorrhages, diffuse white matter lesions	3	3	4	3	3	3.0
2	M	15.4	Traffic accident	4	33	136	68	112	Skull fractures, arachnoid hemorrhages, contusion and punctual hemorrhages (right frontal, temporal, parietal), diffuse swelling	3	2	5	4	3	2.9
3	M	25.2	Traffic accident	4	64	64	34	77	Skull fracture, edema and punctual hemorrhages (cortical), diffuse swelling, and diffuse white matter lesions	3	2	8	6	3	2.7
4	M	8.4	Cerebral hemorrhages	2t	33	81	52	119	Intraventricular and intracerebral hemorrhages, left cortical	—	3	7	7	3	2.6
5	F	18.8	Traffic accident	2t	29	49	7	115	Edema, ischemia, high intracranial pressure, diffuse swelling	2	4	8	4	3	2.4
6	M	17.5	Traffic accident	4	13	44	21	92	Edema, intraventricular and intracerebral hemorrhages, focal lesions (subcortical, brainstem), diffuse white matter lesions	2	3	8	7	6	2.5
7	M	21.8	Traffic accident	5	26	71	11	105	Punctual hemorrhages, intraventricular hemorrhage (left), diffuse swelling, diffuse axonal injury	3	2	4	3	3	2.5
8	F	15.7	Traffic accident	4	30	60	8	99	Subarachnoid hemorrhage (right), high intracranial pressure, edema (right subcortical and brainstem)	1	2	8	5	3	2.4
9	M	17.2	Traffic accident	3	12	80	62	157	Intraventricular hemorrhages (bilateral), multiple punctual hemorrhages, large hemorrhage in basal ganglia, and right frontal, edema (mainly left periventricular white matter)	3	2	5	1	1	2.2
10	F	15.2	Pneumonia + sepsis	3	57	102	11	45	Hypodensity in basal ganglia and cortical temporoparietal, anoxia, cortical and cerebellar atrophy, diffuse white matter lesion	—	2	3	—	—	—

Age, age at injury in years; GCS, Glasgow Coma Scale at admission hospital; T1, time at ICU in days; T2, time before admission EINP in days; T3, time between registration and admission EINP in days; T4, program duration RCL in days; LoC 1, level of consciousness first measurement; LoC 2, level of consciousness end of EINP; DRS, disability rating scale; GOSE, Glasgow Outcome Scale Extended; T5, time of outcome after injury in years.

*Diagnoses based on the medical report in the acute phase.

TABLE 2. Levels of Consciousness

Coma
Eyes are closed all the time. No sleep–wake cycles present
1. All major body functions, such as breathing, temperature regulation, or blood pressure, can be disturbed. Generally, no reactions are noticed after stimulation. Sometimes reflexes (stretching or flexing) are observed as a reaction to strong pain stimuli. No other reactions are present.
Vegetative presentations
The patient shows sleep–wake cycles, but not a proper day–night rhythm. Most of the body functions are normal. No further ventilation is required for respiration.
2. Very little response (hyporesponsive): Generally no response after stimulation. Sometimes delayed presentations of reflexes are observed.
3. Reflexive state: The stimuli often result in massive stretching or startle reactions, without proper habituation. Sometimes these reactions evolve into massive flexing responses. Roving eye movements can be observed, without tracking. Sometimes grimacing occurs after stimulation.
4. High active level and/or reactions in stimulated body parts: generally spontaneous undirected movements. Retraction of a limb following stimulation. Orientation toward a stimulus, without fixating. Following moving persons or objects, without fixating.
Minimally conscious state(s)
Patient remains awake most of the day
5. Transitional state: Following and fixating of persons and objects. Generally more directed reactions to stimuli. Behavior is automatic, that is, opening of the mouth when food is presented, or reaching towards persons or objects. Sometimes emotional reactions are seen, such as crying or smiling towards family or to specific (known) stimuli
6. Inconsistent minimally conscious state: Occasionally obeying simple commands. Total dependency. The patient has obvious cognitive disturbances and is unable to think comprehensively
7. Consistent minimally conscious state: The patient obeys simple commands. Many cognitive disturbances remain. Total dependency.
Consciousness
8. The patient is alert and reacts spontaneously to his/her surroundings. Functional understandable mutual communication is possible, sometimes with technical support. Cognitive and behavioral disturbances can still be present.

7 = mildly to partially disabled (score 1 to 3), and 8 = no disability (score 0).

Data Acquisition and Analysis

The stimuli were 375 pure tones of 1000 Hz (80%, standard) and 2000 Hz (20%, deviant), with an intensity of 70 dB SPL and duration of 75 milliseconds (rise-and-fall time of 10 milliseconds). The tones were delivered binaurally through insert earphones. A random interstimulus interval of 1000 to 2000 milliseconds was used (steps of 1 milliseconds; rectangular distribution). Brain activity was recorded using actively shielded pin electrodes, by means of the ActiveTwo System (BioSemi, Amsterdam, The Netherlands) at a sampling rate of 2 kHz. The total equipment was approved on safety by a Metron QA-90 Safety tester in the Tweesteden Hospital (Tilburg, The Netherlands). The electrodes were placed by using a head cap and electrode gel (Parker Signa) according to the 10/20 system, at F3, Fz, F4, C3, Cz, C4, Pz, and Oz. Linked mastoids served as a reference, which was calculated off-line. Horizontal Electrooculogram (EOG) was recorded from two electrodes placed at the outer canthi of both eyes. Vertical Electrooculogram (EOG) was recorded from infraorbital and supraorbital regions of the two eyes, perpendicular to the pupil. Data analyses were performed using BrainVision Analyzer. The EEG signals were band-pass filtered off-line (0.15 to 30 Hz, 48 dB/octave). The Electrooculogram (EOG) artifacts were corrected by means of a linear regression procedure (Gratton et al., 1983). The raw data were segmented into 375 epochs, including a 100-millisecond prestimulus baseline. Epochs with an amplitude change exceeding ± 200 μV at any channel were automatically rejected. Event-related potentials were averaged separately for the standard and deviant tones.

Peak amplitudes in the individual subject's averaged difference waveforms were scored at the electrode positions Fz and Pz, as the maximum negative or positive value, respectively, in a window of 200 to 1000 millisecond poststimulus.

Experimental Procedure

Nine days after a patient was admitted to the treatment program, the first measurements took place. Patients were examined while they were lying in a bed in a quiet room with a constant temperature (23 $\pm$ 1°C). Every 2 weeks, the 2 oddball tasks were performed at the same time of the day (between 10:30 AM and 11:30 AM). Every 2 weeks also brainstem-evoked potentials (BAEPs) were measured. Brainstem-evoked potentials were present at all measurements in all patients. The oddball tasks, which lasted about 10 minutes each, were always performed in the same order: first the passive and then the active task. The passive task was presented without any warning. After the passive task, the Dutch equivalent of the following instruction was given to introduce the active task: "Pay attention! First we are going to explain what you have to do. You are going to hear a lot of beeps, high beeps and low beeps. Pay attention to the high beeps!"

Every 2 weeks, the rehabilitation physician determined the LoC based on the categories described in Table 2. These assessments were performed until the patient was discharged from the program. The program ended when (1) a patient was qualified for regular rehabilitation because of recovery of consciousness and cognitive abilities or (2) a patient did not show any recovery in a period of at least 6 weeks during the program. These different recovery courses lead to a variation in time span of the patients' participation in the experiment and in the number of measurements.

Long-term outcome was determined by the DRS score at least 2 years after the injury (mean = 2.6, SD = 0.28; see Table 1 for the exact time intervals). A rehabilitation physician performed the interviews by telephone with a close relative of the patients (partner or parent). The healthy control group was measured once, in the same position and location, at different times of the day.

Statistical Analyses

An analysis of variance was used for the data of the healthy control group, in which independent variables were "electrode position" (Pz, Cz, Fz), "tone" (standard, deviant), and "task" (passive, active).

The longitudinal changes of the Auditory Oddball components were analyzed as a function of LoC using a linear Mixed Model Procedure. Level of consciousness was included as fixed factor and the individual subjects were included as random factors. Mixed-effects models use all available data, can properly account or correlation between repeated measurements on the same subject, have large flexibility to model time effects, and can handle missing data appropriately (Francis et al., 1991).

Predictive value of recovery was further evaluated by calculating the relationship between the presence (or the absence)

of a component and the outcome of patients. Positive predictive value for favorable outcome estimates the percentage of patients who will recover when a component is evoked: that is, the number of measurements with the component in patients with favorable outcome (true positives)/total number of measurements with the component in all patients (true positives + false positives). Negative predictive value for unfavorable outcome estimates the percentage of patients who will not recover when no component is evoked: that is, the number of measurements without the component in patients with unfavorable outcome (true negatives)/total number of measurements without the component in all patients (true negatives + false negatives [FN]). Also sensitivity (true positives/[true positives + FN]) and specificity (true negatives/[true negatives + false positives]) for favorable outcome were assessed.

RESULTS

Behavioral Indices of Recovery

At admission, the patients' average LoC score was reflexive vegetative (mean = 3.6, SD = 0.52, range = 3 to 5). The average LoC score increased to the inconsistent MCS (mean = 5.9, SD = 1.9, range = 3 to 8) at discharge. Five patients reached a conscious level (LoC 7 or 8), 2 patients were still in the MCS (LoC 5 or 6), and 3 patients were still in the VS/UWS (LoC 2 to 4) at the end of the program. Overall, these data indicate that during the program the patient group improved on the mean level of consciousness. However, the level of consciousness at discharge of the program could not be predicted based on the level of consciousness at the start of the program. A regression analysis resulted in an equation of $LoC_{discharge} = 5.829 + 0.073 \times LoC_{initial}$ ($R = 0.026$, $R^2 = 0.001$, adjusted $R^2 = -1{,}24$, $F(1,8) = 0.005$, $P = 0.944$).

The long-term outcome scores on the DRS could be obtained for 9 patients. Two to 3 years after the injury the mean score on the DRS was "severely disabled" (mean = 4.4, SD = 2.0, range = 1 to 7). See Table 1 for exact patient information.

Electrophysiological Indices of Recovery

A total of 49 recordings were performed in the patient group. Recordings were sometimes plagued by excessive noise, movements, or general resistance on the part of the patients. The resulting number of measurement that could be successfully analyzed was 47 for passive oddball task and 45 for the active task. In most of the patient data, the peaks and latencies in the individual averages for each measurement were difficult to interpret and score. Additionally, large diversity in responses existed within individual patients. Therefore, we choose to analyze the repeated measurements using 2 different approaches, which in our view are of most added value concerning diagnostic and prognostic aims in the given patient group.

The first approach of our analyses is on overall grand averages of the norm group, patients who did recover, and patients who did not recover to consciousness. Based on Figure 1, statistical analyses are focused on N200 and P300 amplitude.

The second approach of our analyses is based on individual responses during each measurement. Basing on Figures 2 and 3 statistical analyses are focused on P300 and N350.

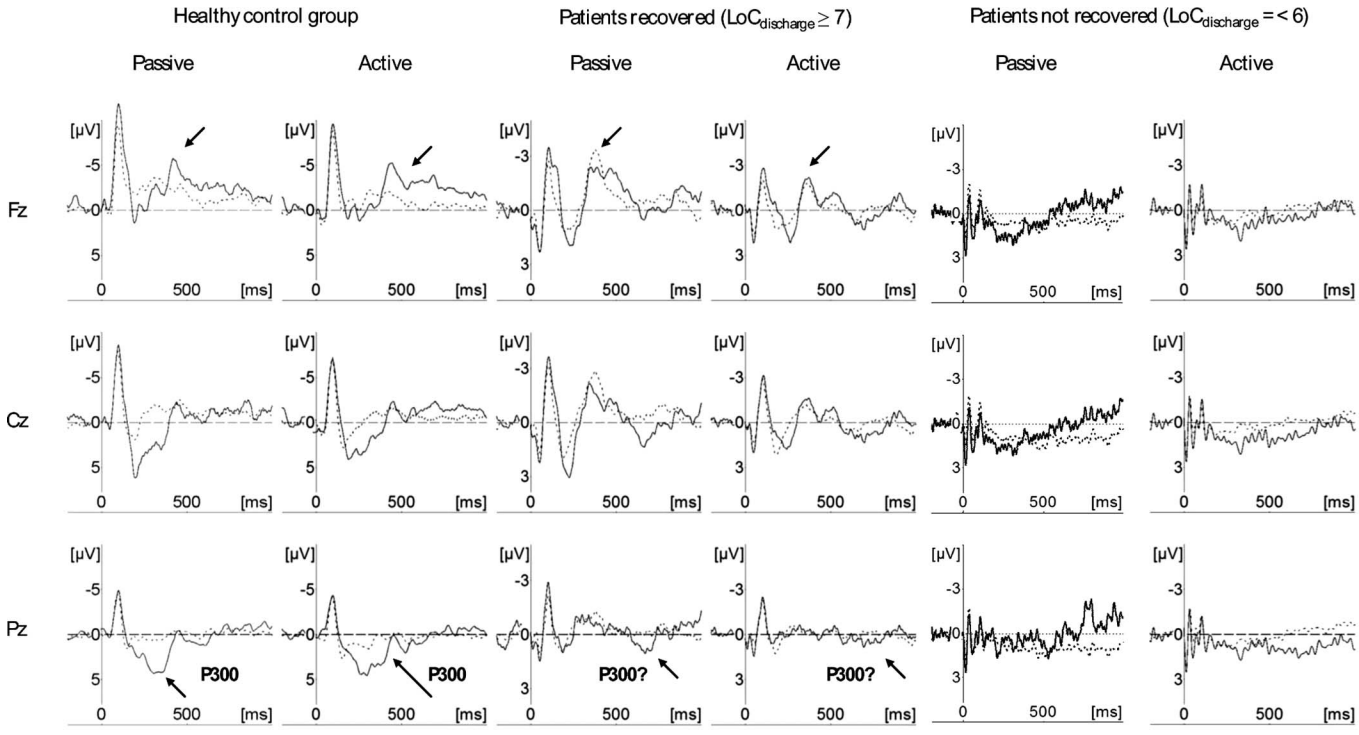


FIG. 1. Grand average event-related potentials in the oddball task, separately for the active and passive tasks, and separately for patients who recovered to consciousness (level of consciousness [LoC] 7 to 8), patients who did not recover (LoC 2 to 6), and normal controls. Each column consists, from top to bottom, of the electrode positions Fz, Cz, and Pz. Note the different y-axis scales for patients and normal controls.

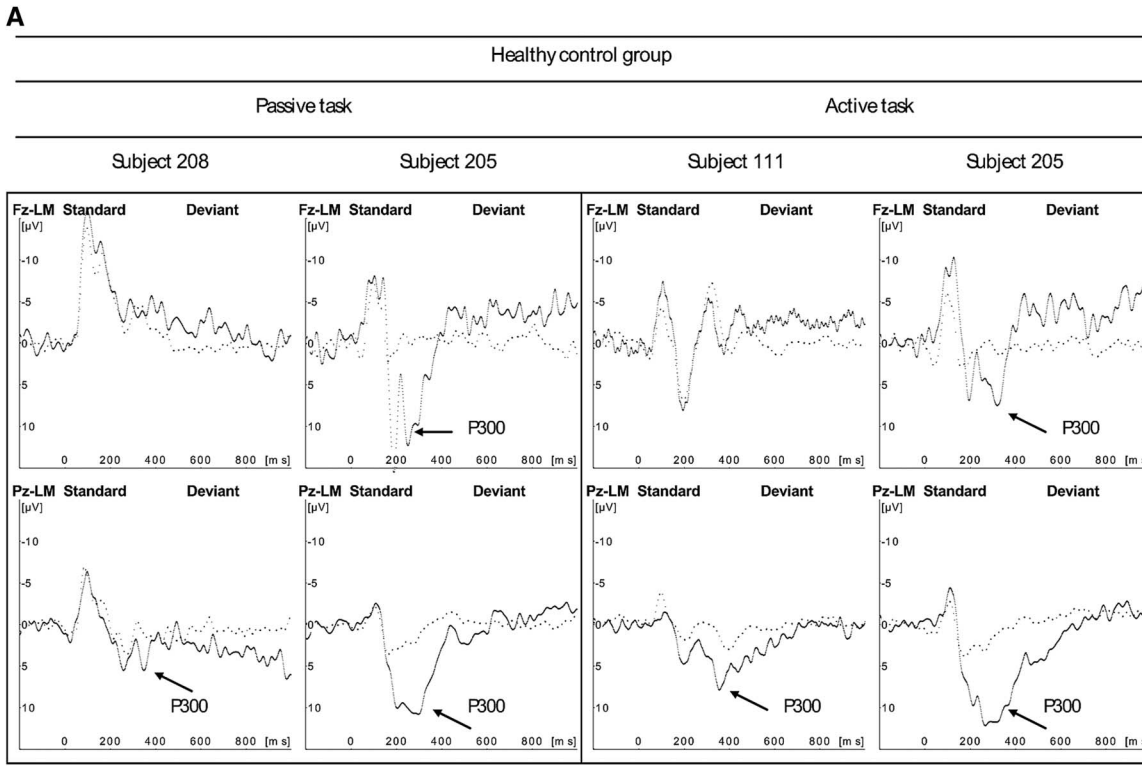
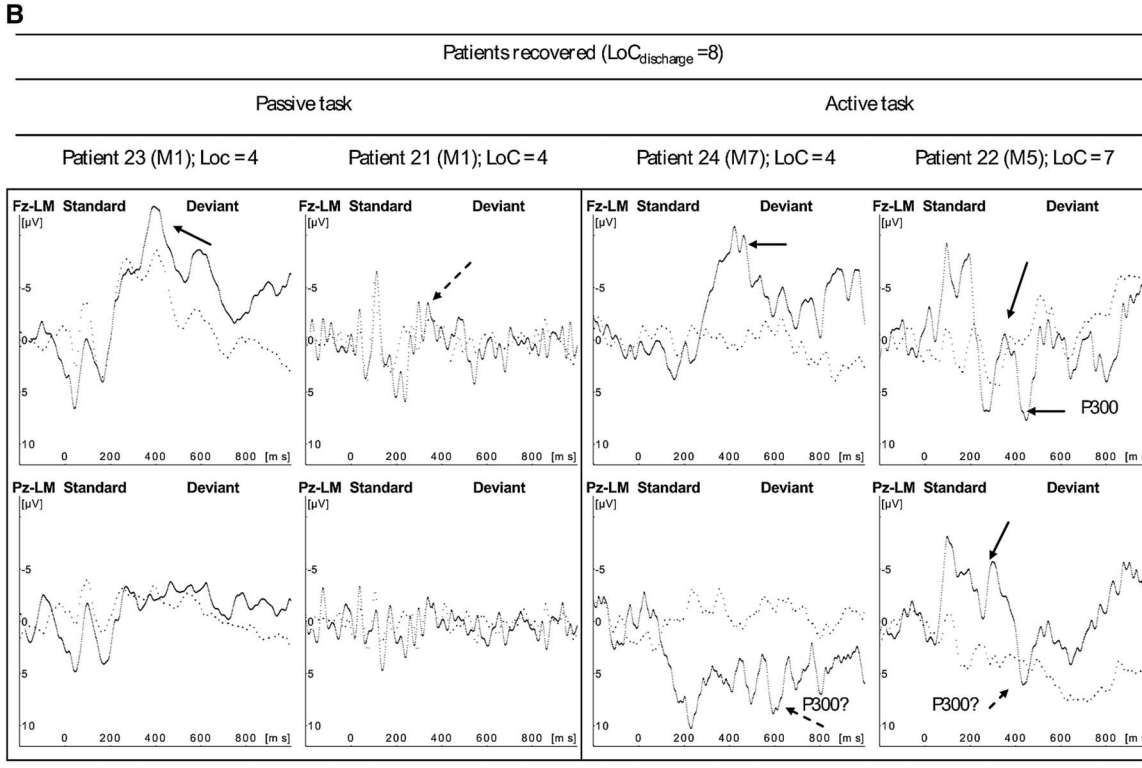


FIG. 2. **A**, Some representative examples of averaged waveforms in the healthy control group. **B**, Some representative examples of averaged waveforms of single measurement (M = measurement number) in patients who recovered to consciousness. **C**, Some representative examples of averaged waveforms of single measurement (M = measurement number) in patients who did not recover to consciousness.



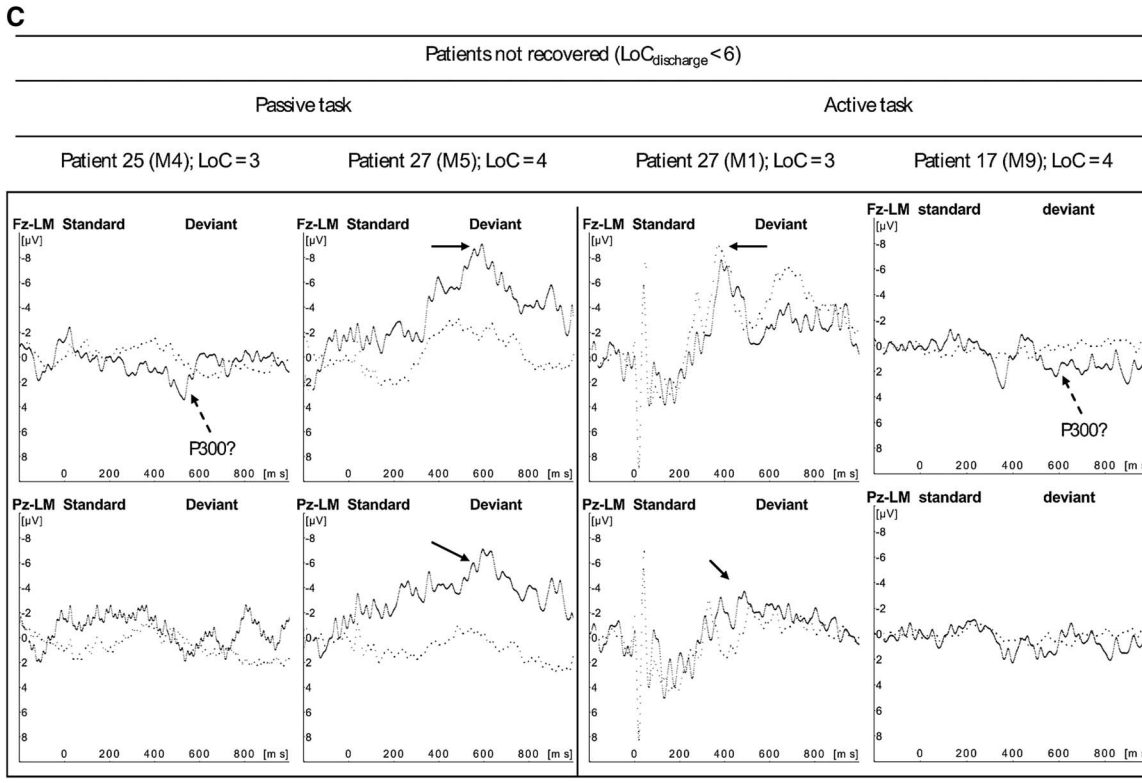


FIG. 2. Continued

Approach 1: Overall Grand Averages of the Norm Group, Patients Who Did Recover and Patients Who Did Not Recover to Consciousness

The patient group was split up into patients who recovered (i.e., reached an LoC score of 7 or 8 at discharge, N = 5) and patients who did not recover (LoC scores 2 to 6; vegetative and MCSs, N = 5) (Fig. 1). Grand averages of these 2 groups are exhibited in Figure 1, along with the averaged waveforms of the healthy control group.

The healthy control group (left panels in Fig. 1) showed a classic P300. Statistical comparison of the peak amplitudes at the Fz, Cz, and Pz electrodes revealed a main effect of electrode position. As can be obtained from Figure 1, amplitude was maximal at the Pz electrode and minimal at the Fz electrode ($F(2,7) = 8.09$, $P = 0.015$). The normal controls also showed the classic oddball effects in which deviant tones elicited greater amplitudes relative to standard tones (main effect of tone: $F(1,8) = 17.63$, $P = 0.003$). The active and passive versions of the oddball task did not result in a statistically distinguishable difference in P300 amplitude (main effect of task: $F(1,8) = 0.27$, $P = 0.617$). Taken together, these data indicate the success of the task manipulation and measurement procedures in normal healthy subjects because the effects are just as expected based on the vast amount of literature about this task and this brain potential.

Quite a different picture emerged in the patients. First of all, the potentials were a lot smaller in the patients than in the controls. In Figure 1 we used different y-axis scales for patients and controls to show the data clearly. However, a close look at the y-axis values reveals that, for instance, the first negative potential (N100) was more than twice the size in the controls than in the patients. Second, the patients who did not recover to consciousness (right panels in Fig. 1) did not show any appreciable potential after the onset of the tones. There seemed to be some sign of a broad positivity, especially at the frontal electrode in the passive task, but there did not seem to be any oddball effect. In addition, the data looked quite noisy in the patients who did not recover compared with the patients who did recover. Yet the groups were of equal size (N = 5) and the total number of measurements for the patients that did not recover was greater (N = 27) than for those that did recover (N = 22).

For the patients who recovered to consciousness, Figure 1 (middle panels) shows a distinct pattern of results. On average, they exhibited a typical P300 to the oddballs, but about 300 milliseconds later than normal—around 650 to 700 milliseconds poststimulus—and clearer in the passive task than in the active task. The maximum of the P300 seemed to be central (Cz), rather than the more typical parietal (Pz) maximum. The P300 was preceded by a large negative potential peaking around 400 milliseconds, the N200, with a maximum at the frontal electrode (Fz). The N200 also seemed more prominent in the passive relative to the active task but did not seem to exhibit an oddball effect.

In sum, patients showed smaller P300 amplitudes than normal controls. Patients who recovered, but not patients who did not recover, showed a small P300, most prominently in the passive task. The most noticeable finding, however, was that patients who recovered showed a large frontal N200 that distinguished them from patients who did not recover.

Based on these grand averages, we defined 2 components N200 and P300 (Squires et al., 1976), of which peak amplitudes in

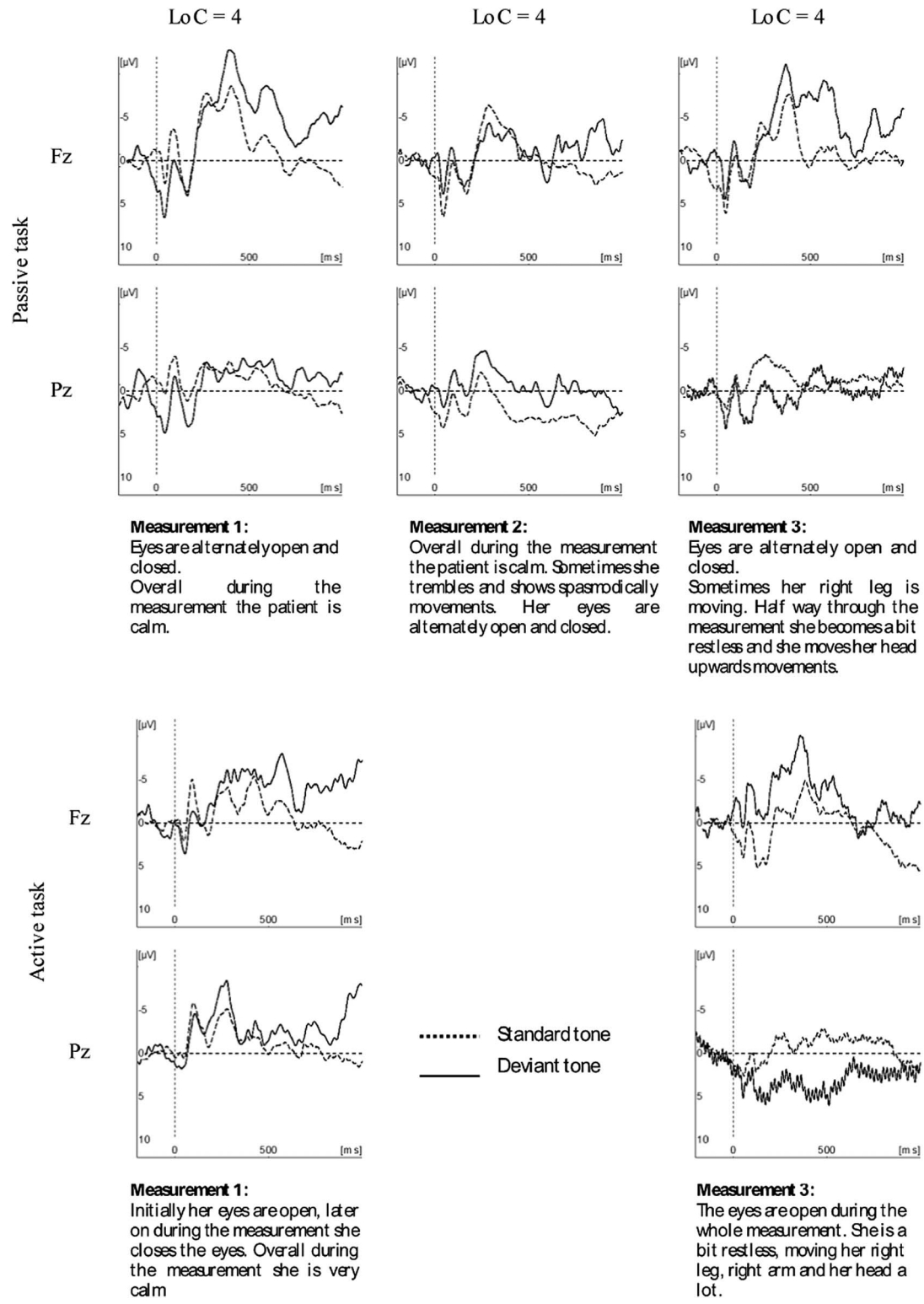


FIG. 3. Tracking the recovery process of patient 9.

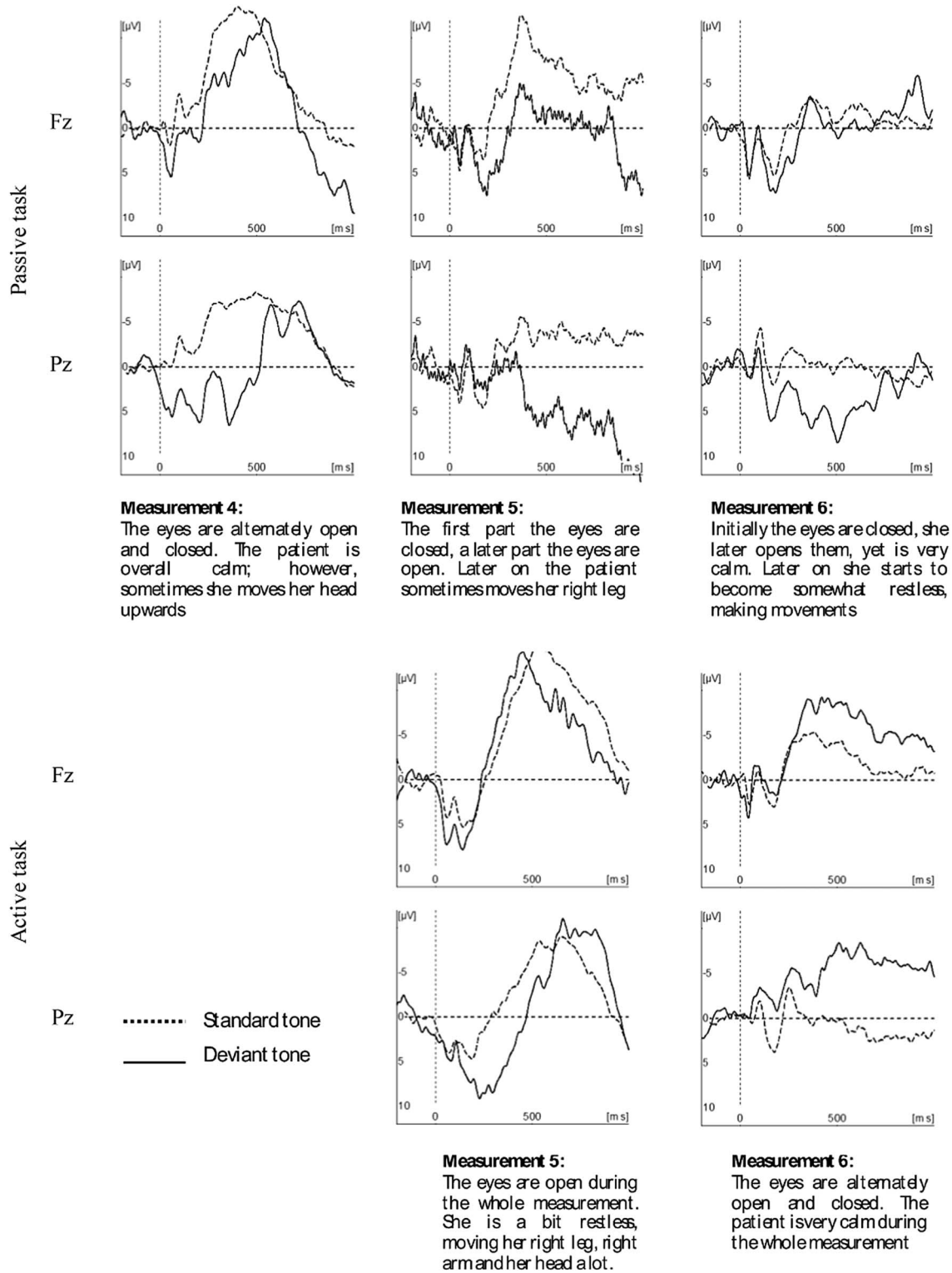


FIG. 3. Continued.

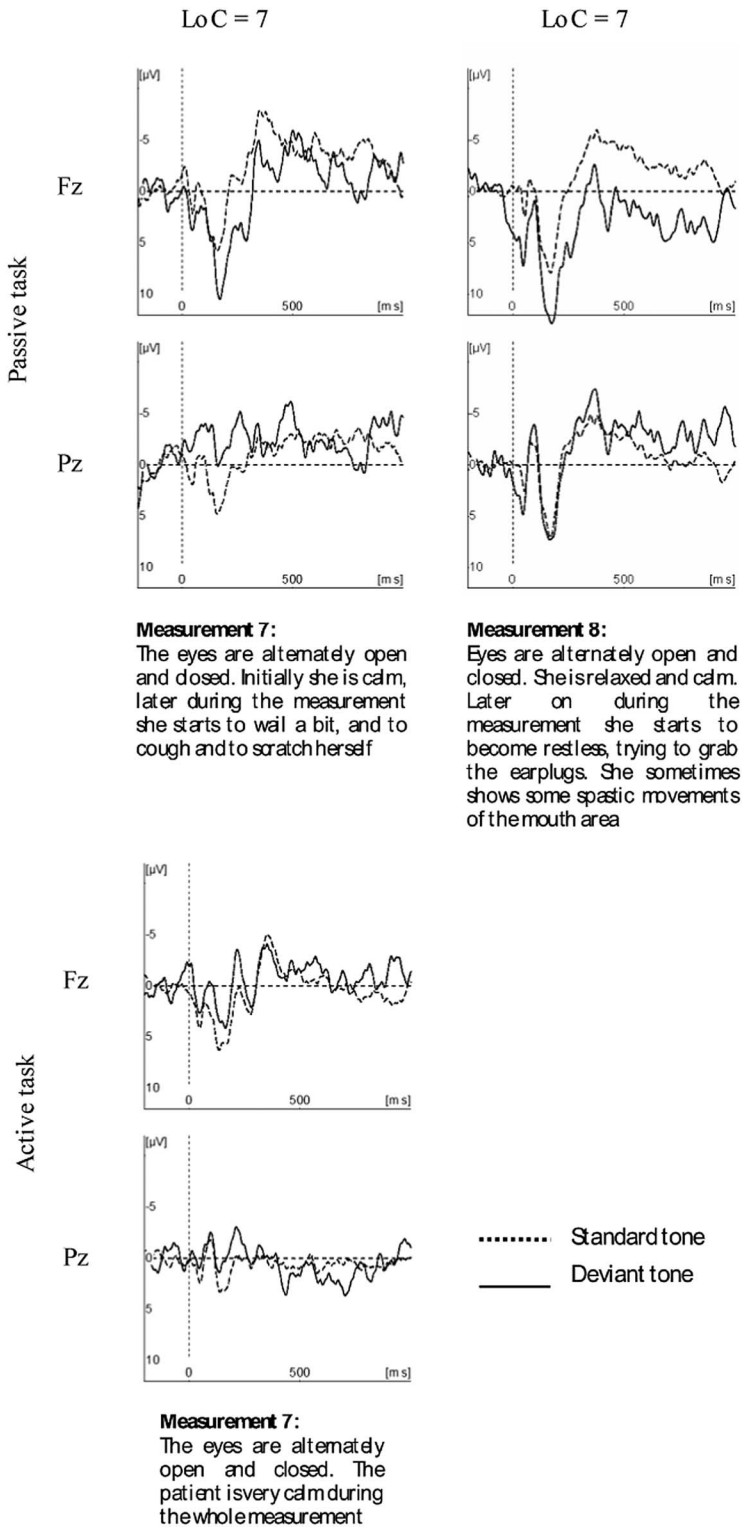


FIG. 3. Continued.

the individual subject's averaged difference waveforms were scored at the electrode positions Fz and Pz, as the maximum negative or positive value, respectively, in a window of 200 to 1000 milliseconds poststimulus. These data lead to the results described below.

Changes in Components of Auditory Oddball Response During Recovery to Consciousness

Table 3 describes the means and standard deviations of N200 and P300 amplitudes for each LoC of the patients and the Norm group. Neither P300-amplitude nor N200-amplitude significantly

changed with increasing LoC ($F(6,15) = 0.41$, $P = 0.86$; $F(6,18)$, $P = 0.69$, respectively).

Significantly larger amplitudes existed during the active task when compared with the passive task for N200 ($F(1,138) = 4.01$, $P = 0.05$) and not for P300 ($F(1,137) = 0.27$, $P = 0.60$).

Finally, outcome did matter: patients who recovered to consciousness revealed significantly larger N200 amplitudes when compared with patients who did not recover to consciousness ($F(1,12) = 17.98$, $P = < 0.001$). P300-amplitude did only marginally differed between the patients who did and the patients who did not recover to consciousness ($F(1,9) = 4.31$, $P = 0.07$).

Approach 2: Individual Responses

Extensive examination of the individual responses lead to another approach of looking at the data: that is to count the occurrence of the negativity and positivity in each measurement, relative to the total number of measurements.

Figure 2 shows some examples of averages based on single measurements for some of the patients and controls. Figure 3 presents the recovery process of one individual patient. As can be seen in these graphs, negativity is often found instead of or after a P300 or a positive component, and therefore something different from a N200. Therefore, it was defined as an N350. In the Discussion section, we will elaborate on the meaning of the negativities found in our analyses.

Figure 2 shows some examples of averages based on single measurements for some of the patients and controls. We selected the particular patients with the intent of showing examples of very clear instances of the potentials, very unclear instances, and something in between. We thus tried to convey an impression of the variability that was present in the measurements. The Cz electrode was not displayed in this figure because the most important effects are present on the Fz and Pz electrode, and leaving out an electrode allowed us to present the data more concisely. Figure 2A shows some examples of healthy control subjects. The P300 was present in all participants, although its size varied considerably across subjects.

Some examples of measurements in the patients who recovered are shown in Figure 2B. In these figures, the P300s at the Pz electrode were difficult to locate, especially at the latency of about 700 milliseconds, where the grand average showed the clearest P300. These data of single measurements show that the P300 in the patients was not small because its latency varied much across patients. If that were the case, large individual P300s would have been found at various latencies, which would become "spread out" in the grand average. It is of course entirely possible that the P300 was small in the patients because of large variability in the single trials, but we had no way of determining single trial latency in this group of patients. The N350 was quite prominent in the patients who recovered, although not in all measurements, as is shown in the second (passive) and fourth column (active task) of Figure 2B. The figure also shows a small (first column) and large (third column) oddball effect on the N350.

As noted above, the data of the patients who did not recover are quite difficult to evaluate. In the average, there were no clearly discernible potentials, but Figure 2C shows some instances of potentials that might be interpreted as N350 or P300, although with less confidence than in the patients that did recover to consciousness.

In sum, the data from the individual measurements displayed in Figure 2 suggest that, although on average a clear pattern of results was present in the waveforms, there was much individual variation across patients and measurements.

Figure 3 describes the recovery process of an individual patient (patient 9; Table 1 and Appendix A) to show the large fluctuations in the amplitude of the N350 and of the P300 during the repeated measurements. For instance, the patient displayed in this figure showed a large N350 in the first measurement when she had an LoC of 4, but the N350 was virtually absent on the seventh measurement when she had an LoC of 7. The same holds for the P300, although a bit less clearly. The P300 was clearly visible in the sixth measurement (LoC 6) but nearly absent in the seventh measurement (LoC 7).

All in all it seems safe to conclude that there was no evidence that the amplitude of the N350 or of the P300 consistently increased or decreased as a function of the level of consciousness. Additionally, EEG frequencies at the different measurements did not systematically differ between those patients who did and those who did not recover to consciousness.

Percentage of Occurrence

When taking into account Figures 2 and 3, it appeared plausible to investigate the probability of the N350 and P300 over repeated measurements and compare patients who did and who did

TABLE 3. Means and Standard Deviations Per Level of Consciousness and the Norm Group: P300 and N200 Amplitudes (μV) for Standards and Deviants in the Passive Task and the Active Task

		N200 (Fz)				P300 (Pz)			
		Amplitude				Amplitude			
		Passive		Active		Passive		Active	
LoC	N	S	D	S	D	S	D	S	D
Norm	11	−3.45 (1.94)	−3.56 (2.27)	−2.80 (2.24)	−3.98 (1.74)	4.20 (2.29)	6.37 (3.77)	3.47 (1.39)	5.75 (2.43)
2	2	−0.02 (0.39)	−0.49 (2.61)	−0.16 (0.24)	−1.41 (1.04)	0.09 (2.01)	7.15 (5.31)	1.60 (0.74)	3.16 (4.99)
3	9	−1.40 (1.39)	−1.56 (1.75)	−2.62 (3.30)	−3.45 (2.67)	2.50 (2.13)	2.17 (1.62)	3.01 (2.81)	2.62 (3.97)
4	16	−1.41 (0.95)	−1.30 (1.93)	−0.97 (1.12)	−1.75 (2.23)	1.89 (1.88)	1.79 (2.67)	1.58 (1.39)	2.19 (3.05)
5	10	−0.76 (1.25)	−1.70 (1.79)	−0.38 (0.84)	−1.68 (3.25)	2.08 (1.28)	2.72 (2.82)	1.47 (2.57)	3.85 (6.89)
6	3	−2.61 (1.82)	−1.75 (2.80)	−1.63 (2.17)	−2.72 (2.31)	3.57 (2.29)	7.68 (4.30)	3.07 (0.70)	−0.46 (3.78)
7	5	−1.06 (0.60)	−2.52 (3.65)	−2.12 (0.20)	−1.66 (0.51)	0.76 (1.09)	2.02 (2.71)	3.03 (0.67)	1.28 (1.01)
8	1	1.31	2.07	3.60	2.55	−2.78	0.27	−1.36	−6.41

LoC, level of consciousness; S, standard tone; D, deviant tone.

TABLE 4. Short-term Outcome: Positive Predictive Value, Negative Predictive Value, Sensitivity, and Specificity, for Recovery to Consciousness

ERP	Task	Site	TP	FP	TN	FN	Pos. PV	%	95% CI	Neg. PV	%	95% CI	Sens	%	95% CI	Spec	%	95% CI
P300	Passive	Pz	10	12	25	2	10/22	45	25–67	25/27	93	74–98	10/12	83	51–97	25/37	69	50–81
	Active	Pz	2	20	22	5	2/22	9	1–30	22/27	81	61–93	2/7	29	5–70	22/43	51	37–68
N350	Passive	Fz	19	3	24	3	19/22	86	64–96	24/27	89	69–97	19/22	86	64–96	24/27	89	69–97
	Active	Fz	15	7	18	9	15/22	68	45–85	18/27	67	46–83	15/24	63	41–80	18/25	72	50–87

ERP, event-related potential; TP, true positives (i.e., number of measurements with the ERP component in patients with LoC ≥7); FP, false positives (i.e., number of measurements with the ERP component in patients with LoC < 7); TN, true negatives (i.e., number of measurements without the ERP component in patients with LoC < 7); FN, false negatives (i.e., number of measurements without the ERP component in patients with LoC ≥7); Pos. PV, positive predictive value for recovery to consciousness = TP/(TP + FP); CI, confidence interval; Neg. PV, negative predictive value for recovery of consciousness = TN/(TN + FN); Sens, sensitivity for recovery to consciousness = TP/(TP + FN); Spec, specificity for recovery to consciousness = TN/(TN + FP).

not recover to consciousness. The predictive values of the measurements of patients participating in this study are displayed in Table 4, separately for the passive and active tasks, and for patients who recovered to consciousness and who did not. The table shows that the predictive value of the N350 was quite high, both in the active and in the passive task. The P300 only appeared to have higher specificity and positive predictive value for recovery to consciousness and a higher percentage of occurrence in only the passive task.

The same pattern can be recognized for long-term outcome when assessed using the DRS. Table 5 displays the proportions of ERP occurrence in the patients who recovered to moderately severe disability or better (DRS ≥ 5) and those who recovered to a worse state of disability (DRS < 5), at 2 to 3 years after injury.

These data can be taken to mean that, if the N350 is present in a patient, then this patient has a high probability of recovering to consciousness. Conversely, if the N350 is absent, then it is more likely that the patient does not recover to consciousness. For the P300, only a high specificity and positive predictive value was found. Another detail that becomes apparent from Tables 3 and 4 is the notion that the passive oddball task seems to elicit the N350 and the P300 more frequently than the active oddball task in the present patient group.

DISCUSSION

Event-related potentials were examined in severely brain-injured patients during their recovery from the VS to consciousness and compared with healthy controls. We wanted to determine whether the ERP components exhibited longitudinal changes corresponding to the behavioral indices of recovery and whether these potentials could be useful in predicting recovery to consciousness.

As expected for an auditory oddball task, normal healthy controls showed a classic P300 potential, which was greater after deviants compared with standards. The potential had a parietal maximum and frontal minimum, which is also in line with numerous findings in the P300 literature (Sutton et al., 1965). These findings, which could be statistically confirmed, indicate that the task manipulation and the recording procedures for collecting the brain potentials were successful. However, in patients it turned out that the ERP components were often difficult to score.

Overall Grand Averages

Event-Related Potentials and Recovery to Consciousness

No changes in N200 and P300 amplitudes were found during recovery to consciousness, which is a result quite different from our earlier finding on another ERP, the MMN. Mismatch negativity amplitude changed during recovery to consciousness: a sudden enhance in MMN amplitude preceded overt communication with the environment (Wijnen et al., 2007). The 2 ERP paradigms differed on 2 important aspects. In the MMN paradigm, we used a fixed interstimulus interval of 500 milliseconds. In the current study, we used a random interstimulus interval of 1000 to 2000 milliseconds. So the most important differences between the 2 paradigms is the length between 2 tones and uncertainty in expectation of tones in time. It seems that especially the uncertainty requires better awareness, in contrast to the more automatic effect of the MMN. Our results resemble the findings of Bekinschtein et al. (2009) and Faugeras et al. (2012). Their global effect mainly differed in expectance uncertainty of the deviant tone and was mainly found in conscious patients in contrary to the local effect that was already found in VS/UWS and sometimes in MCS. In our current study, we sometimes found

TABLE 5. Long-term Outcome: Positive Predictive Value, Negative Predictive Value, Sensitivity, and Specificity for Disability Rating Scale About 2 to 3 years After Injury (Refer Table 1 for the Exact Time Intervals)

ERP	Task	Site	TP	FP	TN	FN	Pos. PV	%	95% CI	Neg. PV	%	95% CI	Sens	%	95% CI	Spec	%	95% CI
P300	Passive	Pz	7	5	30	7	7/12	58	29–84	30/37	81	64–91	7/14	50	24–76	30/35	86	69–95
	Active	Pz	0	7	28	14	0/7	0	0–44	28/42	67	50–80	0/14	0	0–27	28/35	80	63–91
N350	Passive	Fz	11	11	24	3	11/22	50	29–71	24/27	89	70–97	11/14	79	49–94	24/35	69	51–83
	Active	Fz	10	14	20	4	10/24	42	23–63	20/24	83	62–95	10/14	71	42–90	20/34	57	41–75

ERP, event-related potential; TP, true positives (i.e., number of measurements with the ERP component in patients with DRS ≥5); FP, false positives (i.e., number of measurements with the ERP component in patients with DRS < 5); TN, true negatives (i.e., number of measurements without the ERP component in patients with DRS < 5); FN, false negatives (i.e., number of measurements without the ERP component in patients with DRS ≥5); Pos. PV, positive predictive value for recovery of functions = TP/(TP + FP); CI, confidence interval; Neg. PV, negative predictive value for recovery of functions = TN/(TN + FN); Sens, sensitivity for recovery of functions = TP/(TP + FN); Spec, specificity for recovery of functions = TN/(TN + FP).

a P300, which might be indicative of the fluctuations in consciousness level in VS/UWS and MCS.

Outcome did matter. In the patients who did not recover, almost no consistent potentials were found. In the group that recovered, consistent potentials were indeed found, albeit of much smaller amplitude than in the normal controls. The P300 potential showed the classic oddball effect, but it was much delayed compared with the controls, and its scalp distribution was slightly more central. The delay of about 300 milliseconds seemed to be caused by the presence of a large frontal negativity that preceded the P300, which we termed N200. Only this N200 revealed a statistically significant effect: patients who recovered to consciousness revealed larger N200 amplitudes, when compared with patients who did not recover to consciousness.

The presence of the P300 in comatose patients has already been demonstrated in patients who were vegetative or minimally conscious for prolonged periods of time (Kotchoubey et al., 2001, 2005; Laureys et al., 2004), yet no relation of that component to outcome was found. Kotchoubey et al. (2005) did find recovery to be related to the MMN. Our present data extend those findings in that we found the N200 and the P300 to appear only in the majority of the patients who eventually recovered to consciousness. This seems to suggest that the presence of the N200 and the P300 can be of predictive value.

Individual Responses

However, when we counted the frequency of occurrence of the N350 and P300 in individual measurements, both ERP components, yet especially the N350 seemed to be of predictive value. The N350 occurred more frequently, but not always, in patients who recovered relative to patients who did not recover. We take these findings to mean that the presence of the N350 might indicate favorable outcome, and its absence does imply bad outcome. Similar conclusions on sensitivity and specificity have already been drawn for the P300 component of the ERP (Chennu and Bekinschtein, 2012; Daltrozzo et al., 2007; Vanhaudenhuyse et al., 2008). Our present findings suggest that the occurrence of the N350 is more limited to the patients who recover to consciousness.

The N350 as an Index of Recovery to Consciousness

An interesting question concerns the functional significance of the N200/N350. Nielsen-Bohlman et al. (1991) discussed the possibility that the N350 is functionally related to the MMN. Guérit et al. (1999) dismissed that possibility, mainly based on the argument that its latency surpasses that of the MMN in the same patients. The present data also speak against a functional relationship between the N350 and the MMN. First, in our data, the deviant stimuli did not consistently evoke a greater N350 than standard stimuli. Second, we have investigated the MMN in the same patients (Wijnen et al., 2007), and not only was its latency shorter than that of the N350 but the recovery patterns of the MMN and the N350 were also different. Taken together, the available evidence does not seem to suggest any functional relationship of the N350 and the MMN.

Why does the N350 mainly appear in the patients who finally recovered to consciousness? The N350 has been found in sleep stage 2: a state of drowsiness before a person falls asleep. In this stage, a healthy person is not able to (re)act by motor responses anymore (Harsh et al., 1994). However, the person is aware of the inability to act. In the study by Harsh et al. (1994), subjects were asked to push a button to the deviant stimuli in an auditory oddball paradigm. The expected P300 changed into a N350 when the subjects reached sleep stage 2. Subjects reported that at this moment they were not able to push the button anymore. A future study, using instructions to push a button in vegetative patients, should verify the possibility of awareness without being able to act.

Differences Between Passive and Active Oddball Tasks

A final remark should be made about the difference between the passive and the active oddball tasks. Guérit (2005) found that the active task sometimes elicits greater potentials than the passive task, but we were unable to replicate that finding. Only for the N200 significantly larger amplitudes were found during the active task when compared with the passive task for N200.

To the extent that there were differences between the two versions of the tasks, the passive task showed greater amplitudes relative to the active task. Furthermore, counting the occurrence of the potentials suggested that they both occurred more frequently in the passive compared with the active task. It is possible that the active oddball task elicits greater amplitudes when the patients have recovered to a stage in which they are able to "consciously" classify the standard and deviant stimuli and that there was no such patient included in the present study. The overall outcome of the present group was not very favorable.

Our finding also seem to be in contrast with the findings of Schnakers et al. (2008), who found larger P3 in active when compared with passive conditions, only in MCS. However, Schnakers et al. (2008) used different active stimuli, namely, unfamiliar names and the patient's own name randomly, which might have been easier to detect.

It is also possible that effects of task were confounded with time-on-task. The active task necessarily followed the passive task on each occasion, and these tasks were presented after various other tasks had already been administered. Thus, perhaps by the time the active task was administered, the patients were too tired and their processing resources for the classification task were exhausted. This issue clearly warrants further investigation because when consistent differences between active and passive versions of the oddball task can be demonstrated in individual patients, this might be a sign that they understood the task instructions.

SUMMARY AND CONCLUSIONS

Although no evidence was found that the N350 and the P300 changed as a function of recovery, we did find some evidence that outcome can be predicted from especially with the presence and amplitude of the N350. These findings are important because predicting outcome after severe brain injury is not possible based on behavioral indices alone.

More importantly, our methodology is truly unique. It is the first study in which both neurophysiological reactivity and behavioral signs were examined and related with each other in a longitudinal design. The uniqueness and significance of our procedure has already been stated by Kotchoubey (2007). Many existing studies follow either the clinical diagnosis with its strict Vegetative/Minimally Conscious distinction or are based on only physiology. Using behavioral indices, yet taking into account finer gradations both within and between the clinical categories of VS/UWS and MCS, together with "objective" physiologic data will provide a better understanding of specific aspects of the recovery track in these patients.

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REFERENCES

Andrews K. International working party on the management of the vegetative state. *Brain Inj* 1996;10:797–806.

Ansell BJ, Keenan JE, de la Rocha O. *Western neuro sensory stimulation: a tool for assessing slow-to-recover head-injured patients*, Vol. 24. Tustin: Western Neuro Care Centre, 1989.

Bach-y-Rita P, Bach-y-Rita E. Biological and psychosocial factors in recovery from brain damage in humans. *Can J Psychol* 1990;44:148–165.

Bekinschtein TA, Dehaene S, Rohaut B, et al. Neural signature of the conscious processing of auditory regulaties. *Proc Natl Acad Sci U S A* 2009;106: 1672–1677.

Boly M, Garrido MI, Gosseries O, et al. Preserved feedforward but impaired top-down processes in the vegetative state. *Science* 2011;332:858–861.

Bruno M-A, Majerus S, Boly M, et al. Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients. *J Neurol* 2011;259:1087–1098.

Buonomano DV, Merzenich MM. Cortical plasticity: from synapses to maps. *Annu Rev Neurosci* 1998;21:149–186.

Chennu S, Bekinschtein TA. Arousal modulates auditory attention and awareness: insights from sleep, sedation and disorders of consciousness. *Front Psychol* 2012;3:65.

Childs NL, Mercer WN, Childs HW. Accuracy of diagnosis of persistent vegetative state. *Neurology* 1993;43:1465–1467.

Coleman MR, Davis MH, Rodd JM, et al. Towards the routine use of brain imaging to aid the clinical diagnosis of disorders of consciousness. *Brain* 2009;132:2541–2552.

Courchesne E, Hillyard SA, Galambos R. Stimulus novelty, task relevance and the visual evoked potential in man. *Electroencephalogr Clin Neurophysiol* 1975;39:131–143.

Critchley HD, Mathias CJ, Josephs O, et al. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain* 2003;126:2139–2152.

Cruikshank SJ, Weinberger NM. Evidence for the Hebbian hypothesis in experience dependent physiological plasticity of neocortex: a critical review. *Brain Res Rev* 1996;22:191–228.

Cruse D, Chennu S, Chatelle C, et al. Bedside detection of awareness in the vegetative state: a cohort study. *Lancet* 2011;378:2088–2094.

Daltrozzo J, Wioland N, Mutschler V, Kotchoubey B. Predicting coma and other low responsive patients outcome using event-related brain potentials: a meta-analysis. *Clin Neurophysiol* 2007;118:606–614.

Eilander HJ, Wijnen VJM, Scheirs JGM, et al. Children and young adults in a prolonged unconscious state due to severe brain injury: outcome after an early intensive neurorehabilitation programme. *Brain Inj* 2005;19:425–436.

Eilander HJ, van de Wiel M, Wijers M, et al. The reliability and validity of the PALOC- s: a post-acute level of consciousness scale for assessment of patients with prolonged disturbed consciousness after brain injury. *Neuropsychol Rehabil* 2009;19:1–27.

Estraneo A, Moretta P, Loreto V, et al. Late recovery after traumatic, anoxic, or hemorrhagic long-lasting vegetative state. *Neurology* 2010;75:239–245.

Faran S, Vatine JJ, Lazary A, et al. Late recovery from permanent traumatic vegetative state heralded by event-related potentials. *J Neurol Neurosurg Psychiatry* 2006;77:998–1000.

Faugeras F, Rohaut B, Weiss N, et al. Event related potentials elicited by violations of auditory regularities in patients with impaired consciousness. *Neuropsychologia* 2012;50:403–418.

Fischer C, Luaute J, Morlet D. Event-related potentials (MMN and novelty P3) in permanent vegetative or minimally conscious states. *Clin Neurophysiol* 2010;121:1032–1042.

Francis DJ, Fletcher JM, Stuebing KK, et al. Analysis of change: modeling individual growth. *J Consult Clin Psychol* 1991;59:27–37.

Frost RB, Farrer TJ, Primosch M, Hedges DW. Prevalence of traumatic brain injury in the general adult population: a meta-analysis. *Neuroepidemiology* 2012;40:154–159.

Garcia AN, Shah MA, Dixon CE, et al. Biologic and plastic effects of experimental traumatic brain injury treatment paradigms and their relevance to clinical rehabilitation. *PM R* 2011;3:S18–S27.

Giacino JT, Kalmar K. The vegetative and minimally conscious states: a comparison of clinical features and functional outcome. *J Head Trauma Rehabil* 1997;12:36–51.

Giacino JT, Zasler ND, Katz DI, et al. Development of practice guidelines for assessment and management of the vegetative and minimally conscious states. *J Head Trauma Rehabil* 1997;12:79–89.

Giacino JT, Ashwal S, Childs N, et al. The minimally conscious state. Definition and diagnostic criteria. *Neurology* 2002;58:349–353.

Giacino JT, Kalmar K, Whyte J. The JFK Coma Recovery Scale—Revised: measurement characteristics and diagnostic utility. *Arch Phys Med Rehabil* 2004;85:2020–2029.

Gott PS, Rabinowicz AL, DeGiorgio CM. P300 auditory event-related potentials in nontraumatic coma. Association with Glasgow Coma Score and awakening. *Arch Neurol* 1991;489:1267–1270.

Gratton G, Coles MGH, Donchin E. A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol* 1983;55:468–484.

Gross CG. Neurogenesis in the adult brain: death of a dogma. *Nat Rev Neurosci* 2000;1:67–73.

Guérit JM. Neurophysiological patterns of vegetative and minimally conscious states. *Neuropsychol Rehabil* 2005;15:357–371.

Guérit JM, Verougstraete D, de Tourtchaninoff M, et al. ERPs obtained with the auditory oddball paradigm in coma and altered states of consciousness: clinical relationships, prognostic value, and origin of components. *Clin Neurophysiol* 1999;110:1260–1269.

Harsh J, Voss U, Hull J, et al. ERP and behavioural changes during the wake/sleep transition. *Psychophysiology* 1994;31:244–252.

Hebb DO. *The organization of behavior*. New York: John Wiley, 1949.

Jennett B, Plum F. Persistent vegetative state after brain damage. A syndrome in search of a name. *Lancet* 1972;1:734–737.

Kane NM, Butler SR, Simpson T. Coma outcome prediction using event- related potentials: P3 and mismatch negativity. *Audiol Neurootol* 2000;5:186–191.

Kotchoubey B. Event-related potentials predict the outcome of the vegetative state. *Clin Neurophysiol* 2007;118:477–479.

Kotchoubey B, Lang S, Baales R, et al. Brain potentials in human patients with extremely severe diffuse brain damage. *Neurosci Lett* 2001;301:37–40.

Kotchoubey B, Lang S, Mezger G, et al. Information processing in severe disorders of consciousness: vegetative state and minimally conscious state. *Clin Neurophysiol* 2005;116:2441–2453.

Lammi MH, Smith VH, Tate RL, Taylor CM. The minimally conscious state and recovery potential: a follow-up study 2 to 5 years after traumatic brain injury. *Arch Phys Med Rehabil* 2005;86:746–754.

Laureys S, Perrin F, Faymonville ME, et al. Cerebral processing in the minimally conscious state. *Neurology* 2004;63:916–918.

Laureys S, Celesia GG, Cohadon F, et al. Unresponsive wakefulness syndrome: a new name for the vegetative state or apallic syndrome. *BMC Med* 2010;8:68.

Luaute JMDP, Maucort-Boulch DMDP, Tell LM, et al. Long-term outcomes of chronic minimally conscious and vegetative states. *Neurology* 2010;75:246–252.

Lotze M, Schertel K, Birbaumer N, Kotchoubey B. A long-term intensive behavioural treatment study in patients with persistent vegetative state or minimally conscious state. *J Rehabil Med* 2011;43:230–236.

Luria AR. New York: Basic Books, 1973.

Matthews SC, Paulus MP, Simmons AN, et al. Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *Neuroimage* 2004;22:1151–1156.

Merzenich M. Seeing in the sound zone. *Nature* 2000;404:820–821.

Merzenich M, Nelson RJ, Kaas JH, et al. Variability in hand surface representations in areas 3b and 1 in adult owl and squirrel monkeys. *J Comp Neurol* 1987;258:281–296.

Multi-society task force on persistent vegetative state medical aspects of the persistent vegetative state (first of two parts). *N Engl J Med* 1994a;330: 1499–1508.

Multi-society task force on persistent vegetative state medical aspects of the persistent vegetative state (second of two parts). *N Engl J Med* 1994b;330:1572–1579.

Näätänen R, Gaillard AWK, Mäntysalo S. Early selective-attention effect on evoked potential reinterpreted. *Acta Psychol (Amst)* 1978;42:313–329.

Nielsen-Bohlman L, Knight RT, Woods DL, Woodward K. Differential auditory processing continues during sleep. *Electroencephalogr Clin Neurophysiol* 1991;79:281–290.

Perrin F, Schnakers C, Schabus M, et al. Brain response to one's own name in vegetative state, minimally conscious state, and locked-in syndrome. *Arch Neurol* 2006;63:562–569.

Polich J. P300 from a passive auditory paradigm. *Electroencephalogr Clin Neurophysiol* 1989;74:312–320.

Rappaport M, Hall KM, Hopkins K, et al. Disability Rating Scale for severe head trauma: coma to community. *Arch Phys Med Rehabil* 1982;63:118–123.

Rappaport M, McCandless KL, Pond W, Krafft MC. Passive P300 response in traumatic brain injury patients. *J Neuropsychiatry Clin Neurosci* 1991;3: 180–185.

Robertson IH, Murre JMJ. Rehabilitation of brain damage: brain plasticity and principles of guided recovery. *Psychol Bull* 1999;125:544–575.

Schnakers C, Perrin F, Schabus M, et al. Voluntary brain processing in disorders of consciousness. *Neurology* 2008;71:1614–1620.

Schnakers C, Vanhaudenhuyse A, Giacino J, et al. Diagnostic accuracy of the vegetative and minimally conscious state: clinical consensus versus standardized neurobehavioral assessment. *BMC Neurol* 2009;9:35.

Seel RT, Sherer M, Whyte J, et al. Assessment scales for disorders of consciousness: evidence-based recommendations for clinical practice and research. *Arch Phys Med Rehabil* 2010;91:1795–1813.

Squires KC, Wickens C, Squires NK, Donchin E. The effect of stimulus sequence on the waveform of the cortical event-related potential. *Science* 1976;193:1142–1146.
Sutton S, Braren M, Zubin J, John ER. Evoked potential correlates of stimulus uncertainty. *Science* 1965;150:1187–1188.
van Praag K, Kempermann G, Gage FH. Neural consequences of environmental enrichment. *Nat Rev Neurosci* 2000;1:191–198.
Vanhaudenhuyse A, Laureys S, Perrin F. Cognitive event-related potentials in comatose and post-comatose states. *Neurocrit Care* 2008;8:262–270.
Wijnen VJ, Heutink M, van Boxtel GJ, et al. Autonomic reactivity to sensory stimulation is related to consciousness level after severe traumatic brain injury. *Clin Neurophysiol* 2006;117:1794–1807.
Wijnen VJM, van Boxtel G, Eilander HJ, de Gelder B. Mismatch negativity predicts recovery from the vegetative state. *Clin Neurophysiol* 2007;118:597–605.

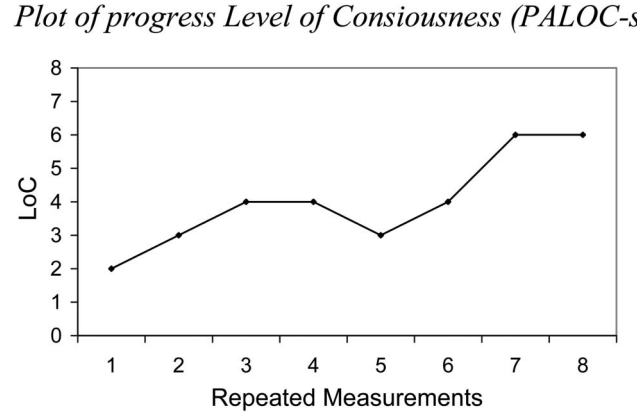
APPENDIX A

Patient 9

Clinical Presentation and Acute Management

Patient 9 was a girl who was 16 years old at the time of her injury. She was injured in a traffic accident. She was found unconscious at the scene of the accident, transferred to hospital, and admitted to the intensive care unit, where her Glasgow Coma Scale was E1M2V1. Initial computed tomography (CT) brain scanning demonstrated edema, multiple contusions, a right subarachnoid hemorrhage, and damage to the brain stem. An intracranial pressure gauge and an endotracheal tube were applied. A later CT scan showed some ischemia. There were fractures to the left femur and left mandible. Patient 9 spent 30 days at the ICU. Patient 9 was transferred to the Rehabilitation Centre Leijpark 60 days after her injury.

Plot of Progress Level of Consiousness



Rehabilitation Program and Progress

Initially, patient 9 responded to all the stimulations, in particular to the touch and pain stimulation. However, when stimulated for longer periods she was no longer motivated, and she would bend her head forward. Later she started to perform tasks and respond to commands. She started to show a yes/no response. However, her acts were inconsistent. She learned to communicate using a talking computer, and she learned to read. She was discharged 99 days after admission, indicated for regular rehabilitation.