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Autonomic reactivity to sensory stimulation is related to consciousness level after severe traumatic brain injury

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Abstract

Objective: To examine changes in the activity of the autonomic nervous system (ANS) that are related to recovery to consciousness in the post-acute phase after severe traumatic brain injury (sTBI).

Methods: Skin conductance and heart rate reactivity to sensory stimulation were recorded every 2 weeks for an average period of 3.5 months in 16 adolescent patients, during the assessment of their level of consciousness (LoC), and their cognitive and functional behaviour.

Results: Both heart rate variability (HRV) and skin conductance level (SCL) in reaction to sensory stimulation changed with recovery to consciousness. Indices of HRV and SCL that represent sympathetic activity of the autonomic nervous system (ANS) increased with recovery, whereas indices that represent parasympathetic activity decreased. In addition, we observed an increase in sympathovagal balance of the ANS with recovery.

Conclusions: Recovery to consciousness determined by clinical observation in sTBI in the post-acute phase is related to changes in SCL and HRV during sensory stimulation. ANS reactivity to environmental stimulation can therefore give objective supplementary information about the clinical state of sTBI patients, and can contribute to decision-making in the treatment policy of unresponsive patients.

Significance: These findings demonstrate that autonomic reactivity can be informative concerning how a severely damaged nervous system reacts to environmental stimulation and how, in a recovering nervous system, this reactivity changes.

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Keywords: Severe traumatic brain injury; Autonomic nervous system; Consciousness; Vegetative state; Minimally conscious state; Sensory stimulation

1. Introduction

Severe traumatic brain injury (sTBI) results in high morbidity and mortality rates. A large number of sTBI patients experience long-term or lifelong disabilities, entailing major costs for family and society. In the United States the incidence of sTBI is 8 times as high as that of breast cancer and 34 times that of HIV/AIDS (CDC Injury prevention, 2004). However, research on recovery patterns is scarce, especially when young adults are concerned, who are known to be a group at great risk of sTBI (Finfer and Cohen, 2001; Jennett, 1996).

The clinical recovery pattern after sTBI, including the different levels of consciousness and characteristics of those

levels, has been extensively discussed (Andrews, 1996; Giacino, 1997; Multi-Society Task Force on Persistent Vegetative State, 1994a; Zeman, 2001). Immediately after severe brain damage the patient is usually in a coma. Patients who do not awaken from coma within a period of about 4–6 weeks may shift into a vegetative state (VS) (Jennett and Plum, 1972; Multi-Society Task Force on Persistent Vegetative State, 1994a), or die. In a vegetative state patients have sleep–wake cycles, autonomic control of blood pressure and respiration are present, while cognitive functioning and consciousness are absent. For some patients such a vegetative state is the final outcome. A subgroup of patients may shift into a minimally conscious state (MCS) (Giacino et al., 2002), also referred to as a low awareness state (Andrews, 1996). Patients then demonstrate discernible but inconsistent evidence of consciousness. This state is often transient but can also be the permanent outcome. When patients react adequately to the environment and when communication is possible (with or without tools), they are assumed to be conscious. The experience of self and the environment, and the stock of knowledge, thoughts and intentions are then present (Zeman, 2001); however, various cognitive impairments might still affect the patient (Multi-Society Task Force on Persistent Vegetative State, 1994a).

In current practice, decisions concerning consciousness rest principally on clinical observations (Chiappa and Hill, 1998). In the acute phase after TBI, the depth of coma is often determined by means of the Glasgow coma scale (GCS, Teasdale and Jennett, 1974). Complementary diagnostic investigation by means of neurophysiological assessment is often carried out, but this is intended mainly to diagnose the extent of brain damage (haemorrhage, oedema, diffuse swelling, intracranial pressure, epileptic seizures, et cetera). In addition, early neurophysiological methods are sometimes used to predict the clinical outcome (Fischer and Luauté, 2005; Fischer et al., 2004; Guérit et al., 1993, 1999; Kane et al., 2000; Wardlaw et al., 2002).

In the post-acute phase, observation scales are also used, examining the recovery to consciousness by observing behavioural skills, such as the Western Neuro Sensory Stimulation Profile (WNSSP, Ansell et al., 1989), the Rancho Los Amigos Scale (Hagen et al., 1972), and the Disability Rating Scale (Rappaport et al., 1982). Yet during this phase neurophysiological assessment is not always considered to be important.

The main purpose of the present study was to examine whether the behavioural changes in the post-acute phase of recovery after sTBI are reflected in physiological reactivity. If so, the examination of neurophysiological features and changes within these features could provide more insight into processes and patterns of recovery during the post-acute phase.

Recently, functional neurophysiological reactivity has been demonstrated in VS and MCS using Event Related Potentials (Kotchoubey et al., 2002, 2005; Neumann and Kotchoubey, 2004), and using fMRI and PET scans (Boly et al., 2004; Jong et al., 1997; Laureys et al., 2004a; Owen et al., 2005; Schiff et al., 2002, 2005).

It appears that external stimuli (such as sounds) can provoke cortical activity in VS (Kotchoubey et al., 2005; Laureys et al., 2004a; Owen et al., 2005; Schiff et al., 2002). This activity is often limited to the isolated activity in certain 'cortical islands' (Menon et al., 1998; Plum et al., 1998; Schiff et al., 1999, 2002), which are not integrated in the entire network of information processing. Therefore, it is still not certain whether any ability to understand is intact (Robertson and Murre, 1999). Results with PET showed that the brain metabolism in VS is reduced by 50% compared to a healthy brain (Laureys et al., 1999, 2002). In addition, in VS brain metabolism of different areas is unrelated, presumably because of the disconnection between these areas (Boly et al., 2004, 2005; Laureys et al., 1999, 2002). Postmortem research showed that in VS often a structurally normal cortex was intact (Adams et al., 2000), however, without any connection to other areas like the thalamus.

In MCS the associative brain areas (secondary and tertiary) are active in response to external stimulation such as sound or pain (Boly et al., 2005). These areas are necessary for the conscious perception of stimuli (Baars et al., 2003). In some studies, it was found that brain activity in MCS in response to sound and pain stimulation was equal to the activity found in a healthy control group (Laureys et al., 2004b; Schiff et al., 2005).

We specifically examined the reactivity of the autonomic nervous system (ANS) to environmental input through different sensory modalities during the recovery from a vegetative state to consciousness. Measurements of the ANS can provide insight into mental activity related to the perception and processing of environmental stimulation, even in the absence of observable behaviour (Öhman et al., 2000). Measuring the function of the ANS in patients with sTBI might be especially informative. According to Plum and Posner (1980), preservation of arousal is required for recovery to consciousness, because conscious behaviour depends on the continuous interaction between cortical systems and the subcortical activating mechanisms (the noradrenergic and cholinergic reticular activation system). Since arousal is mainly mediated by the ANS, we expected that recovery of consciousness is related to, or even dependent on, the functionality and integrity of the ANS.

Spectral analysis of heart rate variability (HRV) and the assessment of skin conductance level (SCL) allowed us to probe the functioning of the sympathetic and parasympathetic branches of the ANS separately. Changes in SCL are influenced primarily by sympathetic elicitation of sweat secretion (Boucsein, 1992). Slow variations of the heart rate mainly reflect the influence of homeostatic control processes, mediated by the sympathetic branch of the ANS (Berntson et al., 1997). More rapid fluctuations reflect processes related to blood pressure control predominantly, but not exclusively, by the sympathetic branch of the ANS

(Akselrod et al., 1981). Very fast fluctuations are related to respiratory activity, primarily controlled by the parasympathetic branch of the ANS (Akselrod et al., 1981; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In addition, the sympathovagal balance can be examined using HRV (Malliani et al., 1998; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

In the current study, adolescents suffering sTBI were admitted to an 'Early Intensive Neurorehabilitation Programme'. Since 1987 the Rehabilitation Centre Leijpark offers this programme for children and young adults in a vegetative or minimally conscious state after acquired brain injury. The rationale of this program is that sensory stimulation and an enriched environment leads to better and faster recovery after sTBI (Rosenzweig and Bennett, 1996). The effect of this programme has not yet been demonstrated, however, Eilander et al. (2005a) showed that patients who participated in this programme had a more favourable outcome than predicted by 'The Multi-Society Task Force on Permanent Vegetative State' (Multi-Society Task Force on Persistent Vegetative State, 1994b).

In order to learn more about recovery processes during the rehabilitation programme, we studied the activity of the different branches of the ANS during a sensory stimulation protocol (WNSSP) (Ansell et al., 1989).

Several studies reported on HRV and SCL in TBI patients, but virtually all were performed in the acute phase and in adult patients. Comatose TBI patients in the acute phase show very low HRV (Lowensohn et al., 1977). An increase in the sympathovagal balance has been found in patients who had recovered from a comatose state in the acute phase (Hildebrandt et al., 1998). Higher sympathetic and lower parasympathetic activity were paired with better scores on the GCS (8–10). Recently, Su et al. (2005) compared HRV with severity of brain damage. In the more severely brain-damaged patient groups both sympathetic and parasympathetic activity were lower in comparison to less severely brain damaged patient groups, and in comparison to a healthy norm group.

Electrodermal reactivity to auditory stimuli is lower or even absent in sTBI patients in a vegetative state compared to healthy controls (Turkstra, 1995). Higher electrodermal activity can be seen in patients recovering from a vegetative state (Turkstra, 1995), together with higher scores on the GCS (8–10) (Hildebrandt et al., 1998). Only one study reported on HRV in the post-acute phase, in which 4 adult sTBI patients were compared with matched controls (King et al., 1997). These patients showed lower power in all frequency bands of the heart rate spectrum compared to controls.

We are not aware, however, of any study in which longitudinal measurements of HRV and SCL are performed in the post-acute phase during recovery to consciousness. In the present report, the relationship is investigated between the reactivity of the ANS to sensory stimulation, behavioural changes during the recovery to consciousness, and cognitive recovery of the sTBI patients. It was expected that during recovery to consciousness patients would become more aroused during the stimulation sessions. Environmental stimuli normally lead to a higher activity of the sympathetic and a lower activity of the parasympathetic nervous system to environmental stimuli: when sympathetic activity increases, the parasympathetic activity decreases (Berntson et al., 1991). Following this pattern, the sympathovagal balance during environmental stimulation would increase with recovery to consciousness as a consequence of reciprocal sympathetic activation (Berntson et al., 1991).

2. Methods

2.1. Patients

Sixteen patients with sTBI who were admitted to the rehabilitation programme between January 2001 and May 2002 were included in the study. Inclusion criteria for participation were: age between 17 and 26 years, no mechanical ventilation, and time between injury and admission no longer than 6 months. Ten (61.1%) were male. Age at the time of the injury ranged from 17.5 to 25 years ($M = 21.5$ years; $SD = 3.0$) (Table 1). Time since injury at admission was at least 4 weeks ($M = 2.3$ months; $SD = 1.6$). The major cause of TBI was a traffic accident (94.4%). All patients participated in this study following informed consent given by a parent, a legal representative, or partner. The duration of the patients' participation in the program ranged from 1.1 to 6.4 months ($M = 3.5$ months; $SD = 1.4$), depending on their recovery rate. The study has been approved by the METOPP (a Dutch Medical Ethics Committee for Research with Patients).

2.2. Observation scales

2.2.1. Level of consciousness (LoC)

For the level of consciousness (LoC) a categorisation was used based on literature on terminology and definitions described by 'the International Working Party Report on the Vegetative State' (Andrews, 1996; Royal Hospital for Neuro-disability, 1996). The categorisation describes a comatose state, 3 vegetative sub-states, 3 non-vegetative sub-states, and a conscious state (see Table 2 for the classification scheme in detail).

The observation scale showed a high reliability and validity (Eilander et al., 2005b). The inter-observer correlations varied between 0.85 and 0.94. The intra-observer correlations varied between 0.95 and 0.96. The inter-observer agreement scores (Cohen's weighted Kappa) varied between 0.82 and 0.95. The intra-observer agreement scores varied between 0.94 and 0.95. The correlations with

Table 1
Patients' details

Ptn	Gender	Age	Ms	iGCS	lGCS	T1	T2	T3	Primary damage*	Secondary damage*	Location (l= left/r=right)*	LoC 1	LoC 2	DRS cat	GOSE	T4
1	F	25;0	2	4	12	14	40	62	Punctual haemorrhages, focal lesions (frontal)	Diffuse swelling, compressed basilar cisterns	Cortical+cerebellum r	6	8	6	5	4;7
2	M	23;1	4	6	12	28	64	78	Skull fracture, punctual haemorrhages, contusion (left temporal)	High intracranial pressure, diffuse axonal injury	Cortical l	6	7	7	5	4;6
3	M	19;9	7	2t	11	62	62	126	Diffuse punctual haemorrhages also in brain-stem, diffuse axonal injury	Diffuse white matter lesion	Cortical+subcortical+cerebellum+brainstem	4	7	6	4	4;4
4	M	24;1	3	6	8	16	42	76	Oedema right parietal and right and left parietofrontal	Atrophy	Cortical	2	7	7	5	4;3
5	M	17;1	6	4t	8	25	40	71	Intraventricular haemorrhage, contusions mesencephalon	Diffuse swelling, compressed basilar cisterns, atrophy	Cortical+subcortical+cerebellum r+ brain-stem	3	7	6	5	4;2
6	M	20;1	10	8	10	23	69	133	Skull fracture, punctual haemorrhages, diffuse axonal injury	Focal lesions and diffuse white matter lesion	Subcortical	2	7	6	3	4;3
7	M	17;7	11	7	11	20	39	195	Skull fracture, hypoxia, punctual haemorrhages, diffuse axonal injury, contusions left parietal, right temporal and right frontal	Diffuse white matter lesion	Cortical+ brain-stem	2	8	5	3	4;1
8	M	18;7	8	5	8	11	31	182	Skull fracture, oedema, multiple punctual haemorrhages and contusions	Diffuse swelling, diffuse white matter lesion	Cortical+subcortical	2	5	4	3	4;0
9	M	25;0	6	6	7	16	38	80	Skull fracture punctual and subarachnoid haemorrhage, contusions right occipital	Diffuse swelling	Cortical+subcortical r+ brain-stem	2	8	6	5	4;0
10	M	25;7	3	2t	10	26	59	118	Skull fracture, punctual haemorrhages and intraventricular, multiple contusions		Cortical+subcortical	6	7	5	3	3;7
11	M	24;1	5	3	8	58	198	77	Skull fracture, oedema and subarachnoid haemorrhage, multiple contusions	Atrophy	Cortical+subcortical	1	2	1	1	4;1
12	M	21;3	7	2t	14	57	57	119	Skull fracture, anoxia, oedema, and multiple contusions	Diffuse swelling, high intracranial pressure	Cortical+subcortical	5	7	6	4	3;7

Table 1 (*continued*)

Ptn	Gender	Age	Ms	iGCS	IGCS	T1	T2	T3	Primary damage*	Secondary damage*	Location (1= left/r = right)*	LoC 1	LoC 2	DRS cat	GOSE	T4
13	F	18;1	8	4	8	31	122	108	Oedema, punctual and subarachnoid haemorrhages, diffuse contusions	Diffuse swelling, high intracranial, pressure diffuse white matter lesions, atrophy	Subcortical + brain-stem	2	2	4	3	3;8
14	M	21;1	3	5	9	16	79	34	Skull fracture, oedema, punctual and intraventricular haemorrhages, multiple contusions	Ischaemia, diffuse swelling, high intracranial pressure	Cortical+sub-cortical	5	8	6	5	3;6
15	M	17;7	9	6	8	21	73	120	Skull fracture, multiple contusions and punctual haemorrhages	Diffuse swelling	Cortical	5	7	5	3	3;6
16	M	25;1	8	2t	8	16	81	111	Skull fracture, left parietal and right frontal subdural haematoma	Intracranial pressure, atrophy	Cortical+sub-cortical	3	2	1	1	3;4

Ptn, patient; F, female; M, male; Age, age at injury in years; Ms, number of measurements with an interval of 2 weeks; iGCS, Glasgow coma scale at hospital admission; IGCS, Glasgow coma scale at hospital discharge; T1, time at ICU in days; T2, time before admission EINP in days; T3, program duration RCL in days; *diagnoses based on the medical report in the acute phase; LoC 1, level of consciousness first measurement; LoC 2, level of consciousness last measurement; DRScat, disability rating scale categories; GOSE, Glasgow outcome scale extended; T4, time of outcome after injury in years.

the WNSSP varied between 0.88 and 0.93, and for the DRS, the correlations varied between 0.75 and 0.88 (Eilander et al., submitted for publication).

2.2.2. Western neuro sensory stimulation profile (WNSSP)

Cognitive and communicative functioning was assessed using the WNSSP (Ansell et al., 1989). The WNSSP was specifically developed to assess the cognitive status and communicative performance in severely impaired head-injured patients. The WNSSP consists of 33 items that assess a patient's arousal and attention, expressive communication, and response to auditory, visual, tactile, and olfactory stimulation. Six subscales (arousal and attention, auditory comprehension, visual comprehension, visual tracking, object manipulation, and expressive communication) and 4 additional observations (response to sound, speech, smell, and touch) have been delineated, which assess specific aspects of a patient's behaviour and provide a means for evaluating a patient's pattern of response. The total score ranged from 0 to 113 (the higher the better).

2.2.3. Stimulation paradigm

Stimulation was provided according to the WNSSP. Care was taken to perform this stimulation as standardised as possible, using the same stimuli, stimulation order, and stimulation intensity during every measurement (see Appendix A for the exact stimuli).

2.2.4. Disability rating scale (DRS)

Changes in arousal and consciousness, and in cognitive, functional, and psychosocial areas were examined using the DRS (Rappaport et al., 1982). The DRS is a quantitative instrument with a continuous 30-point scale for assessing the disability of sTBI patients. It reflects changes in arousal and awareness, and in cognitive, functional, and psychosocial areas. The scale consists of 8 items arranged over 4 categories: (1) arousability, awareness, and responsivity (similar to GCS); (2) cognitive ability for self-care activities; (3) level of dependency, and; (4) psychosocial adaptability. Only the total DRS score was used in this study. The lower the total DRS score the fewer the disabilities, ranging from death (score 30) to no disability (score 0).

2.2.5. Long-term outcome

To determine the long-term functional outcome, the DRS (Rappaport et al., 1982), well as the Glasgow outcome scale extended (GOSE) (Wilson et al., 1998) were used. To make the two scales more comparable, the DRS was reduced to 8 categories and is referred to as DRScat (Rappaport et al., 1982): 1, death (score 30); 2, vegetative state (score 22–29); 3, extremely severely disabled (score 17–21); 4, severely disabled (score 12–16); 5, moderately severely disabled (score 7–11); 6, moderately disabled

Table 2
Levels of consciousness (LoC)

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1. **Coma**
   Patients do not have a sleep–wake cycle (in absence of bilateral third cranial nerve lesion). There is absent control of the basic body functions like respiration, body temperature, blood pressure. Patients may respond to painful stimulation by reflex (extension or flexion), but do not show any other reaction.

Vegetative presentations
   Patients do have sleep–awake cycles, but no day–night rhythm, the basic body functions are generally recovered. No artificial respiration is needed anymore.

2. *Hyporesponsive state*: patients may respond on occasions by a reflex activity in a delayed fashion but are generally unresponsive to stimulation from the environment. They display no detectable signs of awareness.

3. *Reflexive responsive state*: patients generally respond in mass extensor responses or startle responses to stimulation without habituation. This may progress into flexor withdrawal responses. There may be roving eye movements, without tracking. Facial expressions may occur to stimulation.

4. *Retracting reflexive state*: single limb responses to stimulation. Withdrawal or intermittent localisation to painful stimulation may occur by reactivity of the stimulated body part. Eye movements may occur but the patients do not focus on objects or people. Patients may turn to sound or touch.

Non-vegetative states
   Patients are awake for most of the day.

5. *Transitional state*: more definite localizing to visual, auditory or tactile stimulation. Much of the behaviour is automatically, e.g. opening of the mouth when food is seen or felt. Following with the eyes to objects and persons. Sometimes emotional responses as a reaction to family. Patients may smile or cry.

6. *Inconsistent low awareness state*: inconsistent reactivity to simple commands. Patients are completely dependent. Patients have profound cognitive impairments.

7. *Consistent low awareness state*: patients react adequately to simple commands. Patients still show profound cognitive impairments and are completely dependent.

8. **Conscious state**
   Patients react adequately to the environment, and good reciprocal communication is possible (with or without tools). Still profound cognitive impairments can be present.

(score 4–6); 7, mildly to partially disabled (score 1–3); and 8, no disability (score 0).

The GOSE is an extension of the Glasgow outcome scale (GOS) (Jennett et al., 1981). The GOSE is a one-item rating scale including 8 outcome categories (from 'dead' to 'upper good recovery') and can be administered through a structured interview. Compared to the GOS, the GOSE has been shown to be more sensitive to changes in mild to moderate TBI (Wilson et al., 1998). The GOSE consists of 8 items: 1, death; 2, vegetative state; 3, lower severely disabled; 4, upper severely disabled; 5, lower moderately disabled; 6, upper moderately disabled; 7, lower good recovery; 8, upper good recovery. The GOSE was administered in a structured interview as proposed by Wilson et al. (1998).

2.3. Psychophysiological recordings

Heart rate was measured using 3 disposable Ag–AgCl electrodes (diameter of contact area = 15 mm) placed on the sternum and the precordial position V6 (Mulder, 1992). The reference electrode was placed under the right clavicle. The inter beat intervals (IBIs) were stored on a personal computer, using an IBI-trigger which detected the R-waves of each heartbeat with a temporal resolution of 1 ms. Skin conductance, expressed in microSiemens (μS), was recorded from the thenar and hypothenar eminences of the non-dominant hand. Two Ag–AgCl skin electrodes (diameter of contact area = 8 mm) were attached, filled with an electrolyte consisting of 0.05 M NaCl in a unibase cream medium (Boucsein, 1992). An electric circuit applied a constant voltage of 0.5 V. Raw skin conductance values were digitised at a rate of 10 Hz, and stored on a personal computer.

2.4. Experimental procedure

Two days after a patient was admitted to the rehabilitation programme the first measurement took place. Measurements were repeated every 14 days. Depending on their condition, patients were examined either sitting in an upright position in a bed, or sitting in a wheelchair in a quiet room with a constant temperature (23 °C ± 1). Measurements were always recorded at the same time of the day (from 3:00 to 4:00 p.m.), immediately after the afternoon resting period.

First, a 3 min baseline recording of physiological rest-activity was performed (mean duration: 181.4 ± 14.3 s). Then, the WNSSP was administered, and physiological reactivity during stimulation was recorded (mean duration: 913.9 ± 234.6). After stimulation, a second 3 min rest period was measured (mean duration: 181.8 ± 5.6 s). A neuropsychologist scored the patients' behaviour according to the observation scales: LoC, DRS, and WNSSP. This assessment was performed every 2 weeks by the same neuropsychologist until the patient no longer qualified for the rehabilitation programme. The programme was ended when (a) a patient was qualified for further rehabilitation because of recovery of consciousness, (b) a patient did not show any recovery during the programme and therefore was indicated for a nursing home, or (c) a patient deceased. These different recovery courses lead to a variation in time span for patients' participation in the experiment, and in the number of measurements. In addition, patients sometimes could not participate as a result of their medical condition.

Long-term outcome was determined using the DRScat and GOSE scores at least 3.4 years after the injury ($M = 4$, SD = 0.4, 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).

2.5. Analyses

2.5.1. Data analysis

Recordings were divided into 3 epochs, representing the first resting period, the stimulation period, and the second

resting period. Artefacts in the IBI series were detected by outlier analysis based on the mean and standard deviation of the series (Berntson et al., 1995). These parameters were adjusted for each patient (and each measurement) individually, and based on visual inspection of the whole series. Outliers were deleted from the original series and replaced by values resulting from the application of a cubic spline function. IBIs were then converted to a real-time base at 2 Hz, detrended, tapered with a 10% cosine window, and then transformed into the frequency domain (Berntson et al., 1995).

Mean values for the power density in absolute values of power (milliseconds squared) were calculated separately for the LF (≤ 0.04 Hz), the MF (0.04–0.15 Hz), the HF (0.15–0.40 Hz), and total power (TP ≤ 0.40). Because the raw power values are influenced by the length of the data series, which varied in our administration of the WNSSP, we only report the power in normalised units (n.u.), separately for the MF (MFn.u.: MF/[TP$-$LF]$\times 100$) and HF (HFn.u.: HF/[TP$-$LF]$\times 100$). The MF in normalised units is a marker of sympathetic modulation. The HF in normalised units is a marker for parasympathetic modulation (vagal activity). Sympathovagal balance was calculated as MF/HF (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). SCL was determined by averaging all data samples of each of the 3 intervals. This value was referred to a calibrated baseline, which was set at the start of each measurement. Voltages were converted to microSiemens ($\mu S = R$ [calibrated voltage/averaged voltage]$\times 1000$ kΩ).

2.5.2. Statistical analyses

Changes according to the observation scales were examined by comparing the first and the last measurement for each patient, using the Wilcoxin matched-pairs signed-ranks test in the statistical package SPSS 12.0.1. Statistics of behavioural indices of recovery and outcome were calculated.

The longitudinal effects were examined in SAS 8.1, using a mixed-model analysis, with a compound symmetry covariance structure. Mixed-effects models use all available data, can properly account for correlation between repeated measurements on the same subject, have greater flexibility to model time effects, and can handle missing data more appropriately (Gueorguieva and Krystal, 2004; Keselman et al., 2001). In addition, mixed-effects models can be used to model data of ordinal level (Liu and Agresti, 2005). Individual trend-analyses were performed and the mean trend-analysis for all patients was determined (see Francis et al., 1991), in order to examine the shape of the relationship between the dependent variable and the levels of the independent variable. The intercept and the linear (first degree polynomial), quadratic (second degree polynomial) and cubic (third degree polynomial) effects were estimated. For each parameter the strongest effect is reported in the results.

All neurophysiological measurements were analyzed both as a function of time (ordinal measurement) and as a function of the different scores on the observation scales (LoC, DRS, and WNSSP). The individual subjects, ordinal measurement number, and the scores on the observational scales were included as random factors in separate analyses.

A repeated measurement analysis of variance in SAS 8.1 was used to assess the immediate effects of administering the WNSSP. Per patient a mean score was calculated for each resting period (before and after the WNSSP) over all their participated measurements. Overall differences between the two resting periods were analysed, as well as the changes within these differences over time and during recovery.

3. Results

3.1. Behavioural indices of recovery

Sixteen patients participated in the experiment. A maximum of 14 repeated measurements were collected. All patients participated in the first measurement. Fig. 1 presents the amount of patients per measurement and Table 1 presents the number of measurements per patient.

At admission, the patients' averaged LoC score was 3.5 (corresponding to the reflexive vegetative). The LoC score increased to 6.2 (corresponding to the inconsistent minimally conscious state) at discharge. A significant difference was found between the LoC score at admission and the LoC score at discharge ($Z = -3.28$, $P < 0.01$). DRS scores changed from a mean score of 22.1 at admission (corresponding to the level of complete dependence, but yet showing some signs of arousability, awareness, and responsivity), to a mean score of 13.8 at discharge (corresponding to the level in which the ability for self-care activities, but also dependency are present). A significance difference was found between the DRS score at admission and at discharge ($Z = 3.31$, $P < 0.01$). WNSSP scores changed from a mean score of 34.2 at admission (corresponding to an average ability to localize and to follow environmental stimuli), to a mean score of 89.1 at discharge (corresponding to the ability to respond to and to perform simple commands). A significant difference was found between the WNSSP score at admission and at discharge ($Z = -3.36$, $P < 0.01$).

As can be seen in Fig. 1 and Table 3, there was a marked fall-off in the number of patients who underwent the sequential measurements. This irregular distribution of measurements was due to the differences in recovery rate: patients who showed a fast recovery to consciousness were already indicated at an early stage for further rehabilitation. On the contrary, the patients who recovered slowly participated in the programme for longer. Some of the patients did not show any recovery to consciousness, and were indicated for transfer to a nursing home. The decision-
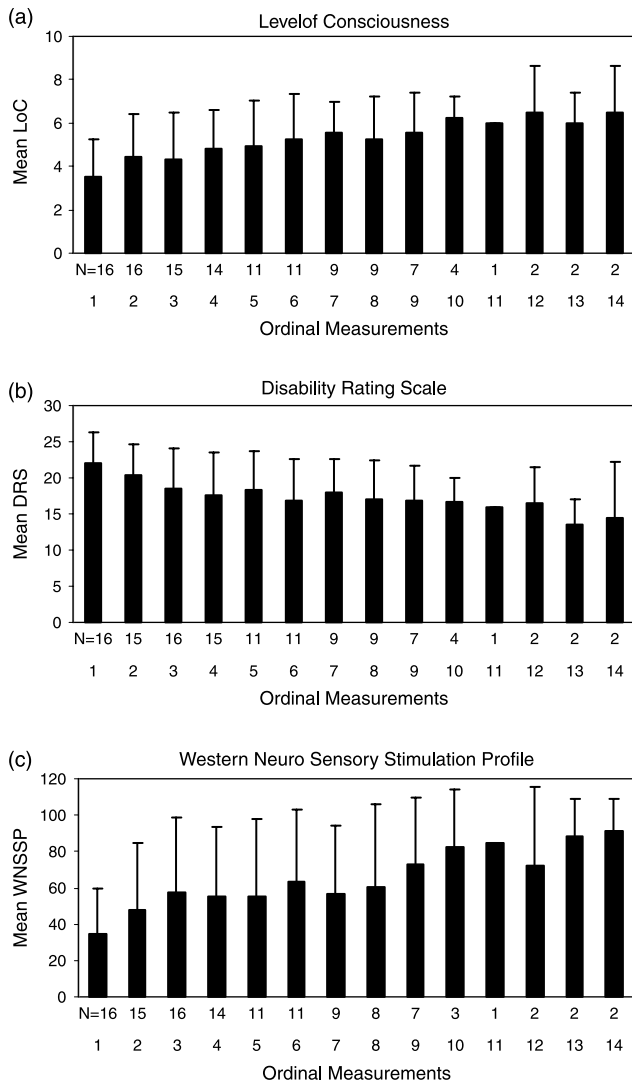
Fig. 1. Behavioural indices of recovery: means and standard deviations over ordinal measurements, which were performed every 2 weeks, and number of patients (N) for each measurement. (a) Level of consciousness. (b) Disability rating scale. (c) Western neuro sensory stimulation profile.

making on the patients' discharge and follow-up destination was influenced by the opinions of relatives or partners, and by waiting lists. In addition, sometimes a measurement could not be performed due to sickness of the patient (e.g. fever).

Overall, these data indicate an improvement trend during the programme for LoC, the DRS, and the WNSSP. From the total number of patients, 3 patients were still vegetative during the last measurement. These 3 patients had deceased in the long-term follow-up measurement. The patients who eventually recovered to consciousness after the rehabilitation programme still showed disabilities about 3.4–4 years after their injury. Two patients were 'mildly to partly disabled' according to the DRScat. Six patients were 'lower moderately disabled' according to the GOSE (see Tables 1 and 4, respectively, for the scores on the observation scales

for each patients, and the descriptive statistics for the first, the last measurement, and the long-term outcome of all patients).

3.2. Psychophysiological correlates of recovery. Changes in heart rate variability and skin conductance level over time: longitudinal measurements

The MFn.u. increased linearly over time ($F(1.86) = 5.22$, $P < 0.05$), whereas the HFn.u. decreased linearly ($F(1.86) = 4.84$, $P < 0.05$). The MF/HF ratio also showed a positive linear trend over the 14 measurements ($F(1.86) = 5.68$, $P < 0.05$). The SCL showed a cubic trend ($F(1.83) = .95$, $P < 0.05$). After an increase during the first 4 measurements, SCL slightly decreased to the 10th measurement. After this a slight increase can be observed.

3.3. Changes in heart rate variability and skin conductance level in relation to the observation scales: longitudinal measurements

See Fig. 2 for the mean HRV (MFn.u., HFn.u., and MF/HF) and the mean SCL for each score on the observation scales. Relations were found between the scores on the observational scales with the HRV and the SCL.

The MFn.u. linearly increased with LoC ($F(1.86) = 24.51$, $P < 0.001$), and with scores on the WNSSP ($F(1.86) = 26.47$, $P < 0.001$), whereas it decreased linearly with scores on the DRS ($F(1.86) = 21.99$, $P < 0.001$). Together, these data show that a linear increase in the MFn.u. was associated with better scores on all observation scales.

The HFn.u showed opposite effects to the MFn.u. With higher LoC and higher scores on the WNSSP, the HFn.u. decreased linearly respectively, $F(1.86) = 22.36$, $P < 0.001$; $F(1.86) = 24.00$, $P < 0.001$. A linear increase of the HFn.u. was observed with decreasing scores on the DRS ($F(1.86) = 19.90$, $P < 0.001$). In sum, a decrease in the HFn.u. was related to better scores on all observation scales.

The MF/HF ratio showed resembling patterns as were found for MFn.u. It showed positive linear trends related to increasing scores on LoC and WNSSP, and decreasing scores on the DRS (respectively, $F(1.86) = 13.41$, $P < 0.001$; $F(1.86) = 13.43$, $P < 0.001$; $F(1.86) = 11.74$, $P < 0.001$). In sum, a linear increase in MF/HF was found with better scores on all observation scales.

SCL also increased with better scores on the observation scales. A positive linear trend was found for SCL related to an increasing LoC ($F(1.84) = 12.66$, $P < 0.001$), to increasing scores on the WNSSP ($F(1.84) = 7.99$, $P < 0.01$), and to decreasing scores on the DRS ($F(1.84) = 8.51$, $P < 0.01$).

Taken together, these results show that the sympathetic activity (SCL, and to a lesser extent MFn.u.) increased with recovery, whereas parasympathetic activity (HFn.u.) decreased with recovery. As a consequence, sympathovagal balance (MF/HF) increased with recovery to consciousness.

Table 3
Distribution of levels of consciousness over the ordinal physiological measurements

Measure-ments	Patients															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	6	6	4	2	3	2	2	2	2	6	1	5	2	5	5	3
2		6	3		3	4	3	3	4	6	1	6	3	7	6	2
3	8	7	5	6	2	3	2	3	5	7	2	6	2	8	5	2
4		7	6	7	5	5	4	3	6		2	6	2		6	2
5		7			7	4	3	3	7		2		2		6	
6		7			7	5	4	4	8				2		7	2
7		7				6	5	5				7	3		7	4
8		7				6	5	5				6	2		6	2
9		7				7	5					7			7	2
10						7	6									
12							8	5								
13							7									
14							8									
Total Ms	2	4	7	3	6	10	13	13	6	10	5	9	8	3	9	8

Table 4
Descriptive statistics of the first and the last measurement, and of the long-term outcome

	First measurement			Last measurement			Long-term outcome	
	LoC	DRS	WNSSP	LoC	DRS	WNSSP	DRScat	GOSE
Mean (SD)	3.5 (1.8)	22.06 (4.2)	34.3 (25.7)	6.2 (2.2)	13.8 (6.4)	89.1 (40.6)	5 (1.8)	3.6 (1.4)
Median	3	23.5	24.5	7	11	109.5	6	3.5
Min–Max	1–6	14–29	0–91	2–8	6–24	8–113	1–7	1–5

LoC, level of consciousness; DRS, disability rating scale; WNSSP, western neuro sensory stimulation profile; DRScat, disability rating scale categories; GOSE, Glasgow outcome scale extended.

3.4. Immediate effect of administering WNSSP

Differences in autonomic reactivity between the first and the second resting period were found for all measurements. Compared to the first resting period, during the second resting period the MFn.u. was higher ($F(1.15)=14.18$, $P=0.002$) and the HFn.u. was lower ($F(1.15)=14.68$, $P<0.002$). In addition, the MF/HF ratio and the SCL were higher in the second resting period ($F(1.15)=6.32$, $P=0.02$; $F(1,14)=7.08$, $P=0.02$).

These results show that after the stimulation period, sympathetic activity was higher and the parasympathetic activity was lower, compared to the situation before the stimulation period. A longitudinal trend was only found for the sympathovagal balance over measurements ($F(1.85)=4.93$, $P<0.05$). For other variables no trends were found: the differences between the two resting periods remained equal for each (longitudinal) measurement ($P_s>0.05$), as well as during recovery ($P_s>0.05$).

4. Discussion

The activity of the ANS in adolescents with sTBI in the post-acute phase was examined during sensory stimulation. The changes in the reactivity of the ANS during each administration of the WNSSP were examined, as well as the longitudinal changes related to recovery in the post-acute phase. We related the longitudinal changes in ANS activity to the recovery of consciousness. The results of our study are clear-cut: changes in autonomic reactivity during recovery were related to recovery to consciousness. Fig. 2 demonstrates a strong monotonous relationship which appears linear.

The reactivity of the sympathetic branch of the ANS is best reflected in the SCL (Boucsein, 1992), which showed a cubic trend over time, and an increase when related to the observational scales. It appears that sympathetic reactivity increases with recovery from a vegetative state to consciousness, and with better performance on the DRS and the WNSSP.

The spectral analysis of HRV provides insight into the sympathovagal balance, as well as into the health and functioning of the ANS. The MF is largely attributable to sympathetic nervous activity (Akselrod et al., 1981; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Therefore, the increase of the MFn.u. might reflect an increased sympathetic reactivity. Since the HF is indicative of vagal outflow (Akselrod et al., 1981; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), the decrease of the HFn.u. component of HRV can be interpreted as a sign of decreased parasympathetic
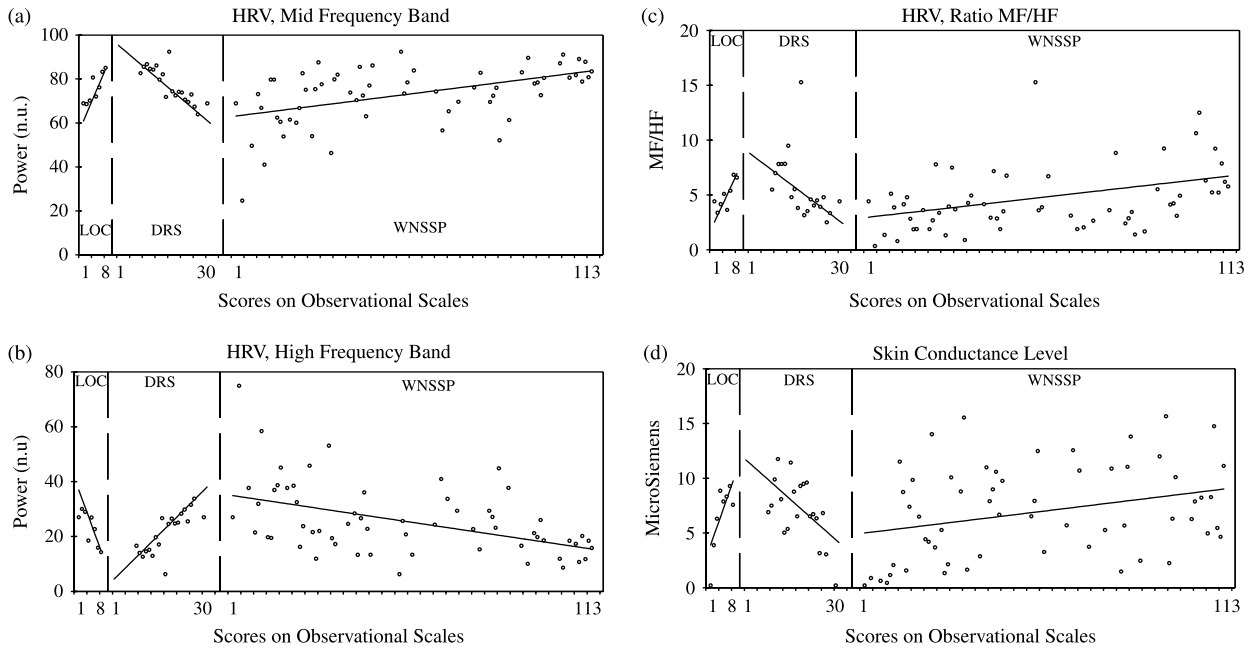
Fig. 2. Longitudinal measurements for observation scales: mean scores on 'Level of Consciousness' (LOC), disability rating scale (DRS), and western neuro sensory stimulation profile (WNSSP), for: (a) Mid frequency band in normalised units. (b) High frequency band in normalised units. (c) Ratio MF/HF: level of consciousness. (d) Skin conductance level.

reactivity. As a result, a clear increase in sympathovagal balance (MF/HF) by means of reciprocal sympathetic activation was found (Fig. 3).

We conclude that sympathetic reactivity increased and parasympathetic reactivity decreased during recovery from a vegetative state to consciousness in adolescents, following sTBI. Our results in the post-acute phase resemble and extend previous results in the acute phase. For instance, Hildebrandt et al. (1998) found the same pattern in adult patients recovering from a comatose state (defined by a GCS of 8–10) within the acute phase (Hildebrandt et al., 1998). In these patients, MF, SCL, and LF/HF were higher in patients scoring better on the GCS. In addition, recovery in the acute phase is related to an increase in SCL, and hence to increased sympathetic reactivity (Turkstra, 1995). It appears that the recovery of ANS function and integrity is an important first step for recovery to consciousness. The DRS scores reveal that most of the conscious patients were still severely disabled at the end of the rehabilitation programme, and even 3–4 years after their injury.

A second unequivocal result from the present study was that ANS reactivity was different after the administration of the WNSSP, and that this difference neither changed over time nor with recovery to consciousness. This could be an important finding in the light of the current paucity of methods for an accurate diagnosis of a non-responsive patient: even when no overt behaviour can be observed, physiological reactivity to environmental stimulation could already be detected. Some form of sensory information processing might still be present. The changes in autonomic activity within each measurement resembled the changes in

autonomic activity over time and the changes related to recovery. However, only the patients who did recover, and therefore shifted to higher scores on LoC and the WNSSP, and lower scores on the DRS, showed the longitudinal changes in ANS reactivity. Of course, because there was no control group, it remains uncertain whether these changes in ANS reactivity during recovery were caused by the stimulation administered, or were the result of spontaneous recovery.

During the first period of recovery the parasympathetic part of the ANS was highly activated, accompanied by a very low activity of the sympathetic part. With recovery to consciousness patients became more aroused, as a result of decreasing parasympathetic and increasing sympathetic activity. This pattern of ANS reactivity supports the notion of Plum and Posner (1980): preservation of arousal is required for recovery to consciousness. The increased arousal to environmental stimulation found in our study was not due to increased motor responses. The later vegetative states (LoC 3 and 4) and the transitional state (LoC 5) are characterised, respectively, by involuntarily movements, and agitated, emotional behaviour. If the results found were caused by purely motor responses we would have found a quadratic trend with increased arousal followed by a decrease in the later LoCs. The hypofunction of the ANS in the acute phase after TBI might be associated with the dysfunction of the reticular activation system (Lehrer et al., 1989). The hyperfunction of the ANS when recovering from sTBI might be associated with affected cortical inhibition centres (Lehrer et al., 1989; Plum and Posner, 1980), and thereby releasing reticular and
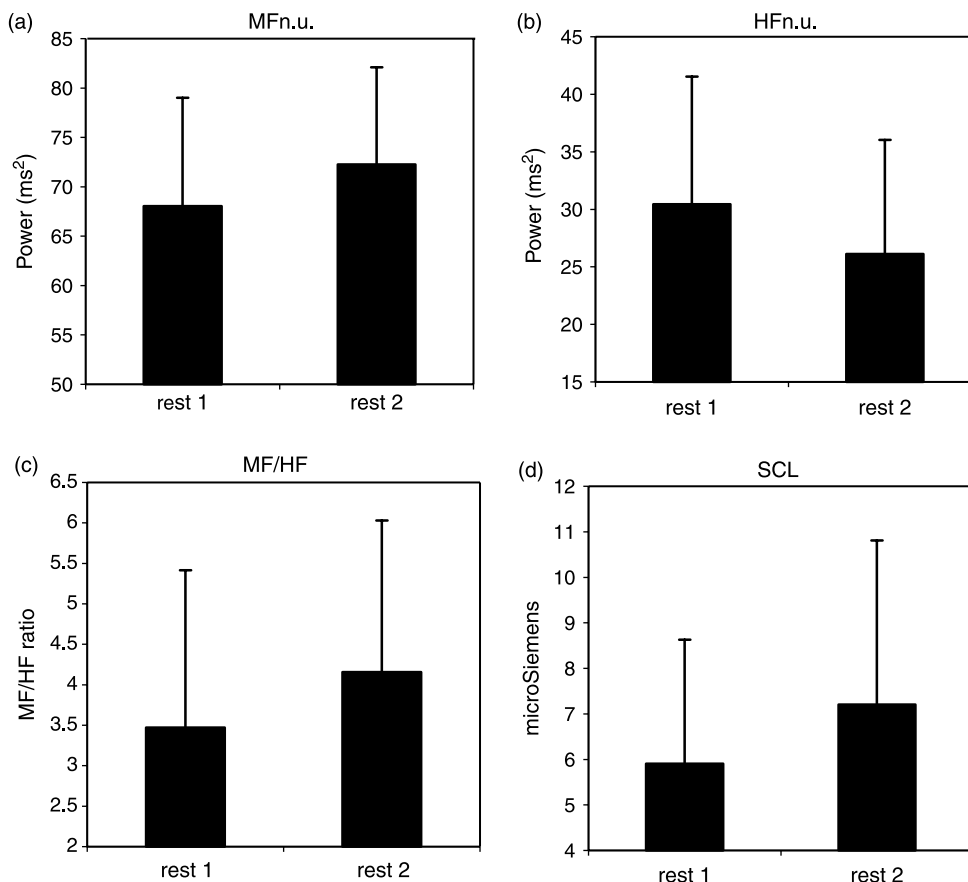
Fig. 3. Effects of administering the WNSSP, a comparison between the pre and post rest periods (means and standard deviations): (a) Mid frequency band in normalised units. (b) High frequency band in normalised units. (c) Ratio MF/HF. (d) Skin conductance level.

autonomic networks from normal tonic cortical inhibition. For instance higher skin conductance levels but impaired performances were found in TBI patients in comparison to a healthy control group with cognitive tasks (Plum and Posner, 1980).

Recovery of consciousness depends upon the brain's capacity to repair. In most of the patients involved in our study this capacity appeared to be present. It could be that damage to 'higher' cortical structures that regulate and control the ANS (such as the anterior cingulate cortex, and the insula), and medial temporal lobe structures (such as the amygdala and hippocampus) (Critchley et al., 2002, 2003; Matthews et al., 2004), caused the ANS to be dysfunctional after severe traumatic brain damage. In addition, abnormalities in cholinergic and adrenergic functioning might lead to a dysfunction of the ANS. The neurotransmitters involved in autonomic activity have been suggested to be involved in (cognitive) deficits after sTBI (Lyeth, 2001; Salmond et al., 2005). It is possible that the changes in ANS during recovery to consciousness were due to the recovery of these higher cortical structures controlling the ANS, and nuclei releasing the neurotransmitters (e.g. the locus coeruleus and the basal nucleus of Meynert) involved in the ANS. Furthermore, an overactivity of the sympathetic branch

could still exist, caused by a disturbed inhibition regulation of cortical structures.

To draw conclusions on this issue and to rule out the possibility of an overactivity of the sympathetic branch during recovery in the post-acute phase, patients need to be compared with a healthy norm group.

Differences in brain activity in response to environmental stimuli in vegetative and minimally conscious patients in the acute phase have been reported previously (Boly et al., 2004; Kotchoubey et al., 2002, 2005; Laureys et al., 1999, 2000, 2004a; Schiff et al., 2002). In our study, differences within ANS reactivity were shown both within the vegetative and the minimally conscious state, during recovery to consciousness. These differences appear to be quantitative rather than qualitative. Viewed from the perspective of ANS reactivity, in the post-acute phase recovery appears to be gradual and continuous. This is interesting in light of the discussions about the various stages of recovery (Andrews, 1996; Giacino and Whyte, 2005). The 8 sublevels of consciousness that we used could provide additional information in research concerning direct brain activity, especially when examining the shift from a vegetative into a minimally conscious state. The current research results justify the question whether VS and MCS

represent true 'states' or levels. It is plausible that a continuum exists extending from the deepest level of coma to the highest level of consciousness. When levels need to be used it is advisable to divide the VS and MCS into sublevels. These two diagnoses are definitely not static ones, and recovery takes place with slow steps. Sometimes even a slight regression can be seen during the recovery process. A longitudinal within subject design using ERPs, fMRI or PET might provide more insight into both the structural and functional changes involved in recovery to consciousness.

Our findings could be helpful during the rehabilitation of patients suffering sTBI. The ANS activity after a stimulation period does not change with recovery. However, the ANS reactivity during stimulation changes over time for patients who recover. This might be helpful for identifying the patients who are recovering: stimulation appears to directly arouse them more and more over time.

Recognising the possibility for recovery is important for rehabilitation management, as well as for filling the gaps in our understanding of recovery patterns in the post-acute phase. In a preliminary experiment, we found differences in phasic skin conductance and heart rate responses between patients who did or did not recover to consciousness (Wijnen et al., 2005). The reactivity of the ANS to the environment may therefore be a promising topic in future research focusing on early recognition of recovery possibilities.

In addition, early recognition of under- or over-activity within the different branches of the ANS could lead to adjustments in the treatment of sTBI patients. The longitudinal measurements over an extended period of time allowed us to follow the individual patients during their stages of recovery, albeit with a limited number of adolescent patients. Future work involving larger groups of patients of different age categories will be aimed at confirming and extending the present conclusions.

Acknowledgements

Appendix A
Stimulation protocol according to the WNSSP: mean duration and standard deviation of the rest and the stimulation periods for the total patient group

	Stimuli	Mean duration (SD)
Rest period	None	181.4 (14.3)
Auditory stimulation		257.1 (114.5)
	Sound (bell)	
	Verbal commands	
	Shake my hand	
	Open/close mouth	
	Stick out tongue	
	Open/close eyes	
	Raise eyebrows	
	Move (body part)	
Visual stimulation		319.1 (121.5)
	Mirror following (horizontal and vertical tracking)	
	Picture following (horizontal and vertical tracking)	
	Person following (horizontal tracking)	
	Visual commands	
	Shake my hand	
	Open/close mouth	
	Stick out tongue	
	Open/close eyes	
	Raise eyebrows	
	Move (body part)	
Tactile stimulation		143.3 (80.1)
	Touching body	
	Touching mouth with eartip	
	Object manipulation	
	Spoon	
	Comb	
	Pencil	
Olfactory stimulation	Four different odours	188.3 (47.6)
Rest period	None	180.8 (5.6)

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