Instrumental Methods in the Diagnostics of Locked-in Syndrome

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Number of words: abstract 122; text: 6110 words; 21 pages; 6 Figures

Key words: locked-in versus vegetative syndrome, consciousness, MRI, EEG, CCT, vigilance.
Abstract

The locked-in syndrome (LiS) is typically characterized by a paralysis of almost all body muscles combined with intact cognitive functions. In practice, there are often additional brain damages besides the one directly causing LiS. These damages can lead to cognitive impairment, which substantially complicates the diagnosis of LiS. At the level of behavior, therefore, the disease can be confused with akinetic mutism, vegetative state (syn. unresponsive wakefulness state) and some other conditions. Using instrumental methods in addition to behavioral diagnostics helps to avoid diagnostic errors and to improve prognosis of rehabilitation of such patients. These methods, which include measurements of brain electric or magnetic fields, electrical potential of muscles, blood flow and oxygen consumption in the brain, are reviewed in this paper.
Introduction

The term “locked-in” describes the pathologic state in which movement abilities completely (or almost completely) disappear whereas sensory and cognitive abilities still persist. The patient is behaviorally characterized as quadriplegic or quadriparetic, aphone or hypophone and should show sustained eye opening (if ptosis is ruled out as a complicating factor) and vertical or lateral eye movements (ACRM, 1995). This condition can result from different neurological diseases, for instance an acute inflammation of the efferent fibers in the peripheral nervous system caused by a Guillain-Barré syndrome, or the degeneration of motor neurons in the cortex and the ventral horn of the spinal cord in the amyotrophic lateral sclerosis. But the most important and most investigated cause is the injury of the anterior part of the Pons varolii. This site contains the main descending tracts that conduct motor impulses from the cortex to the brain stem and spinal cord. Damage in this part of the brain can lead to the ventral pontine syndrome, the locked-in syndrome (LiS) in the narrow sense. Such an acute brain injury is most frequently caused by a thrombosis in the basilar artery or by less common diseases (e.g., local cerebral hemorrhage, encephalitis, or trauma) (Patterson and Grabois, 1986). This acute emergency case is one of the most urgent situations in Neurology, with a lethality of 90% which needs immediate diagnostic and therapeutic intervention. An angiography and (if the thrombosis is diagnosed) a following lysis is initiated if at least two of the following symptoms are observed: (1) progressing decrease of consciousness; (2) disturbance of eye movements (oculomotorius impairment, acute asymmetry of the eye bulb, nystagm or anisocoria), (3) hemi- or tetraparesis or hypasthesia, (4) vertigo, ataxia or dysarthria (Ringelstein and Nabavi, 2007). For the chronic situation of a patient who is obviously not moving and might be unconscious for days, we need optimal and standardized diagnostic procedures to confirm the diagnosis.
Although the definition of the LiS is unequivocal, the diagnosis can be difficult. As already said, one of the most important criteria is the intact cognitive function despite the severe motor impairment. But in fact there is almost no LiS patient who does not have any dysfunctions in sensation, perception or thinking, at least in the acute phase of the disease. That is why the diagnosis of LiS does not aim to rule out all cognitive disorders (otherwise this diagnosis would never be set), but to identify them as temporary phenomena or secondary consequences in contrast to the completely different syndromes such as the vegetative state (Jennett, 2002) or akinetic mutism (Ackermann and Ziegler, 1995), in which cognitive impairment comes to the fore. As already mentioned, both can easily be mistaken with LiS (Andrews et al., 1996; Schnakers and Zasler, 2007), although their clinical picture is largely determined, not by motor disorders, but by disorders of consciousness.

Typically, three different stages of LiS can be distinguished (Patterson and Grabois, 1986): the “complete” or “total” LiS, in which all motor functions are lost; the classical LiS, in which the patient remains in possession of a single voluntary movement (most frequently a movement innervated by the oculomotor nerve, i.e., a vertical eye movement) leaving the only possibility to communicate; and the partial LiS, in which very few movements (but more than one; e.g., small finger movements) are still under the patient’s voluntary control.

There are several reasons of why cognitive disorders occur during LiS. The lesion is not always localized exactly in the anterior part of the pons, as it is shown in textbooks, but can also spread out in the posterior or rostral direction. Thus sensory pathways or a portion of the reticular formation, regulating vigilance and alertness, can also be affected. Moreover, in exceptional cases LiS can result from an extrapontine brain injury like bilateral damage to the white matter of both cerebral hemispheres (Chia, 1984), or the cerebral peduncles (Chia, 1991; Park et al., 1997).
The acute phase of stroke is often accompanied by swelling of the surrounding brain tissue, which can result in the loss of function of brain areas not directly affected by the infarction. This secondary dysfunction can initially be more severe than the primary cell necrosis due to the local hypoxia. Furthermore, LiS can be a manifestation of a basilar vasospasm as a secondary consequence of a subarachnoidal hemorrhage (Lacroix et al., 2012).

Furthermore, it still remains unknown how the sudden and profound paralysis can cause secondary malfunction of sensation, perception or cognition. The pontine catastrophe results in the abrupt loss of all the information about the position and the vectors of forces in muscles, joints and tendons, naturally available at each moment of our life.

Sometimes, several infarctions simultaneously appear in several brain areas. One of them, localized in the pons, yields a LiS, while additional lesions may lead to parallel deficits of other brain functions. The same pattern can occur in a traumatic LiS (Schnakers et al., 2008). The clinical diagnosis of those extra deficits is extremely complicated or even impossible due to the massive impairment related the LiS.

An informative, functional diagnosis of LiS should, at least in an ideal case, include not only the present status of the patients but also a prognosis of the disease. This is especially important for the partial LiS, in which minimal but noticeable abilities to move and therefore to communicate besides the vertical eye movements persist. The correct assessment of cognitive disorders in LiS is of particular importance because these dysfunctions, being secondary as compared with the motor paralysis, can be corrected in the first line in the course of rehabilitation. This cognitive rehabilitation can then form a basis for the following motor rehabilitation. In contrast, in total LiS, in which no motor function remains, the main problem
is the differential diagnosis from severe disorders of consciousness such as coma or vegetative state.

All the factors mentioned above – atypical location of the lesion or secondary lesions in the acute state; additional injuries in remote brain regions, undiagnosed on the background of LiS; functional disorders of consciousness caused by the sudden loss of the motor function with the corresponding lack of the proprioceptive feedback; problems in diagnosis and communication with patients suffering from complete LiS; and finally the necessity for a dynamic-functional prognosis and not only a static diagnosis – all this explains why clinical-behavioral methods may not be sufficient to describe a patient with LiS.

In this chapter important methods used in the diagnostics of LiS and recent findings of latest methodology are considered. After this overview we shall summarize how far these methods can help to answer the principal questions of the diagnosis of LiS or to overcome the main difficulties of this diagnosis. Finally, we shall try to imagine how an ideal functional diagnosis of LiS should look like, what methods should be developed and what obstacles can be met.

We concentrate on methods having yielded results specifically related to LiS. These are methods capable of demonstrating structural changes in the brain, such as cranial computer tomography (CCT) or magnetic resonance imaging (MRI) and those which can visualize the vascularization of the brain (angiography). Functional methods directly measure functional activity of the nervous tissue, such as electroencephalography (EEG), electromyography (EMG) and magnetoencephalography (MEG). Other techniques record changes in the structure of the brain, in blood flow (positron emission tomography, O$_{15}$PET), in brain metabolism (Glucose-PET) or blood oxygenation (functional MRI = fMRI).
Of course, there are many other methods that may be helpful in the diagnostics of the LiS. Laboratory tests, for example, can provide useful information about an inflammation accompanying brain injury. Virtually all medical instrumental methods might contribute for the diagnosis or decision of the best therapy of LiS. However, these methods are unspecific in relation to the nature of the syndrome. That is why these methods are not discussed in this paper.

**Electrophysiology**

*Rhythmic electrical activity of the brain*

Electroencephalography (EEG) refers to rhythmic oscillations of brain potentials, recorded from the scalp. Both EEG and evoked potentials (see below) measure functional activity of cortical neurons directly. Their advantages are a high temporal resolution (i.e., processes are recorded on-line), low costs, easy recording and data analysis, as well as the ability to be applied in virtually all patients. In contrast, the main disadvantage is a poor spatial resolution. Furthermore, the EEG is limited to the measurement of superficial cortical activity, whereas the activity of subjacent sulci and subcortical brain structures is only indirectly reflected in EEG waves.

For the diagnostics of LiS the EEG has been used systematically since the 1950ies, even before LiS was described as a nosological entity (Baldy-Moulinier et al., 1977; Hawkes and Bryan-Smyth, 1974; Markand, 1976; Watson and Adams, 1951). The typical results of these and following studies were a normal oscillation pattern with well-defined alpha-waves and distinct reactions to sensory stimulation (mostly as alpha-blockade with an increase of rhythmic beta-waves) and to cognitive load (Bauer et al., 1982; Chia, 1991; Kamondi and Szirmai, 1993; Rusinov V.S., 1990; Towle et al., 1989). More recent studies employing more sophisticated EEG techniques (e.g., Low-Resolution Electromagnetic Tomography: (Pascual-
Marqui et al., 2002) reveal, however, moderate EEG slowing that may probably be regarded as a consequence of the altered general condition of the patient suffering from the severe immobility rather than the direct effect of LiS (Babiloni et al., 2010). A deceleration of the basic rhythm (Towle et al., 1989) and an attenuated coherence between the single sites (Rusinov V.S., 1990) have also been reported. Interestingly, sleep wave patterns seem to be unaffected in LiS (Oksenberg et al., 1991; Rotenberg and Kobrin, 1985).

The remaining reactivity to stimulation seen in the EEG is regarded as an indication of conscious perception of the stimulus. However, this seems to be not completely true (Brenner, 2005) since EEG-changes to external stimuli do only prove the intact function of the most important brain loops between cortex and thalamus responsible for the maintenance of the waking state, habituation and orienting response. In an interesting but little known work Jacome & Morilla-Pastor (Jacome and Morilla-Pastor, 1990) reported three LiS patients, who could still communicate ‘yes’ and ‘no’ with a wink but had no EEG-activation change to external stimuli. Güting and colleagues (Güting et al., 1996) also observed non-reactive EEG in three of their five patients with LiS. Therefore, it can be concluded that neither reactive changes in EEG rhythm strictly prove conscious perception, nor the lack of such reactions is an argument for a severe disorder of consciousness.

Another possibility would be to instruct the patient to imagine movements of hand or feet. In healthy subjects this imagery task shows a reduction of power of µ- (7-13 Hz) and β- (13-30 Hz) frequency bands over the scalp positions of the appropriate motor cortex (for right hand movement imagery for instance C3) of the EEG during motor imagery (Kubler et al., 2005). In a recent two-center study this bedside test identified three of 16 patients with the diagnosis of vegetative state who were capable to modulate their EEG according to motor imagery (Cruse et al., 2011). The authors used a linear support vector machine classifier, which was trained with filtered and artifact rejected data of a 25 channel EEG to classify trials
of either hand or toe motor imagery. Obviously, the patients were misdiagnosed as vegetative state, while in fact, they had LiS.

**Evoked and event-related potentials**

Evoked potentials (EP) are variations in EEG wave pattern time-locked to a stimulus. Earlier (up to 20-50 ms after stimulus) and later (up to 500-800 ms) components of EP are usually distinguished. The early potentials represent the activity of sensory pathways from the sensory input to cortical areas. Their amplitude and latency provide information about the occurrence and the velocity of information transmission from the sensory presentation to the central nervous system. The late potentials refer to further processing of the stimulus predominantly in the cortex and can therefore be used to detect higher cognitive functions (Kotchoubey et al., 2002) and consciousness (Kotchoubey, 2005, 2007). Another common distinction is that “EP” refers to the early components whereas the later components are termed “event-related potentials” (ERP).

Early EPs are commonly used in clinical neurology. One might assume that in LiS these components should be normal because the typical lesions of the anterior pons should only affect the descending motor tracts while the ascending sensory tracts, running posterior to the lesion site, remain unaffected. Contrary to this expectation, however, abnormalities of auditory or somatosensory EPs are a frequent though instable finding in LiS examinations (Chia, 1991; Ferbert et al., 1988; Krieger et al., 1993; Landi et al., 1994; Towle et al., 1989). The frequency of abnormal auditory EPs concurs with the fact that a vertebrobasilar occlusion, which usually leads to LiS, sometimes becomes evident as a sudden hearing disorder: Huang (Huang et al., 1993) observed this symptom in seven out of 503 patients with vertebrobasilar insufficiency. Six of these seven had severe abnormal or lacking auditory EPs, and four of them developed a LiS later on.
Another reason for abnormal EPs in LiS might be a secondary swelling in the acute state (Krieger et al, 1993). In those patients, who were in the chronic LiS, and who nevertheless showed different abnormal EPs (Gutling et al., 1996; Towle et al., 1989), the damage due to an infarction of the basilar artery is obviously not restricted to the anterior pons but spreads to adjacent regions. Figure 1 shows the association between the morphology of the pons injury and the EP pattern of a LiS patient. Both factors (i.e., the size of the lesion and the acute edema) can cause the apparently paradoxical finding of Krieger et al. (Krieger et al., 1993) that the level of abnormality of sensory pathways correlates with the following restoration of motor functions.

----Insert Figure 1 about here-----

Later cortical components (event-related potentials, ERP) of LiS patients were less frequently described. The most common of these components is a late positive P3 wave with a latency of 300 - 400 ms. This wave is typically elicited in healthy individuals if two stimuli with different occurrence (e.g., 20% and 80%) are presented as a brain response to the rare stimulus (Donchin, 1981) (see Figure 2A).

----Insert Figure 2 about here-----

This characteristic positive wave is also observed after presentation of stimuli with subjective importance. For instance, it appears during memory tasks on trials with correct recognition of familiar objects (but not on trials with correct rejection of unfamiliar objects; (Onofrj et al., 1997)). According to the current knowledge, the P3 reflects the ability of the brain to differentiate stimuli and objects and to respond properly and selectively to significant events in the environment. Onofrj and colleagues (Onofrj et al., 1997) performed the two experiments described above (i.e., the presentation of rare versus frequent stimulus, and a memory recognition task) with four LiS patients. The authors showed a distinct P3 in both
experiments in each patient already one week after lesion. However, one of these patients had no detectible P3 in the first days of the disease. An additional patient, who had nicely reproducible EPs, showed no late ERP even weeks after the pontine infarction (Onofrj et al., 1996). These late components returned after two months and were interpreted by the authors as an indication for the relocation of the cortical function in other adjacent regions.

Another late ERP wave useful for assessment of cognitive functions is the N400, which is a typical brain response to violation in a meaningful context (review (Kutas and Federmeier, 2011) see Fig. 3B). We found an N400 response in two LiS patients demonstrating their ability to semantic differentiation.

Myography und motor evoked potentials (MEP)

Electromyography (EMG) is a technique to measure and record electrical activity of skeletal muscles at rest and at contraction. In LiS patients the most frequently recorded potentials are the so-called motor evoked potential (MEP). While sensory evoked potentials are recorded from the scalp in response to external stimuli, the MEPs are recorded from muscles in response to transcranial magnetic stimulation (TMS) of the motor cortex. Talking in the previous section about EPs we expected that they should remain intact in a typical LiS patient. However, this was, generally, not the case. In contrast, we might expect that the MEPs should completely disappear, because the syndrome results from a complete disruption of all or almost all descending motor pathways. This is mostly true but not always (Facco et al., 1989; Landi et al., 1994). Indeed, normal MEPs are never recorded in LiS patients but weak oscillations with prolonged latency can be recognized, indicating that parts of the corticospinal tract are preserved (Cincotta et al., 1999; Facco et al., 1989). Measurable MEPs can predict a good motor prognosis (Bassetti et al., 1994). Interestingly, residues of voluntary movement control can be detected if active motor imagination of the patient leads to
normalization of the MEP (i.e., to the increasing amplitude and decreasing latency) (Cincotta et al., 1999). This observation shows that the routinely performed tests may underestimate the abilities of LiS patients. More active tests including task instructions may therefore indicate a possible direction of rehabilitative interventions.

**Morphology of the brain and its vessels**

*Angiography*

The most common etiology of a LiS is the thrombosis of the basilar artery. An angiography provides important information about (1) the presence of an occlusion, (2) its localization, (3) the morphology of the blood vessels which may have caused the thrombosis and (4) the present blood vessel pathology. In most cases the angiographic findings show a typical interruption of blood flow in the basilar artery (Ferbert et al., 1988; Malm et al., 1999). A bilateral thrombosis of the vertebral arteries, prior to the site where they join to form the basilar artery, is extremely rare (Cabezudo et al., 1986). An incomplete occlusion can lead to a transient LiS, which can also be diagnosed best by an angiography (Krieger et al., 1990). Figure 3 shows angiographic data as well as MRI scans of a LiS patient.

----Insert Figure 3 about here-----

Angiography, in addition, can shed light on morphological abnormalities of the vessels predisposing to a thrombosis, e.g., a pathologically expanded basilar artery (Al-Sardar and Grabau, 2002) or the high entry point of the vertebral artery into the transverse foramina ascending the cervical spine. Normally, the artery enters the transverse foramen of the sixth cervical vertebra (C6), in rare cases C5 or even C4. The higher is the entrance, the higher the risk for a circulatory disorder, which might be caused by an impaired blood flow or a
developing embolus. In two case reports a development of LiS was observed in patients whose vertebral artery entered the spinal cord at C3 (Fujiyama et al., 1994; Jackson et al., 2000). After a traumatic brain injury angiographic data can help to differentiate between a coma and a LiS due to a traumatic injury of the basilar artery (Fox and Lavin, 1991; Odabasi et al., 1998). This kind of injury may be rare because the artery is protected from mechanic exposure. On the other hand, if the spinal column is pathologically deformed in the neck area the bone protection can become a risk factor. In this case even a microtrauma (e.g., during chiropractic manipulation) might result in a disruption of the blood flow and to LiS (Krieger et al., 1990). It has to be mentioned here that transcranial (color-coded) sonography (TCCS) of the basilar artery has the potential to diagnose intracranial vertebrobasilar arterial disorders, too (Toyoda et al., 2005). It is less invasive than angiography and can detect the direction of blood flow.

Cranial computer tomography (CCT)

Brain imaging via x-ray computed tomography has been successfully used in the diagnosis of brain stem lesions for several decades (Liu et al., 1983). Usually, the CCT in LiS shows an area of low tissue density in the anterior pons, sometimes with an extension to the tegmentum, the mesencephalon or the cerebellar peduncles (see Figure 4). The method is also important for visual detection of additional brain injuries which can be clinically masked by the severe symptoms of LiS (Facco et al., 1989; Onofrj et al., 1997). Thajeb (Thajeb et al., 1993) examined 22 patients with severe occlusive defects in the area of the basilar artery using both CCT and angiography. CCT scans in this acute phase of infarction showed no pathologic changes because a hypodensity only slowly develops during the first two weeks after the infarction. Two of the 22 patients showed no visible lesion until the 21st day post
infarction although they had clear clinical symptoms and positive angiographic findings indicating the acute impairment of circulation in the brain stem.

These two patients showed a good recovery of the motor abilities in their follow up. Six of the 20 patients with positive CCT scans showed no injury of pons or mesencephalon but single hypodense regions in subcortical structures. These patients also had a relatively benign course. In contrast, in four patients a complete involvement of the area supplied by the basilar artery including brainstem, cerebellum, the temporal and occipital lobe was observed. All of them died despite of active anticoagulation therapy. LiS developed over time in those patients who showed circumscribed lesions in the upper brainstem, although other structures as the thalamus or the cerebellum might also be affected (Thajeb et al., 1993).

*Magnetic resonance imaging (MRI)*

MRI is the most sensitive method in the diagnosis of structural disorders in LiS. It allows description of the exact localization of the pontine lesion, typical for the syndrome, as well as additional accompanying lesions (Benitez et al., 1994; Dollfus et al., 1990; Onofrj et al., 1997). The MRI is especially important for atypical forms of injury such as a non-vascular LiS due to mechanic trauma or intoxication (Patterson and Grabois, 1986). Thus Blumbergs et al. (Blumbergs et al., 1991) reported a patient who suffered from a traumatic avulsion of the pons from the medulla oblongata diagnosed via MRI imaging. In a case of disseminated encephalomyelitis, MRI not only helped to diagnose the causing factors but also provided information important for the assessment of the prognosis and the development of the LiS (Axer et al., 2005). In two other cases this technique helped to detect an acute demyelination in the area of the upper anterior pons and the lower mesencephalon due to hepatic insufficiency (Martin and Young, 1995; Morlan et al., 1990).
MRI can be extremely important in the case of an unusual localization of brain injury, e.g., below the pons (Latronico et al., 1993) or bilaterally in the mesencephalon (Chia, 1991), in a LiS with an atypical clinical course (Wali, 1993) or atypical etiology (Luxenberg et al., 2009). Furthermore, MRI can often reveal a distinct brain lesion also in LiS patients with negative CCT scans (Durkin, 2003; Martin and Young, 1995). The faster development of characteristic changes in MRI as compared to CCT allows an intervention within the critical timeframe (Zaidat et al., 2005). Nevertheless, MRI scans may also sometimes show no pathological changes during the first days of the disease and repeated examinations are necessary. In single cases of brain stem infarct even repeated MRI scans showed no pathological changes (two of 105 patients with brainstem or cerebellar stroke; (Malm et al., 1999)). Even more promising are MRI techniques, which are optimized to visualize and quantify lesions of the white matter such as diffusion weighted MRI (DWI; (Samaniego et al., 2009)). In acute basal artery occlusion DWI has been demonstrated to be the strongest predictor of outcome (NIHSS-score and Coma duration; (Cho et al., 2009)). However, group data for using diffusion imaging in LIS patients in the chronic state are not yet available.

Overall, MRI is a very promising tool for diagnosis of LiS since it can combine the specification of lesion location with high spatial accuracy, the specification of white matter lesions by diffusion imaging and the verification of a thrombosis of the basilar artery by MR-angiography (Mortimer et al., 2011). Additionally, it is capable for functional imaging, which will be described in the next chapter. Examples of MRI scans of LiS patients are presented in Figure 5.

--- Insert Figure 5 about here ----

**Functional Imaging**

*Positron Emission Tomography (PET)*
PET offers a highly reproducible method for measuring glucose consumption or blood flow in the brain. Both can be used for evaluation of resting state, highly characteristic for the level of consciousness (Laureys et al., 2004). PET has been acquired quite early in the differentiation between VS and LiS (Levy et al., 1987) and has shown high metabolic levels in the brains of patients in a locked-in syndrome compared to those in a vegetative state. Moreover, no supratentorial cortical areas show a significantly lower metabolism in patients in a locked-in syndrome when compared with healthy controls (Laureys et al., 2004). Especially the H$_2^{15}$O-PET seems to be promising for the measurement of brain activity with respect to possible impairment of consciousness. Most recent data show that using support vector machine algorithms in fluorodeoxyglucose PET considerably improves the differentiation between VS and LIS patients (Phillips et al., 2010).

fMRI (functional magnetic resonance imaging)

In contrast to the morphologic MRI tomography depicted above, fMRI measures the „blood oxygenation level-dependent“ (BOLD) signal and thus, like PET, delivers information about the metabolic activity of local brain regions. During the last decade it is progressively used to differentiate between LiS patients and patients with severe disorders of consciousness. Owen et al. (Owen et al., 2006) applied a mental imaginary task which allowed them to find out that a female patient wrongly diagnosed as “vegetative state” was, in fact, in LiS. A normal pattern of BOLD-responses in this task was also reported by Bardin et al. (Bardin et al., 2011) in a typical LiS patient. The same imagery task was applied in a large group of patients with the diagnoses MCS or VS (Monti et al., 2010). In five of the 54 patients significantly increased modulation in the supplementary motor area for motor imagery in comparison to spatial imagery was observed. Four patients showed increased hippocampal activation during spatial imagery than during motor imagery. This method could also be utilized for communication via BOLD-response using yes for motor imagery (SMA) and no
for spatial imagery (hippocampus), which might be performed by means of real-time fMRI (deCharms, 2008).

All these patients who were diagnosed as VS but were, in fact, in “functional locked-in state” revealed by fMRI, suffered from the consequences of traumatic brain injury. Contrary to it, Kotchoubey et al. (Kotchoubey and Lang, 2011) reported a patient having both a basilar thrombosis (which typically leads to LiS) and secondary massive ventricular hemorrhages (which might have caused VS). Using functional tests with fMRI, the authors came to the conclusion that the severe motor paralysis prevailed over the disorders of consciousness, and the follow-up time course confirmed this prognosis.

Another field of fMRI application is the measurement of functional activation in resting state (see Figure 6, (Noirhomme et al., 2010; Vanhaudenhuyse et al., 2010) Healthy subjects and LiS patients exhibited a characteristic fronto-parietal activation (best visualized in the midsagittal view) which is gradually decreased with increasing impairment in consciousness. Possibly, this default network is highly characteristic to conscious resting brain activity (Boly et al., 2008). Brain connectivity evaluation can further evaluate characteristic functional connectivity between the thalamus and the frontal or parietal cortex (Noirhomme et al., 2010).

--- Insert Figure 6 about here ----

**What do we expect from instrumental methods? – an outlook**

The instrumental examination demonstrates that LiS is not a pure motor disorder, in which all functions besides of the motor system remain normal, as it is described in schoolbooks. In contrast, disorders of perception and attention are not exceptions in LiS and
can lead to a wrong diagnosis (Andrews et al., 1996). Despite massive motor and possibly accompanying sensory disorders, higher cortical functions such as gnosis and abstract praxis (beyond its motor expression) are preserved in LIS patients. These higher functions are often related to consciousness and are usually assessed by means of well-established neuropsychological tests. However, these tests in general rely on the measurement of motor and speech motor responses, which limits their utility in LiS. Indeed, recently neuropsychologic tests had been adjusted to the needs and capacities of the severely paralyzed (Kotchoubey, 2005; Kubler et al., 2005; Neumann and Kotchoubey, 2004), but in many cases of the classical LiS, and particularly in the complete LiS even modified tests cannot be used. The results of the various measurement techniques presented in the previous section show that neuropsychological analyzes of mental functions have to be developed further on.

The results of instrumental methods show that there is no one-to-one correspondence between brain structure and remaining functions in LiS. In some patients, despite CCT and MRI findings clearly indicated the diagnosis of LiS, careful and repetitive testing does not reveal the remaining cognitive functions expected to be normal. This repeatedly occurring divergence demonstrates how much we need functional tests, which not only describe the structural pathology, but show that, notwithstanding the severe paralysis, the patient’s brain works during neuropsychological tasks – and how it works.

One of these functional methods mentioned in the previous part is the measurement of ERP during cognitive tasks. These tasks can be hierarchically structured so that the next one requires more complex cognitive abilities than the preceding one (Kotchoubey et al., 2002; Kotchoubey et al., 2003). In the work of Onofrj (Onofrj et al., 1997), for example, the P3 wave was recorded, first, in an oddball-experiment, which assumes a relatively simple discrimination and estimation of stimuli, and then in a memory recognition test, thus checking
up a complex memory function. In our studies also semantic abilities of LiS patients were assessed with the help of ERP.

This method is easy, cheap and flexible, and can be applied immediately at the bedside. However, it lacks sufficient spatial resolution, and, moreover, the resolution is still worse if we need to record the activity of subcortical structures. These disadvantages can be compensated by using other functional imaging techniques. Magnetoencephalography (MEG) possesses not only the same high temporal resolution as the EEG, but also much higher spatial resolution. However, the MEG, like the EEG, mainly records processes within the cerebral cortex, whereas the activity of subcortical structures remains undisclosed. Because the magnetic field of the brain is extremely weak, the MEG requires a very complex apparatus, which hinders the clinical application of this technique. A few years ago the first report about a MEG analysis from a LiS patient was published, in which no explicit benefit of this method could be shown (Silver et al., 2006).

In contrast to the above methods, PET can reach all regions of the brain, both cortical and subcortical and even the brain stem. This procedure is not completely non-invasive because a radioactive material has to be injected so that its metabolism in the brain can be recorded. But the radiation dose of the patient is usually kept to a minimum so that its harmful effect can be ruled out. Another advantage of PET is the size of the observed effects, which is particularly important for individual patient diagnostics. Therefore, PET can successfully be used for differentiation between LiS and an apparently similar condition, the akinetic mutism (Tengvar et al., 2004). PET data show that the metabolic rate in LIS is consistently higher than in the vegetative state (Levy et al., 1987).
By using more sophisticated ways of PET data analysis and a support vector machine trained on data of healthy individuals and patients with VS, all eight patients with LIS were correctly classified as conscious (Phillips et al., 2010).

Functional MRI also yields information about the intensity of metabolism in different brain regions and thus indirectly about these regions’ functional activity. Despite certain similarities to the processes registered with PET, both spatial and temporal resolution of fMRI are higher. Present fMRI techniques have a temporal resolution of a few seconds, compared with milliseconds in EEG and tens of seconds in PET. If, however, patients need artificial respiration, an MRI-investigation may be a problem unless a specialized MRI-compatible respiration device is available.

There are very promising new functional (automatic pattern classification using a support vector machine) and structural (diffusion imaging) measurements available for the diagnosis of LIS. Future technical progress will hopefully lead to strengthening the extant advantages and to minimizing the disadvantages of each method. However, it would be a mistake to think that the mere measurement of electrical brain activity or nuclear magnetic resonance would substantially improve the diagnostics of LiS. This measurement should be integrated into goal-directed neuropsychologic tasks. These tasks should be adjusted so to permit the recording of neurophysiological data (ERP, PET, fMRI). Examples are the hierarchic assessment of cognitive functions using ERP (Neumann and Kotchoubey, 2004) and fMRI (Owen et al., 2006; Owen et al., 2002). It is only in context of appropriate stimulations and instructions where the physiologic markers acquire their functional meaning; without this context their informative value is limited.

According to the presently prevailing view, it is critical for the diagnosis that an active instruction is given to the patients, and that their brain activity demonstrates that they are able
to understand and follow this instruction. Thus Kotchoubey et al (Kotchoubey et al., 2003) asked a patient to imagine movements of the left and right hands and recorded the EEG over the right and left motor hand areas; Owen et al. (Owen et al., 2006) instructed their patient to imagine playing tennis versus navigation in her apartment and recorded fMRI activation quite different for these two tasks; other authors asked patients to count a particular stimulus (Schnakers et al., 2008) or to solve a mental task (Cruse et al., 2011). Whatever kind of brain responses is recorded, it has to be a response that could not emerge without following the instruction. The disadvantage of this methodology should also be kept in mind: it can result in false negative findings, because some LiS patients, though conscious, may be unable to follow instructions, e.g., due to problems with attention span or working memory (Bardin et al., 2011; Kotchoubey and Lang, 2011).

Proposal of a standardized diagnostic procedure in the diagnosis of LiS

The procedure of diagnosis is of course dependent on the severity and acuteness of symptomatology. A patient with an acute decline of motor function and signs of basilar artery thrombosis has to be delivered to a therapeutic intervention immediately after angiographic evidence of a thrombosis. For more chronic patients, MRI seems to be the most promising diagnostic tool. It is capable to localize the lesion and differentiate it from edema. It can distinguish bleedings and detect lesions of the white matter by diffusion weighted imaging techniques. Additionally, MR-angiography is capable to demonstrate a characteristic occlusion of the basilar artery. Furthermore, an analysis of the resting state functional imaging can reveal a characteristic default network distinguishing LiS from severe disorders of consciousness.

For patients with contraindications for MRI, a combination of CCT with angiography might also serve for ascertain the diagnosis together with functional investigations on the
basis of EEG/ERP or PET. At least some of these functional tests should involve active instructions, e.g., to imagine some response or to count some stimulus.

As regards the methods of recording brain activity, the EEG (including EP and ERP) is the only method used at the patient’s bedside. fMRI presents probably the best combination of a good spatial and temporal resolution, but it is also the most complex technique. Both spatial and temporal resolution of PET is lower than those of fMRI; on the other hand, the advantage is much scanning of patients with artificial respiration. For both PET and fMRI the patient has to be transported to an imaging center.

The functional direction of the necessary instrumental methods, whose two pillars are the comprehensive system of neuropsychological tasks and the recording of brain activity during these tasks, would contribute not only to the better diagnosis but also to the rehabilitation of LiS patients. As mentioned above, the rehabilitation of cognitive dysfunction in LiS can provide the basis for motor rehabilitation. This can be called “top down rehabilitation”. Therefore, good diagnostics is not limited to assessment of the current status but includes the patient’s perspectives as well. An example is the above mentioned study of Cincotta (Cincotta et al., 1999) in which the recovery of MEPs of a patient during the functional activation through active movement imagination heralded clinical improvement. Other mental imagery procedures (Kotchoubey et al., 2003; Monti et al., 2010; Owen et al., 2006) can also contribute to the integration of functional brain imaging and cognitive rehabilitation.
Acknowledgment

We like to express our sincere gratitude to these colleagues, who were welcome to provide us with the data of their patients. The CCT in Figure 4 are from Dr. M. Onofrij (Chieti, Italy). The MRI in Figure 5 is from Prof. N. Latronico (Brescia, Italy). The Figure 6 is printed with kind permission from Noirhomme et al., 2010. ML was supported by the German Research Association (DFG; LO 795/7).
Literature


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Figure 1:

The relation between the morphology of the lesion and the EPs (from Towle et al., 1989). Findings in electrophysiology as well as in the CCT indicate a damage in the right medial lemniscus and the upper olive.
Figure 2:

A: A P3 wave of event related potentials in a typical group of healthy individuals (left) and a locked-in patient (right), at a frontal (Fz), central (Cz) and parietal (Pz) lead. A rare (.2) target tone should be counted, and a frequent (.8) tone should be ignored. B: The same in an experiment in which 100 semantically related and 100 unrelated word pairs were presented. The N400 indicates the ability to semantic analysis of verbal stimuli. Negativity is plotted upwards. Bars indicate stimulus presentation.
Figure 3:

Angiography (top left) and MRIs of a patient four weeks post-infarction. The black arrow on the left panel marks a sudden stop of supplying branches of the basilar artery. In the sagittal T1-weighted MRI a large hypodense area is located in the anterior to medial pons. In the T2-weighted axial MRI a left-sided hyperintensity in the pons can be detected.
Figure 4:
Cranial computed tomography (CCT) of two patients with locked-in syndrome.

A: CCT of a 39-year old man some weeks after infarction. In the lower layers hypodense areas in the inferior pons and the left cerebellar hemisphere can be detected. In the posterior cranial fossa (upper row) strong artifacts do complicate an exact evaluation.

B: CCT of a 31-year old woman with complications due to a gestosis during pregnancy. The CCT scan performed some days after the event shows hypodensities in the caudal pons, left-sided hypodensities around the rostral pons and bilateral hypodensities in the cerebral peduncles. Clinically she was awake but tetraplegic with intact vertical eye-movement and eye closure.
Figure 5:

Three examples for MRI scans of patients with LiS. A: A 32-year-old patient whose history began with a fulminating headache and visual impairment, followed by a tetraparesis and stupor within 12 hours. Three days later a caudal pontine ischaemic lesion was seen in CCT scans. At this point he was awake and could move his eyes in all directions. The MRI 14 days after the accident shows in the sagittal T1-weighted scan (left, hypointense) and in the axial T1- and T2-weighted scans (hyperintense, right) a well accentuated large pons lesion (R > L), which was probably caused by an infarction of the basilar artery. B: A 39-year-old patient with attacks of a basilar-type migraine with severe headache and right arm paresis in the anamnesis. The stroke started with symptoms of dysarthria. After six hours a left sided facial nerve paresis developed whereas the initial paresis of the left arm decreased. In course of time tetraparesis and stupor were present. After five days awareness, he showed an intact blink reflex but a spontaneous decerebrate position. Vertical and left-sided ocular movements were intact. In the MRI in T1-weighted as well as in T2-weighted scans a hyperintense, new zone of infarction in the junction of the lower pons to the medulla oblongata is visible. C: 40-year-old patient with severe acute abdominal pain with suspected mesenteric infarction. Over time stupor and tetraparesis occured. In the initial CCT hypointensities in pons is visible. After three days of being awake the patient showed spontaneous rising of the left eye and eye lid. In the T1- and T2-weighted MRI scans a few days after infarction a big, almost bilaterally equal pontine lesion was definable.
**Figure 6:**

The internal network of patients with brain death, coma, vegetative state (VS), minimally conscious state (MCS), and Locked-in syndrome (LIS). The network was extracted with ICA. The black and white contour represents a template of the internal network extracted from eleven awake healthy subjects with ICA. Yellow and orange colors represent the areas which activities positively correlate with the time course of the internal network. The figure is based on data from Boly et al. (Boly et al., 2008) and Vanhaudenhuyse et al. (Vanhaudenhuyse et al., 2010). With kind permission from Noirhomme et al., 2010.