Stimulus-induced gamma activity in the electrocorticogram of freely moving telemetric implanted rats: the neuronal signature of novelty detection.

Dissertation

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A mon Père,

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Maty,

Benoît,

Et Olivier.

« Brains are characterized by every property that engineers and computer scientists detest and avoid. They are chaotic, unstable, nonlinear, nonstationary, non-Gaussian, asynchronous, noisy, and unpredictable in fine grain, yet undeniably they are among the most successful devices that a billion years of evolution has produced. »

Walter J. Freeman.

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Abbreviations

%	Percent
α	Alpha frequency range (8-13 Hz)
β	Beta frequency range (14-30 Hz)
γ	Gamma frequency range (30-80 Hz)
δ	Delta frequency range (0.5-4 Hz)
θ	Theta frequency range (4-7 Hz)
ACh	Acetylcholine
ADC	Analogue-to-Digital Conversion
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
AP	Action Potential
Ca^{2+}	Calcium
CCD	Charge Coupled device
cm	Centimetre
DSP	Digital Signal Processing
ECoG	Electrocorticogram
EEG	Electroencephalogram
EPSP	Excitatory Post Synaptic Potential
g	Gram
GABA	γ-aminobutyric acid
h	Hour
Hz	Hertz
i.p.	Intra-Peritoneal
IR	Infrared light
K^{+}	Potassium
LED	Light Emitting Diode
LFP	Local Field Potential

LTP	Long Term Potentiation
m	Metre
mg	Milligramme
min	Minute
ml	Millilitre
ms	Millisecond
NA	Noradrenaline
Na^+	Sodium
NMDA	N-methyl-D-aspartic acid
PCB	Printed Circuit Board
POm	Medial division of the posterior nucleus of the thalamus
REM sleep	Rapid Eye Movement sleep
s	Second
S2	Secondary somatosensory area
SC	Subcutaneous
VGND	Virtual Ground
VPM	Ventral Postero Medial nucleus of the Thalamus

Figure legends

1 Introduction

1.1 Object recognition processes

Learning is maybe the most valuable and intensively studied function of the brain. Without this ability our daily life would be an eternal restart with the impossibility to use our past experiences. The faculty of memorisation requires a capacity of the system to distinguish the different events from others and object recognition is a part of this process.

1.1.1 The binding problem

The binding theory raised from the experiments performed on the visual system and in particular by the work of Christoph von der Malsburg and his team (1981) who first discussed the “binding problem” or “binding theory” as a general phenomenon which could be summarised in the following way: “the representation of the various attributes of the visual world by distributed neuronal assemblies can be bound together harmoniously in the time domain through oscillatory synchrony” (Buzsáki, 2006). Already decades ago, the Gestalt psychology stated that an object is defined by the spatial and/or temporal coherence of the parts. A simple ball is characterised by a colour, a size, a texture, a movement pattern that distinguishes it from other objects. These features are processed in different parts of the cortex and have to be bound together to form an image in the brain to recognise it as a ball even if the perceptual conditions and its characteristics diverge from the model in mind.
A fundamental problem of neuroscience is how the coordination of many brain areas coding for the same object is achieved. The first theoretical solution to this problem was the model of “cardinal neurons” which stated that complex

information is represented in the brain by convergence of the processing stream to highly specified assemblies or even single neurons (Fell *et al.*, 2003). However this model failed to explain the recognition of new objects and would be very costly in terms of neuron quantity to code the external environment. Because of these disadvantages another theory has been proposed relying on temporal coding.

1.1.2 The temporal binding

The “temporal binding” or “binding by synchrony” has been proposed has a solution to the binding problem. The binding by synchrony is a response to the “classical neural networks” theory’s weaknesses highlighted by Frank Rosenblatt with his now classic example (Fig. 1). He has shown that this model did not have the means of combining elementary symbols into more complex ones, a common brain activity (von der Malsburg, 1999). Following this observation many experiments have shown that transient synchronized oscillations were present in the signal after the presentation of a sensory stimulus. Wolf Singer's team was one of the first who highlighted this phenomenon in the cat's visual cortex (Gray *et al.*, 1989). In these experiments they have shown that the presentation of coherently moving bars to an anaesthetized cat enhanced gamma (γ) “burst” activity (30-80 Hz) in local field potential (LFP) recordings as compared to independently moving patterns. These transient oscillations were rarely observed spontaneously whereas they were clearly induced by the visual stimuli. Since this time the controversy is still open whether this internal coordination of spike timing serves a function in cortical processing or whether it is an epiphenomenon.

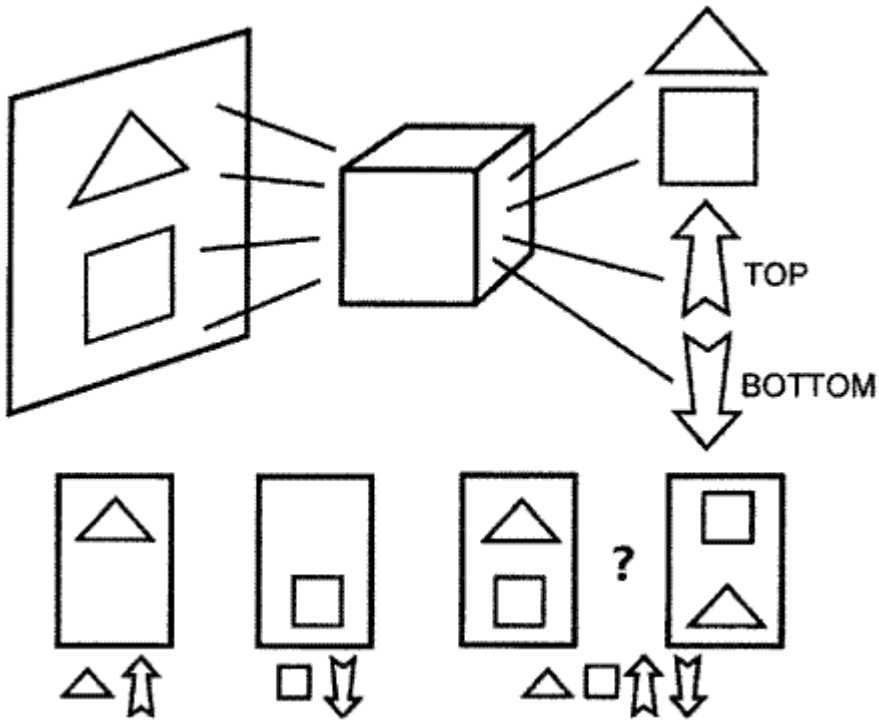


Figure 1: Rosenblatt's example. Imagine a specific neural network for visual recognition, which can produce four solutions, each of them coded by one output neuron. Two neurons recognize objects, a triangle or a square, both generalising about position. The other two indicate the position of objects in the image: in the upper half or in the lower half, both generalising over the nature of the objects. When showing single object to the network it responds adequately, e.g. with (triangle, top) or (square, bottom). A problem arises, however, when two objects are present simultaneously. If the output reads (triangle, square, top, bottom) it is not clear whether the triangle or the square are in the upper position (from von der Malsburg, 1999).

The arguments in favour of synchronization as a part of the coding process is that synchronized excitatory postsynaptic potential (EPSP) salves are more effective in triggering postsynaptic spikes than temporally dispersed inputs and may therefore have a privilege role in synaptic transmission. It has also been shown that cortical network can distinguish synchronous from non synchronous events with a high temporal resolution (Lumer *et al.*, 1997) resulting in the idea that synchronicity may serve as a tag of related events

(Singer, 1999). On the other hand, arguments against temporal binding state that this theory does not explain how synchrony could determine which of the inputs carry signals worthy for further computation and why synchrony should be seen in primary sensory cortices since it is believed that binding is a high-level process (Shadlen & Movshon, 1999). Furthermore these authors remark that considering the number of inputs a cortical neuron receives the chance to have synchronous spikes is really high except in a very narrow time window and that these neurons may lack the biophysical mechanisms to detect precise coincidence at a millisecond range. However, Csicsvari *et al.* have proved that population synchrony of CA1-CA3 regions exceeds values expected from random coincidence of uncorrelated spike trains (Csicsvari *et al.*, 2000).
"Finding correlation patterns even if their occurrence is context dependent proves that the brain can reliably process temporal patterns but does not prove their relevance to the binding issue" (von der Malsburg, 1999). Nevertheless "oscillations do exist in the brain and these will generate synchronous interactions, the brain is not a casino" (Csicsvari, personal communication). Moreover a growing set of results showed that the γ rhythm could be the most probable candidate to the binding problem and therefore a strong argument for the binding by synchrony.

1.1.3 The γ rhythm

The γ rhythm (30-80 Hz in mammals), like most oscillations, is shaped by inhibitory interneuron activity. A particular role is played by the fast $GABA_A$ receptors (Wang & Buzsaki, 1996) which have a time constant in the range of the γ frequency band. Two types of γ band activities in response to sensory stimuli have been observed. First, an evoked response up to 150 ms after the stimulus which is time-locked on it. Second, an induced response in a time

range up to 400 ms non phase-locked on the stimulus onset (Fell *et al.*, 2003).

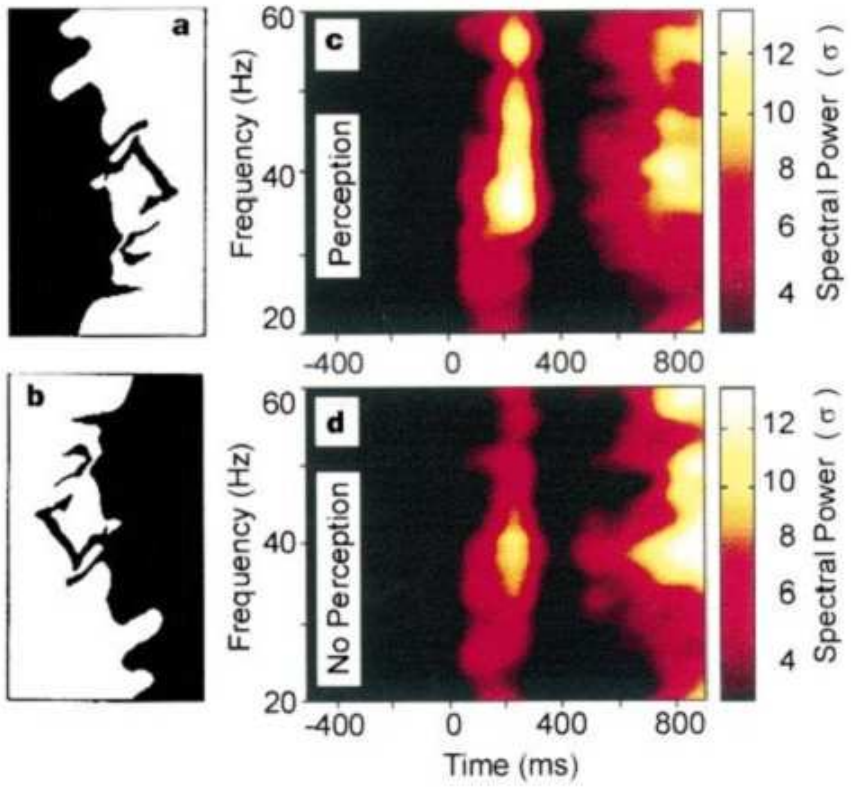


Figure 2: Stimulus induced γ oscillations. The presentation of "mooney faces" to human subjects induced broadband γ burst oscillations in EEG recordings 200 ms after stimulus onset especially strong when the stimulus was meaningful (from Rodriguez *et al.*, 1999).

The evoked γ band activity as been shown to be enhanced for example after odour presentation in honeybee antennal lobe (Stopfer *et al.*, 1997). In this experiment the authors have shown that the abolition of this synchronized activity (20-30 cycles in insects) by the injection of picrotoxine (a $GABA_A$ antagonist) impaired the discrimination of molecularly similar odorants. With regard to the induced γ band response, it has been shown to be selectively enhanced in different brain areas during perception tasks (Buzsáki, 2006; Rodriguez *et al.*, 1999; Tallon-Baudry *et al.*, 1996; Tallon-Baudry *et al.*, 1997; Tallon-Baudry & Bertrand, 1999) (Fig. 2) and involved in neuronal processes

such as attention, learning and memory (Buzsáki, 2006; Jensen *et al.*, 2007; Kaiser & Lutzenberger, 2003). Furthermore, the γ rhythm presents all the characteristics to forward messages in the most effective way from neuronal populations to downstream neurons. Harris *et al.* proposed that cooperative activities in a time window of 10-30 ms was the most appropriate timescale for information transmission and storage in cortical circuits (Harris *et al.*, 2003). This time window corresponds to the γ frequency range and this time resolution has been shown to be the most effective to induce synaptic plasticity (Magee & Johnston, 1997). Finally it has been proposed that synchronized neuronal firing in the γ frequency range may be crucial to produce a coherent object representation (Buzsáki, 2006; Singer *et al.*, 1997).

All these studies provided strong arguments for the possibility that the γ frequency band may play a role in the novelty detection process as well. To investigate it we have been interested in the rodents' whisker system.

1.2 The barrel cortex

1.2.1 Anatomy of the whiskers to barrel pathway

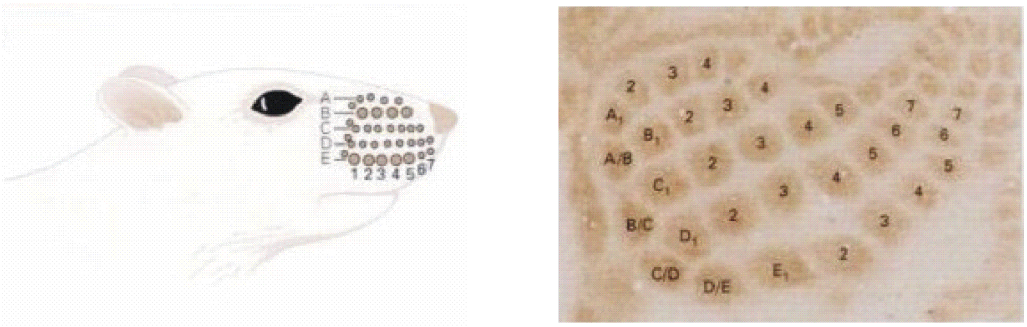


Figure 3: the cortical representation of whiskers in rodents (adapted from Kandel *et al.*, 2000).

In rodents, the vibrissae or whiskers, long hairs organized in 5 rows on the snout, have the most prominent representation in the somatosensory cortex. The arrangement in arrays of the vibrissal pad is maintained in the brainstem (barrelettes), the thalamus (barreloids) to finish with a perfect somatotopic representation in the cortex with the so-called barrels (Figs. 3 and 4). These barrels are cellular aggregates formed by the cell body of layer IV pyramidal neurons which respond to tactile inputs principally from a single whisker (Kandel *et al.*, 2000).

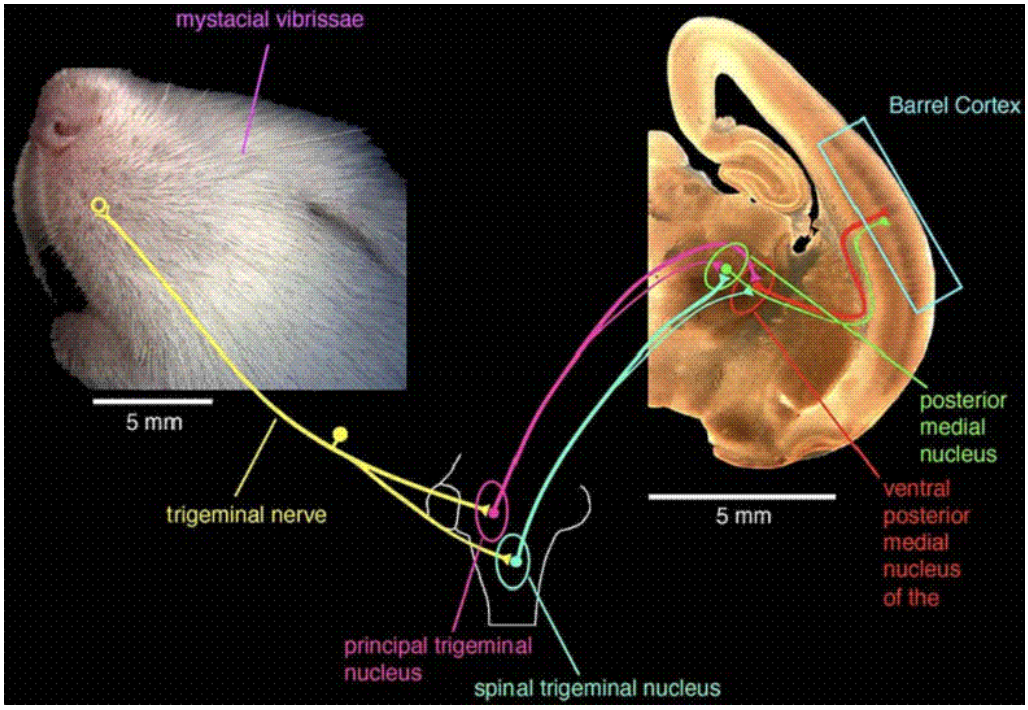


Figure 4: Vibrissal sensory system of the rat (from Lübke & Feldmeyer, 2007). Brainstem nuclei that receive vibrissal primary afferents include the principal trigeminal nucleus (Pr5) and all sub-divisions of the spinal trigeminal complex (Sp5). Each of these subnuclei contributes axons to the trigeminothalamic tract, but the main stream of ascending fibers rises from the Pr5 and the interpolar division of the Sp5 (Sp5I). Trigeminothalamic fibers innervate the VPm, POm and intralaminar nuclei of the contralateral thalamus, and each of these nuclei projects to the somatosensory cortical areas (Deschenes *et al.*, 1998).

The anatomical connections from the whiskers to the rodent barrel cortex form

two parallel pathways (lemniscal and paralemniscal). The lemniscal pathway of the rat trigeminal system ascends through the ventral postero medial nucleus (VPM) of the thalamus to the barrels in layer 4 of the cortex and to layer 5b and 6. The paralemniscal pathway ascends through the medial division of the posterior nucleus of the thalamus (POm) to layers 1 and 5a and to the septa between the barrels in layer 4 (Ahissar *et al.*, 2000).

1.2.2 Signal processing from whiskers to cortex

Ahissar *et al.* have shown that the two pathways were involved in different processing tasks (Ahissar *et al.*, 2000). They recorded output responses of the brainstem, thalamus and barrel cortex neurons for the two pathways after air pulse stimulations of one or two whisker rows in anaesthetized rats. The brainstem neurons simply relayed primary afferent responses in both pathways to thalamic neurons which in contrast exhibited specific transformation of signals. An adaptation process was observed in the VPM with a decrease of the spike count after a number of repetitions of the same stimulus until the system reached a stable state. In the POm the latency of the response onset increased until it stabilised resulting in a decrease of the response duration due to the unchanged offset latency. The response of the barrel cortex neurons showed similar variations for the 2 pathways. The authors showed that the paralemniscal pathway and not the leminiscal one coded different whisker stimulation frequencies with an increase of the onset latency response correlated to the increase of the stimulation frequencies. The variable latencies and effective cortical feedback in the paralemniscal system can serve the processing of temporal sensory cues, such as those that encode object location during whisking. In contrast, fixed time locking in the lemniscal system is crucial for reliable spatial processing.

1.2.3 Cortical column organisation of the barrel cortex

A basic feature of sensory cortices is their organization in functional, vertically oriented columns, recruiting modules of signal processing and a system of transcolumnar long-range horizontal connections (Lübke & Feldmeyer, 2007). In the barrel cortex a cortical column contains approximately 10,000-20,000 neurons distributed over 6 laminae divided into 2 main categories. The excitatory neurons with pyramidal and spiny stellate cells form the majority of the population and the rest is composed of inhibitory GABAergic interneurons. Most of the synaptic connections in the barrel cortex are local (intracolumnar) and intralaminar (within a respective cortical layer). There is a preferential direction of synaptic signaling from the thalamic input layer 4 to supragranular cortical layers which return from there to infragranular layers.

1.2.4 Anatomical connections of the barrel cortex

In addition to the thalamocortical projections described above, the barrel cortex is reciprocally connected to cortical and subcortical structures that are involved in processing sensory information from the vibrissae and in motor control of whisking behaviour (Jones & Diamond, 1995). The barrel cortex massively projects back on the thalamus and it has been shown that these projections follow the principle of parity since these latter projections are much stronger than the thalamocortical ones (Deschenes *et al.*, 1998). The barrel cortex is also strongely bidirectionaly connected with the ipsilateral secondary somatosensory area (S2), the motor cortex and the perirhinal cortex. Despite the ipsilateral connections preference, the barrel cortex projects also on these structures on the contralateral hemisphere. Finally the barrel cortex receives modulatory inputs from the basal forebrain with cholinergic afferents and from

the locus coeruleus with strong noradrenergic projections.

1.3 Novelty detection

The “novelty detection” concept developed in this thesis includes the prediction that a specific neuronal activity may happen in the brain when the subject is exposed to an object or a situation for the first time. The ability to detect and respond to novel events is crucial for survival in a rapidly changing environment. Not surprisingly the brain areas shown to be involved in novelty detection are also associated with perception, attention, learning and memory such as the prefrontal cortex, perirhinal cortex and hippocampus (for a review see (Ranganath & Rainer, 2003). Two kinds of novelty have been distinguished so far, the stimulus novelty and the contextual novelty. Recently, γ as well as β oscillatory activities have been shown to accompany the presentation of a novel auditory stimulus in the human EEG (Haenschel *et al.*, 2000). In addition, in human auditory detection task it has been shown that novel sounds elicited a scalp recorded potential that peaked around 200-300 ms after the stimulus presentation, the so called “novelty P3” (Barcelo *et al.*, 2006; Ranganath & Rainer, 2003) (Fig. 5). This novelty related activity is largest over the central and frontal areas and has been observed in many different species. Furthermore, β2 oscillations (23-30 Hz) are transiently and selectively increased during the active exploration of a novel environment in the hippocampus of freely moving mice (Berke *et al.*, 2008). In primates using single-unit recordings it has been possible to establish a relation between the firing rate of prefrontal cortex neurons and novel object presentation in a visual task (Rainer & Miller, 2000). In these experiments the authors have demonstrated that familiar objects activated a smaller population of prefrontal cortex neurons than novel objects did, also accompanied by a firing rate

increase. Another crucial structure in the novelty detection process is the perirhinal cortex which has been shown to play an important role in recognition memory and in particular for the discrimination of novel and familiar individual objects (Barker *et al.*, 2007).

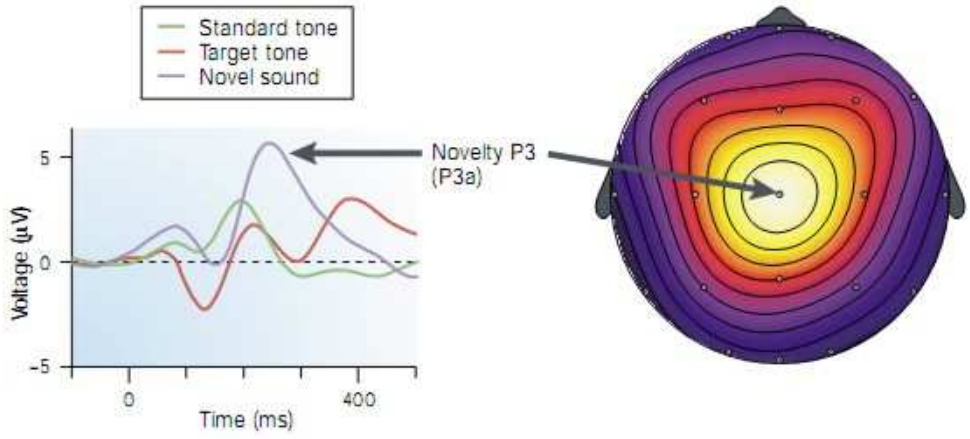


Figure 5: The novelty P3 event related potential in humans. In an auditory detection task a novel sound elicits a cortical potential particularly large over frontal and central areas in humans (modified from Ranganath & Rainer, 2003).

All these observations indicate that distinct oscillatory patterns correlate with the detection and perception of a novel object in a wide range of brain areas. However no study reported any novelty related electrical pattern in primary areas such as the barrel cortex. Some studies have used the expression of inducible transcription factor such as c-fos as a marker of neuronal activity and shown that the exploration of a new environment induced a prominent and highly specific increase in expression levels in the somatosensory cortex in rodents (Montero, 1997; Staiger *et al.*, 2000).

The brain regions associated with novelty detection are numerous and it has been stated that the coordination between them could be achieved by the effect of neurotransmitters such as acetylcholine (ACh) and noradrenaline (NA). Both tend to enhance stimulus-evoked activity and promote NMDA-receptor

dependent plasticity and have been shown to increase in cortical level in response to novel object (Ranganath & Rainer, 2003).

The working hypothesis of this thesis was then that a specific induced oscillation, most likely in the γ band frequency, would be detected in the rat barrel cortex following the exploration of a new object by the animal. In order to investigate this hypothesis the electroencephalography in freely moving rats has been chose.

1.4 Electroencephalography

At the end of the 19th century Richard Caton has been the first to observe electrical impulses from the surface of living animals' brains using a galvanometer. A major contribution to this field has been performed by Hans Berger who published a study in 1929 using a method called electroencephalography on human subjects. In this study he described the different network rhythms present in the brain and opened a complete new area of research in neuroscience.

1.4.1 Electroencephalogram signal's theory

The electroencephalogram (EEG) as well as the electrocorticogram (ECoG) are recordings of the electrical activity of the cerebral cortex, through electrodes placed on the scalp or on the dura respectively (Bromfield *et al.*, 2006). While the ECoG has a ten times better signal to noise ratio in comparison to the EEG, it can obviously only be used in animal experiments or during a neurological operation. In the following EEG and ECoG will be referred as EEG.

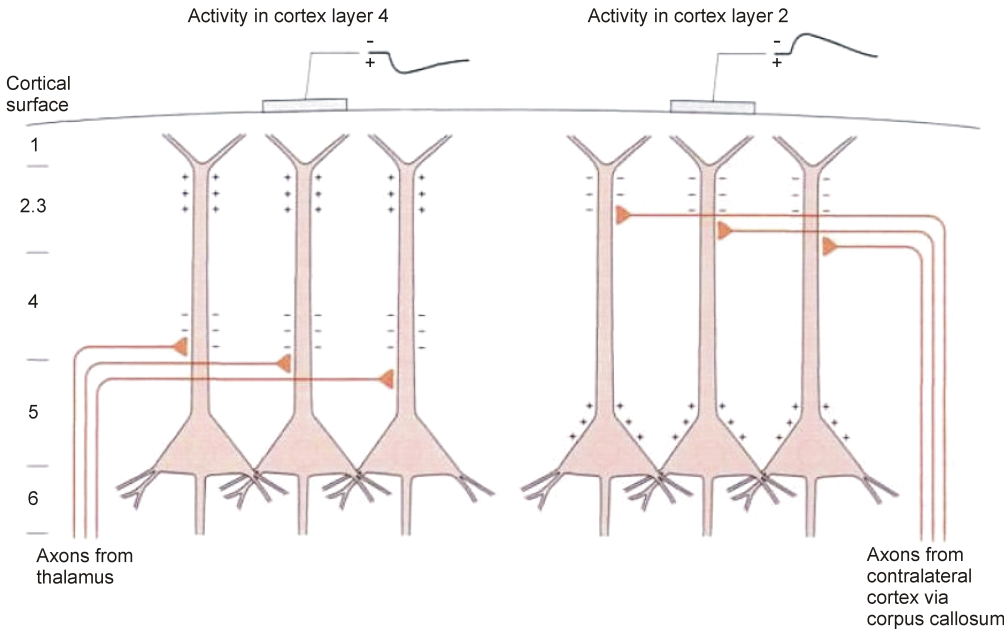


Figure 6: The EEG signal. An incoming excitatory signal at a dendritic synapse induces an inward flux of positive charges leaving a relatively negative charge in the extracellular space. The inward current must flow in a closed loop and then leave the dendrite at a distant site. Thereby, a dipole is created. An extracellular electrode placed on the skull near the apical distal end of the dendrite detects a positive potential if the activated synapse is situated proximally (left panel) and a negative potential if the activated synapse is situated distally (right panel) (modified from Kandel *et al.*, 2000). By convention, the additional negative extracellular charge is shown as an upward deflection, while the presence of a source and an additional positive charge is shown as a downward deflection.

The EEG measures primarily the electrical potentials of cortical neuronal dendrites near the brain's surface (Fig. 6). At an excitatory synapse, an incoming action potential (AP), mainly mediated through Na^+ influx and K^+ efflux via voltage-gated channels, releases neurotransmitter from the pre-synaptic element into the synaptic cleft. The released glutamate, the most commonly used neurotransmitter in excitatory synapses, binds to specific receptors, such as AMPA and NMDA, on the post-synaptic element. This binding gives rise to a post-synaptic potential resulting from positively charged Na^+ ions rushing into the cell (Kandel *et al.*, 2000). This ion movement leaves

a relatively negative charge in the extracellular space in the vicinity of the synapse. Current by the movement of electrons or ions, must flow in a closed circuit (Kirchoff's law). Therefore the same amount of positive current that entered the cell at the synapse (inward current referred to as the "sink") must also leave the cell at some other distant site (outward current referred to as the "source") (Freeman, 2001). An outward flow of positive charges leaves to a relatively positive charge in the extracellular space at the synapse. In this case, a dipole is created outside the dendrite, with a relatively negative charge at the distal part and a positive charge closer to the cell body. Thus, an extracellular electrode placed near the end of the dendrite detects a negative potential.

The deflections recorded at the surface of the brain are strongly influenced by the type of cortical inputs. Sensory inputs from the thalamus reach the pyramidal cells at the level of their soma and proximal regions in layer 4 and then produce a positive deflection on an EEG trace (Fig. 6, left panel). In the case of associative cortical inputs toward the distal dendrite in the layers 5 and 6 a negative deflection can be seen in the recording (Fig. 6, right panel).

Due to the small potential in amplitude and the big distance from the cell to the scalp surface it is not possible to record electrical changes in a single neuron. However, EEG recordings are made possible by the distinct orientation and polarity of cortical pyramidal neurons. In fact these cells are mostly organised parallel in the cortex and perpendicular to the brain surface. This structural organisation associated with the synchronous synaptic activation of these neurons permits the summation of thousands of dipoles and thereby the recording of the EEG signal.

Due to the underlying mechanisms of EEG, it is difficult to precisely localise the origin of the observed activity especially in human brains where the cortex is formed of gyri and sulci resulting in a very complicated summation of dipoles. It is important to highlight again the fact that EEG recordings are not due to AP activity but that they reflect the synaptic input into the cortex with a non homogenous contribution of all cells to the signal. The model presented

above summarises the theory behind the EEG signal but the reality may be different. As already pointed out, the cortical pyramidal cells are not always nicely organised in parallel, not perpendicular to the skull and pyramidal cells are not the only cortical cells. An unknown factor is the effect of glial cells on the signal that can be captured at the surface of the skull.

1.4.2 EEG signal and glial cells

In mammalian brains, glia represent the biggest population of cells with a one to ten ratio when compared to neurons. In contrast to the latter, the function of glial cells is not well understood so far. For a long time considered to be the glue of the brain and logistic support for neurons. Glial cells are divided into two main categories: the microglia and the macroglia. Microglia is mainly implicated in macrophage like activity. On the other side oligodendrocytes, the cells forming the macroglia with the astrocytes, are responsible for the generation of the myelin sheath insulating neurons (Alberts *et al.*, 1994), which greatly increases the rate at which an axon can conduct an AP. Moreover the astrocytes are intimately associated with synapses but have been traditionally viewed as passive elements in synaptic function (but see Perea & Araque, 2005). This has been the common view about glial cells for decades but during the last ten years an important amount of data showed that oligodendrocytes as well as astrocytes may be directly involved in information processing. It has been observed in a recent study that in the white matter area of the cerebellum of post-natal day 7 rats half of the NG2+ cells (oligodendrocyte precursor cells expressing the proteoglycan NG2) fire AP (Karadottir *et al.*, 2008). Even if the validity of this study has been challenged this concept opens new perspective regarding information processing in the brain (Fields, 2008). Not less surprising NG2+ cells form direct synaptic junctions with both glutamatergic

and GABAergic neurons (Paukert & Bergles, 2006). Furthermore, evidence has been provided for a bidirectional communication between astrocytes and neurons via non synaptic glutamate release and Ca2+ excitability as a key element in this information exchange (Perea & Araque, 2005).

The signal that can be recorded at the surface of the brain could hence be far more complex than described by the simplified dipole model above and since the impact of glial cells on EEG recordings is unknown a part of the system method may be lacking to understand the working system.

1.4.3 EEG rhythms

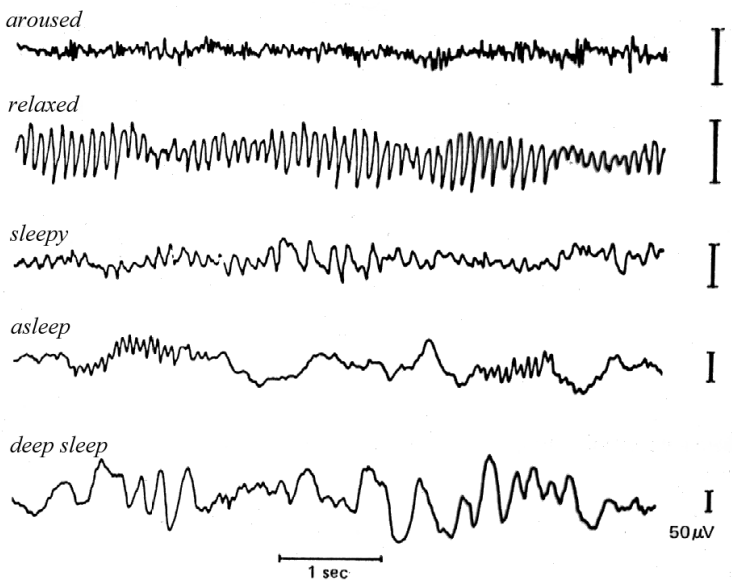


Figure 7: The EEG rhythms in humans. Depending on the brain's state the EEG differs in amplitude and frequency and can be used in clinical medicine as a diagnostic test in the evaluation of patients with seizure disorders, sleep disorders, etc., and can help to localise certain infections (from http://neurocog.psy.tufts.edu/images/eeg_states.gif).

The EEG waveforms are divided into five major frequency bands: delta (δ; 0.5-4 Hz), theta (θ; 4-7 Hz), alpha (α; 8-13 Hz), beta (β; 14-30 Hz) and gamma (γ;

30-80 Hz). Each frequency band has been associated with one or several brain states such as arousal, sleep, attention, etc. (Fig. 7). The α rhythm has been observed first. It is associated with relaxed wakefulness and enhanced in the occipital cortex when the eyes are closed. β waves are observed in frontal areas during intense mental activities and are associated with δ activity during slow wave sleep activity with a so called synchronous EEG (Kandel *et al.*, 2000). The θ rhythm is present in awake mammalians and is particularly strong during rapid eye movement (REM) sleep. Finally the γ has been observed during attention, learning, perception.

1.4.4 EEG recordings in animal research

Animal research is required to understand the brain's working properties and EEG recordings have been used in many species such as monkeys, cats, rodents, in anaesthetised, head restrained as well as freely moving animals. The rest of this manuscript will only deal with this latter method. Traditionally performed with a direct connection of the animal to the recording apparatus, e.g. a cable connecting the electrodes on the animal´s head with the recording amplifier (left panel of Fig. 8), this technique represented the only approach to study the correlation of animal´s brain activity and behaviour under relatively natural conditions. Because this method is not devoid of disadvantages telemetric recording systems have been developed to enable the subject's normal behaviour.

1.5 Electrophysiological recordings in freely moving animals

1.5.1 Tethered versus Telemetry

The tethering method is widely used to perform electrophysiological recordings in freely moving animals (Fig. 8, left panel). Despite the lack of direct measurement of stress hormones levels in comparison it to telemetric implantations, this technique has been attested to be a stress generator (Brockway & Hassler, 1992) whereas wireless technology has been shown to minimize the stress artefacts unavoidable in traditional electrophysiological recordings (Kramer & Kinter, 2003) (Fig.8, right panel).

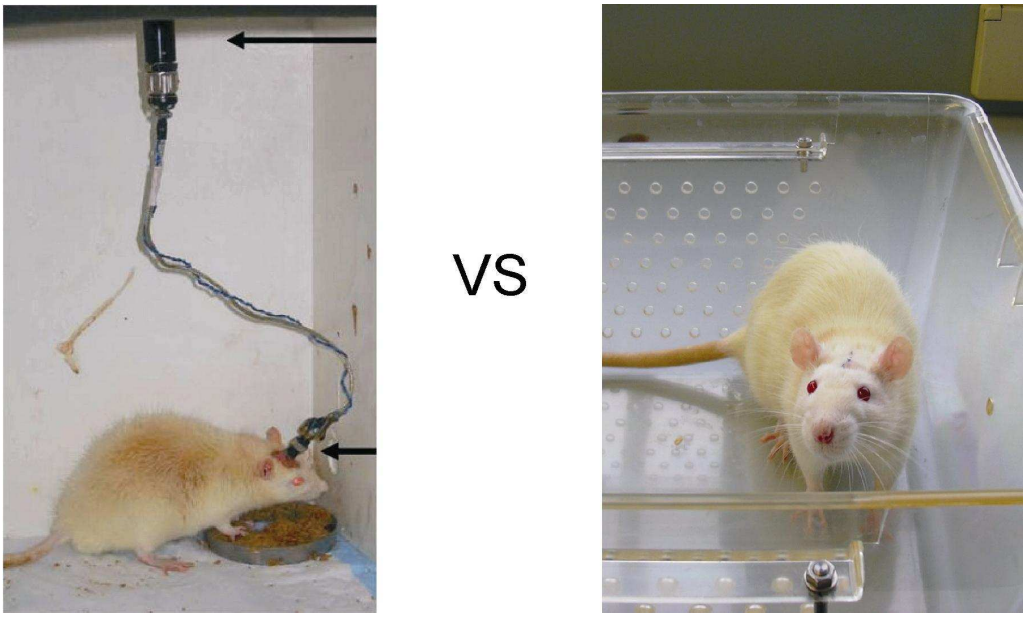


Figure 8: Tethered versus telemetric method (modified from Williams *et al.*, 2006 and Lapray *et al.*, 2008).

Some studies have shown an increase in rodents (Bohus, 1974) or non-human primates heart rate (Adams *et al.*, 1988) during standard *in vivo* recordings. It

has been also documented that sleep diurnal ratio in B6 mice was smaller in fully implanted animals compared to tethered ones (Tang & Sanford, 2002) due to either greater or relatively more sleep in the dark period. The authors of this paper stressed that cable systems in rodents - animals known to be fast climbers - were even more restricting than in big animals. The risk of infections and the recording of movement artefacts produced by wires and connectors are also minimised with implantable telemetry. Furthermore animals can be under continuous recording protocols for days or during shorter periods of time without technicians' interventions. No maintenance of the system is required and telemetric devices can be turned on and shut down by external controls.

1.5.2 Why developing a new telemetric system ?

Two main categories of telemetric systems are currently available on the market: the backpack and the fully implantable units.

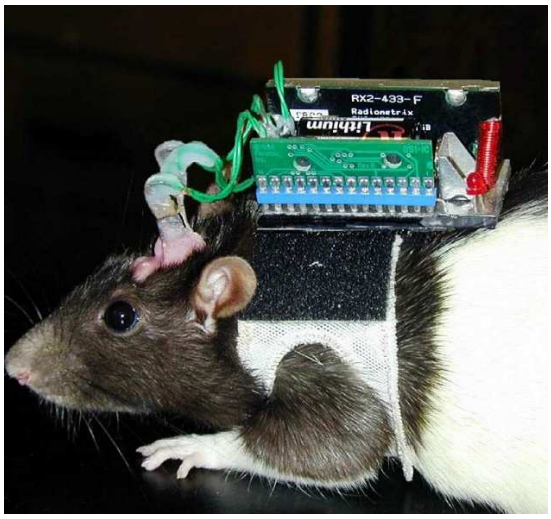


Figure 9: The backpack style unit. In this method the animal carries the transmitter directly on its back with the disadvantage of reduced free movements (from Xu *et al.*, 2004).

Some have externally fixed transmission devices, e.g. "backpack" style units (Obeid *et al.*, 2004). The external units (Fig. 9) have disadvantages and limitations since the animal is always mechanically impaired by the unit and therefore the behaviour will be comparable to the tethered model. The connection between the electrodes and the transmission unit penetrate the skin and the affected area is more sensitive to injuries especially in the case of social interaction studies.

On the other hand the available fully implantable units still have many disadvantages: High costs as well as a limited distance between transmitter and antenna far too small to allow real behavioural experiments such as openfield exploration.

Therefore we designed a fully implantable telemetric system which was well suited for behavioural experiments.

1.5.3 Telemetric system's properties

A telemetric recording system should fulfil a number of requirements (Brockway & Hassler, 1992; Lapray *et al.*, 2008):

- Implantable devices should be as small and light as possible to minimise or better prevent stress and discomfort to the animal.
- The system and wires should be surrounded by fully bio-stable and bio-compatible material in order to allow recordings over long periods of time.
- The energy consumption should be as low as possible with the opportunity to switch the system on and off from distance or by recharging the battery.
- The signal should be transmitted over at least a few meters in order to allow free exploratory behaviour of the animals in a defined environment.
- Internal components should be re-usable and the external recording system should be as simple as possible to minimise the costs.

- All external components of the telemetric system (e.g. the receiver) should be as small as possible and portable to allow experiments outside the conventional laboratory settings.
- In order to allow maximal flexibility in the experimental design and in the recording protocols, the properties of the signals sent out from the transplanted transmitter should be adjustable from the outside.

1.6 Data acquisition and signal analysis

1.6.1 Data acquisition

Telemetry recording signals like any biological signals are continuous in amplitude and time and therefore have to be converted in digital data in order to be analysed by computers. This process called analog-to-digital conversion (ADC) transforms a continuous signal following 2 steps: first the amplitude scale (dependent variable) is made discrete by a “quantization” and then the time scale (independent variable) is made discrete by “sampling” the continuous wave at a given interval (van Drongelen, 2006). Both of these digitalisation steps restrict how much a digital signal can contain. This method is comparable to the application of a grid on to the signal, the smaller the mesh, the more information is gained, but also more space is required for storage. A good ADC is when an equilibrium is found between the information that needs to be retained and the amount that can be “lost”.

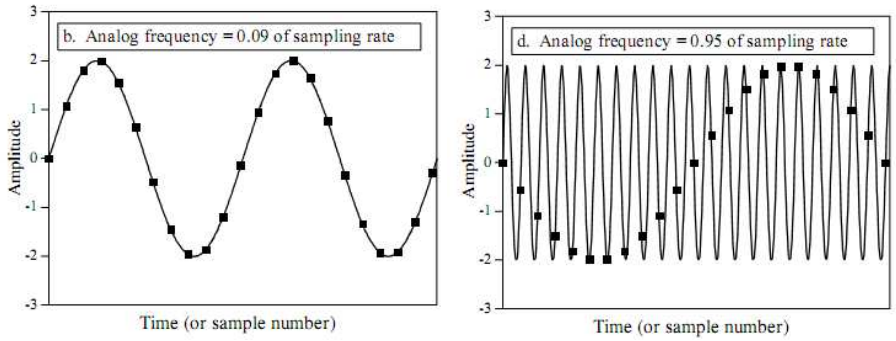


Figure 10: Nyquist-Shannon sampling theorem. The left panel shows a proper sampling in contrast to the right panel with the creation of an alias, e.g. a wrong representation of the original analog signal (from Smith, 1997).

The “quantization” can be defined in most cases by the addition of a specific amount of random noise to the signal and uses the bit as unit (Smith, 1997). Unlike the “quantization”, the “sampling” is well known by electrophysiologists and can be the origin of wrong representations of the original analog signal. To avoid this phenomenon the Nyquist-Shannon sampling theorem as been developed. It states that a digital signal cannot contain frequencies above one-half the sampling rate. This is why the sampling rate of a signal should be at least twice as high as the highest frequency of interest. The result of an improper sampling rate would be the creation of an *alias*, a frequency which does not exist in the original signal (Fig. 10).

1.6.2 Digital signal processing

As defined by Steven W. Smith, digital signal processing (DSP) deals with a specific area of computer science using signals, in most cases originating from sensory data from the real world such as brain activity, seismic vibrations, sound waves, etc (Smith, 1997). DSP implies the mathematics, the algorithms, and the techniques used to manipulate these signals after they have been

converted into a digital form. In electrophysiology most of DSP are based on the Fourier analysis named after Jean Baptiste Joseph Fourier (1768-1830) and his publication claiming that any continuous periodical signal could be represented as the sum of properly chosen sinusoidal waves (Smith, 1997). This statement is the basis of many mathematical tools used by neuroscientists. The Fourier Transform has been one of the first methods applied to brain waveforms which transforms the time domain into a frequency domain. When studying brain event related activities this method has the disadvantage of losing the notion of chronology. Therefore sliding windows Fast Fourier Transform, Wavelet Transform have been developed.

The development of our own telemetric recording system in addition to the modern mathematic tools available should allow the exploration of the novelty detection mechanism, a particular learning process, in unrestricted animals.

2 Materials

2.1 Chemicals

Acetic Acid 0.1%	
Actril	Minntech, Minneapolis, USA
Atropine	Sigma-Aldrich, Steinheim, Germany
Baytril® 2.5 %	Bayer Vital, Leverkusen, Germany
Chloral hydrate 99%	Sigma-Aldrich, Steinheim, Germany
Conductive glue	Chemtronics, USA
Grip Cement	Dentsply Caulk International, Milford, USA
Hydrogen Peroxide	Primoplast, E.Leclerc, Issy-les Moulineaux, France
Iodine	Braun, Melsungen, Germany
Isoflurane	Abbott GmbH, Wiesbaden, Germany
Ketamine	Ratiopharm, Ulm, Germany
Korsolex AF	BODE Chimie, Hamburg, Germany
Meliseptol	Braun, Melsungen, Germany
NaCl	Braun, Melsungen, Germany
Rimadyl	Pfizer, Karlsruhe, Germany
Silicone, Elastosil N2010	Wacker Chemie AG, Munich, Germany
Water soluble separating agent	PVA, R&G, Waldenbuch, Germany

2.2 Equipment

Binocular Wild M5A	Spectra Services Inc., NY, USA
CCD-IRIS Camera	Sony, Berlin, Germany
CCD Camera M10SX-C	JAI, Stemmer Imaging, Germany
CED	Cambridge Electronic Design, Cambridge, England
High Speed Micro Drill	Fine Science Tools GmbH, Heidelberg, Germany
Hybrid transceiver system	TR1001, RF Monolithics®, Dallas, USA
Infra Red spotlight	Kemo-Electronic, Langen, Germany
Interface chip FT232R	IC1, FTDI, Glasgow, UK
Microcomputer PIC12F675	Microship Technology Inc., Chandler, USA
Microcontroller PIC16F876A	Microship Technology Inc., Chandler, USA
Non-absorbable polyamide monofilament sutures 4-0	Resorba, Nürnberg, Germany
Picolo frame grabber	Euresys, Angleur, Belgium
Receiver	Institute of Physiology, Mainz, Germany
Silver-oxide batteries	Renata 399, 9.5 x 9.6 mm, 55 mAh
Stainless steel wires with HiFlex nylon coating, 0.3 mm	Griffin, Schramberg, Germany
Stereotaxic apparatus	Wild MSA,
Telemetric recording system	Institute of Physiology, Mainz, Germany

2.3 Software

Corel Draw 12	Corel GmbH, Unterschleiβheim, Germany
Delphi	Cupertino, CA, USA
Ethovision version 2.3	Noldus, Lilienthal, Germany
Excel MS Office 2000	Microsoft, Redmond, USA
JEDI Visual Component Library	JVCL, http://jvcl.sourceforge.net/
Matlab 7	The MathWorks Inc., Natick, USA
Matlab SON Library	KCL, London, UK
Reference Manager Pro 10	Reference Manager, USA
Spike2 version 5.16	Cambridge Electronic Design, Cambridge, England
Systat version 10	Systat Software, Erkrath, Germany
Time-Frequency Toolbox	http://tftb.nongnu.org
Word MS Officec 2000	Microsoft, Redmond, USA

3 Methods

The Methods part below is divided into two main parts: the validation of the new telemetric recording system (chapter 3.1) and the novelty detection experiments (chapter 3.2).

3.1 The telemetric recording system

3.1.1 Overview

The complete system (Lapray *et al.*, 2008) consists of an implantable transmitter which amplifies and performs the ADC of the recorded signal and communicates bidirectional with a receiver via radio transmission over a distance of up to several meters (Fig. 11).

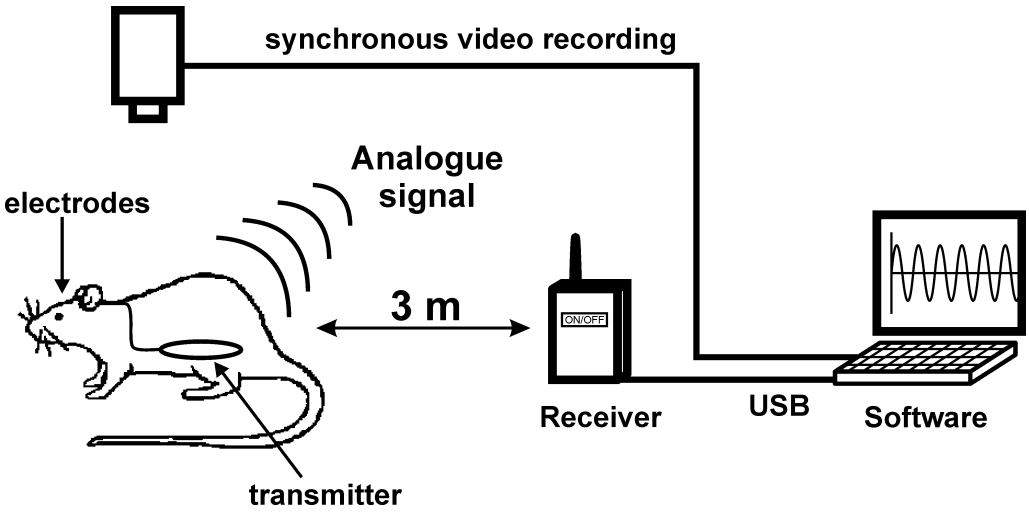


Figure 11: Overview of the telemetric recording system's main components (modified from Lapray *et al.*, 2008). The system is composed of the implantable transmitter, a receiver connected to a computer which displays and records the signal synchronously with the video of the freely moving animal.

The receiver is connected to a computer via an USB connection and the data are displayed and saved by a software developed for this purpose. A commercially available CED and Spike2 software can also be used to display and store the data on the host computer.

3.1.2 Implanted System

The implanted part of the telemetric system (Fig. 12) consists of a microcomputer (PIC12F675), an analogue input amplifier and a transceiver. An operational amplifier is also present to generate the virtual ground potential and a power switch in order to reduce the power dissipation during the standby mode. The system is assembled with two stacked printed circuit board (PCB) connected by wires. The power supply is composed of two small silver-oxide batteries, hold together by conductive glue and connected to the PCB by stainless steel wires. The first stage of the analogue input path is an instrumental amplifier with a ten times amplification. The input voltage is measured as a differential signal between the positive and the negative input. The reference potential (virtual Ground VGND) has a level of half of the battery voltage and is connected to the skull of the animal. The second stage is a standard non-inverting operational amplifier with a 500 times amplification and is DC-decoupled by a 2.2 µF non polarized capacitor. To avoid resonance effects a small capacitor with a value of 470 pF is connected in parallel to the resistor in the backward path. The analogue signal is internally digitised in the microcomputer with a resolution of 10 bits and a conversion time of 2 µs. The microcomputer controls the system status “running” and “standby”, the digitalisation, reception and transmission of radio signals. It contains a non-volatile EEPROM memory of 128 bytes where the parametric data are stored. The implanted system acts as a receiver and a transmitter following the

protocol illustrated below (Fig. 12 C).

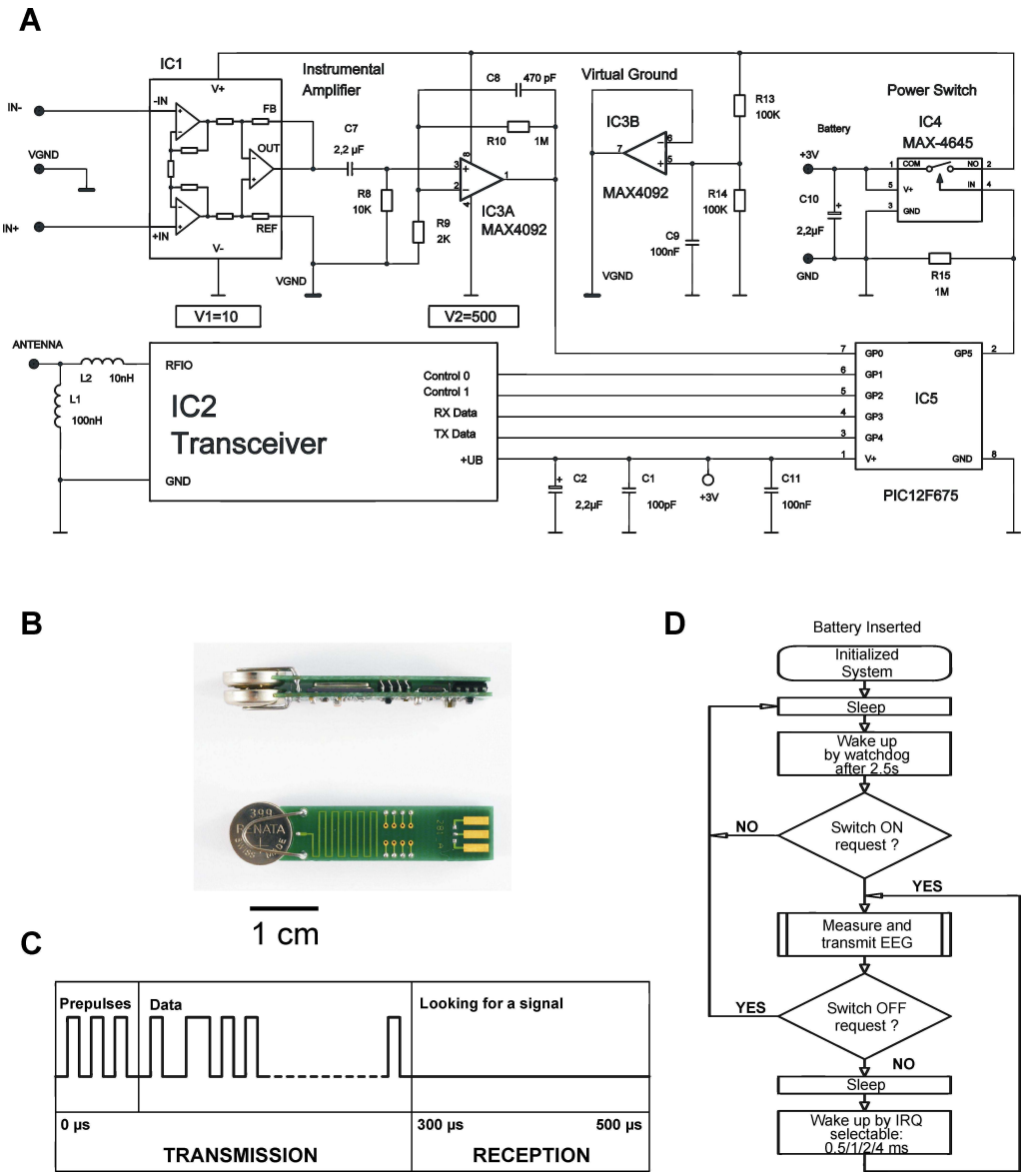


Figure 12: Properties and function of the implanted unit. (**A**) Electronic circuit diagram of the implanted unit. (**B**) Photograph of the telemetric device. (**C**) Illustration of the protocol, which starts with initiating pulses to adjust the automatic-gain-control of the receiver and subsequently transmits the data. After 300 µs the transceiver switches to the reception mode and can also detect other transmitters using the free ISM band. During this period instructions can be received to modify the system´s status. (**D**) Flowchart of the machine program (from Lapray *et al.*, 2008).

The hybrid transceiver system is available for European (868.35 MHz) and other ISM-band frequencies (e.g. USA 916.5 MHz). This part has a unique rapid switching time between reception and transmission of 20 µs in combination with a transmission data rate of 115.2 kb/s. After inserting the battery the system remains in a standby status with a low dissipation power of less than 2 µA. The implanted system "wakes up" every 2.5 s, switches the receiver ON and scans for an instruction to start. This operation takes less than 10 ms. If no signal is received, the system falls back to standby status. After receiving an ON signal the unit starts to measure and transmit the data until it receives an OFF instruction. The acquisition rate can be pre-selected by software from 250 Hz up to 2 kHz. In order to minimize the size of the system most of the parts are **S**urface **M**ount **D**evices with a size of 0402.
Before implantation, electrode wires (0.3 mm stainless steel wires) with small connectors at their tip are plugged on stainless steal pins on the transmitter and a small layer of epoxy is applied on the connectors to protect them. These wires can easily be replaced to reuse the system for new implantations. The transmitter is then covered with two layers of a biocompatible silicone. For this the system is dived in the silicone and left one night to dry. Between the two layers of silicone and the system one layer of water-soluble separating agent is first applied with a brush in order to facilitate the cleaning of the transmitter after extraction from the animal at the end of the experiments. The shape of the silicone is made as round and smooth as possible with a cotton bud to avoid discomfort and distress of the animals.

3.1.3 Control System

The receiving and control system is composed of a personal computer and an interface box connected via standard USB (Fig. 13). The receiver of the

interface is the same as in the implanted units except some modifications to adapt the high frequency power and the modulation depth. According to the received data rate the implemented microcontroller (PIC16F876A) is connected by a high speed serial interface (2.5 mbps) to a special interface chip (FT232R) which converts the serial data stream into the complex USB protocol. The program is written in machine language to optimise the usage of memory space and processing speed. Because the microcontroller has the ability to write on its own program memory the software can be updated by the PC.

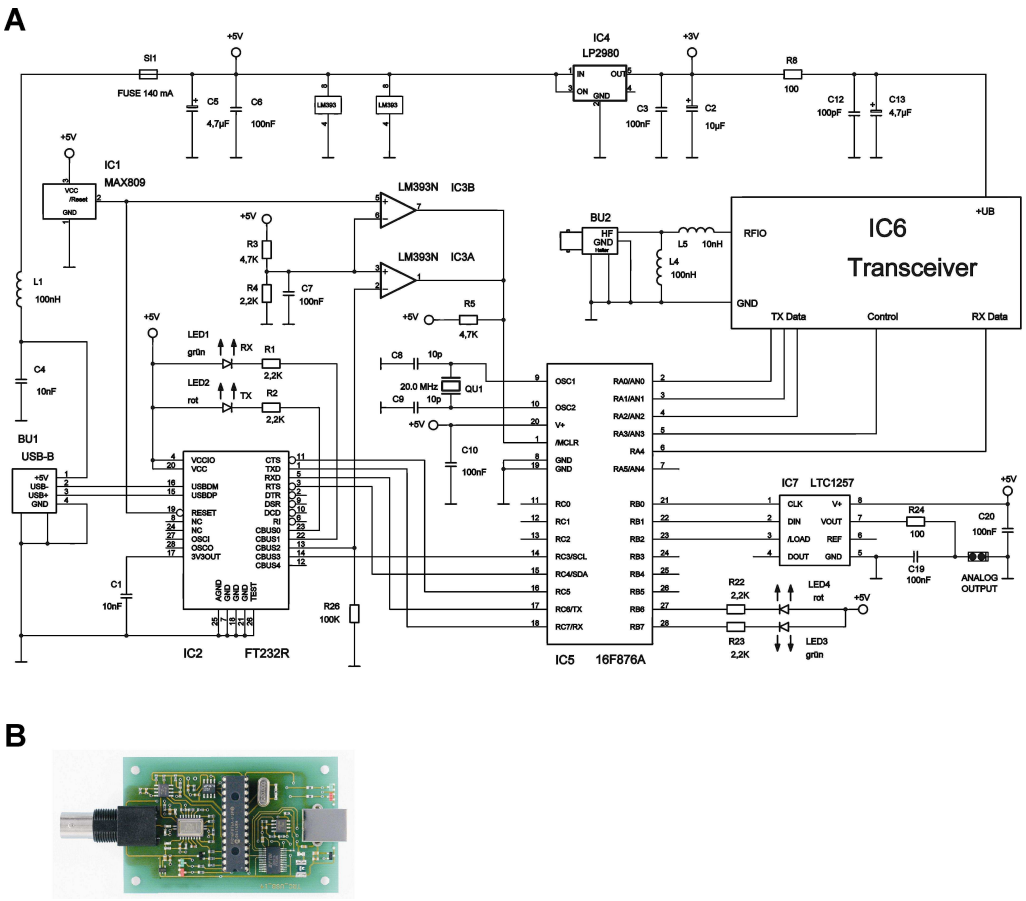


Figure 13: The receiver. (A) Electronic circuit diagram of the control interface and (B) photograph of the USB unit (from Lapray *et al.*, 2008).

3.1.4 PC software

The PC software consists of three independent modules for (1) capturing data, (2) replaying/analysing data, and (3) software servicing the control system and the implanted devices. In the capture module the user has to define the parameters of the capture session (e.g. sampling rate) and whether parallel to the EEG data acquisition video recording data will be collected. The user can also define a detailed time schedule describing the points of time and the duration of each recording session. After initialisation of the control device the software starts the data transmission of the implanted system. All data will be sent as a continuous stream in the predefined sample rate. For quality insurance each data block contains a unique identification and a successive numbering so that a loss of single data packages during wireless transmission can be identified. The data will be written on the PC hard disk in a binary format together with a timestamp. In parallel, the received data will be displayed on the PC screen in scope-like fashion. In the case that a video camera is attached to the PC via an USB port the software is capable to capture a video stream synchronously to the EEG data recording on the PC hard disk. Due to the large amount of data in this mode the rate of the real-time EEG data display on the scope is reduced. However, due to extensive buffering a loss of data can be ruled out. Terminating the capture session can be initiated either manually or automatically by the predefined time schedule. For this reason repetitive recordings over a long period of several days without the presence of a human operator are possible which may be advantageous in the case of long-term behavioural studies. The replay software module reads the EEG data together with the stored video stream and displays them synchronously on the screen. With a time mark the user can indicate time intervals of interest. The accompanying EEG data of these time frames can either be stored separately on the hard disk or be exported in a format compatible for use with other

analysis software (e.g., Spike2, MatLab). In this module the incorporation of individual specific analysis routines is also possible.
The service module of the software can be used to parameterise (e.g. by setting the sample rate) the implanted devices. The whole software package is written in Delphi using modules from the JEDI Visual Component Library. Besides the large number of EEG data, video streaming produces extensive file sizes. For this reason, a suitable size of the hard disk is necessary.

3.1.5 Surgical implantation

3.1.5.1 Transmitter disinfection

Before any implantation, the transmitter was dived in a cold sterilising solution (Actril) for 5.5 h at room temperature. Thereafter, the transmitter was rinsed three times with sterile saline and then soaked in sterile saline for a minimum of 5.5 h. The transmitter could be left in the sterile saline until implantation within 48 h (http://www.datasci.com/information/technotes/392-0027-020.asp).

3.1.5.2 Surgery

All experiments were conducted in accordance with the national and European (86/609/EEC) laws for the use of animals in research and were approved by the local ethical committee (Landesuntersuchungsamt Koblenz, 23 177-07/G07-1-001). Six and 5 male Wistar rats, respectively, weighing 250-400 g were used for testing the running system and the novelty detection experiments. The animals were housed individually in standard plastic cages (42x26x20 cm^3) under a 12 h light-dark cycle (lights on at 7 am). The room temperature was

maintained at 21±2°C and relative humidity at 50±5%. Standard rodent food and tap water were available *ad libitum*.

The surgery was performed after relaxing the animal with isoflurane and then a deep anaesthesia was induced with an intra-peritoneal (IP) injection of chloral hydrate (400 mg/kg). This anaesthetic agent is known to be a good myorelaxant but has a pretty bad analgesic effect, therefore it was followed after 10 min by the i.p. injection of ketamine (1 ml/kg, ketamine 500 mg/10 ml) and by the subcutaneous (SC) injection of atropine (0.8 mg/kg). This latter was used to decrease the effect of ketamine on the heart rate and to prevent secretions in the throat of the animal. The anaesthesia was maintained by ketamine injections during the whole surgery. For a better recovery all rats were given an antibiotic (i.p.) (0.1 ml Baytril® 2.5 %) and an analgesic treatment (SC) (0.05 ml Rimadyl®). Used for canine osteoarthritis and post-operative pain management, Rimadyl has a strong effect on rats for a period of 48 h.

The area from the part between the eyes, back between the ears and across the neck as well as the abdominal skin were clipped and cleaned with iodine. The animal was then placed on a heating pad and an incision was made in the left part of the abdomen's mid-line, 1 cm caudal to the xyphoid cartilage, and the transmitter was placed in the peritoneal cavity (Fig. 14A). The unit could be sutured to the body wall with non-absorbable polyamide monofilament preventing any risk of torsion of the system around the gut. After being secured in the stereotaxic apparatus a 4 cm mid-sagittal incision was made on the scalp and the skin reflected with hemostats to expose the entire skull. A way for the leads was made between the skin and muscles by moving aside connective tissue and transmitter leads were slipped subcutaneously from the abdomen to the incision made on the head. The skull was then cleaned and dried with sterile saline and hydrogen peroxide and ridged with a blade to allow a good contact with the grip cement (Fig. 14B). The Bregma was marked according to the coordinates (Paxinos & Watson, 1998) and 3 holes were made through the

skull with a driller fixed on the stereotaxic apparatus in order to control the descent and avoid any brain damage or bleeding. The recording electrode was placed above the somatosensory cortex (L=β+5.5 mm, AP=β-2.3 mm), the reference and the ground electrodes were placed above the cerebellum always at the same coordinates (Fig. 14C). Three stainless-steel screws (0.5 mm diameter) were used as electrodes and soldered to the leads. The assembling was anchored in place with grip cement. Both incision sites were closed using 4-0 Resolon, cleaned with sterile saline and iodine. Surgery lasted a maximum of 3 h from induction of anaesthesia and the success rate was 100% for recovery from anaesthesia and surgery.

The animals were weighed daily throughout the experiment as an indicator of general health.

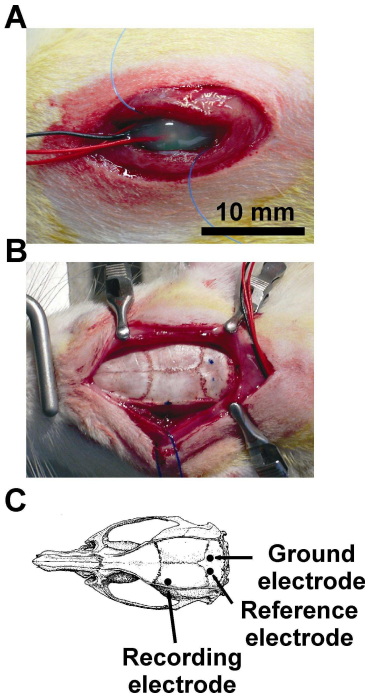


Figure 14: The surgery. A) The system is placed in the abdominal cavity and the wires are slipped through the skin up to the head incision where the B) skull was cleaned and dried. C) The recording electrode was placed above the barrel cortex whereas reference and ground electrodes were placed above the cerebellum (from Lapray *et al.*, 2008).

3.1.6 Data recordings for the testing system

After surgery, a recovery period of 5 days was given to the animals before starting the first recording session, corresponding to the time needed to gain the pre-surgical weight again. The rats were placed in the recording field that could be a cage, an open field, a labyrinth or a water maze. The recording system was switched ON through the receiver and the signal directly observed on a computer's screen. The signal was recorded with our software at a sampling rate of 500 Hz and saved on the hard-disk with the synchronous video recording. Data were then imported to MatLab 7 for further analysis. Frequency analysis was performed by the use of Fast Fourier Transform (“fft.m”, one epoch of 100 s) with a band-pass filter between 1 and 80 Hz (Butterworth 3rd order filter, “butter.m”).

3.1.7 Statistics for the first set of experiments

Statistical analyses were performed with Systat Version 10. Values throughout this thesis are given as mean ± SEM. For statistical comparisons, an unpaired samples t-test was performed.

3.2 Novelty detection experiments

3.2.1 Habituation procedure

It is well known that stress can be reduced by repetitive contacts with the stress generator (Whishaw, 2004), therefore the animals were handled and weighted twice a day for 2 days (first session always between 09:00 and 11:00 and second between 14:00 and 16:00, see experimental protocole Fig. 15) in the animal facility.

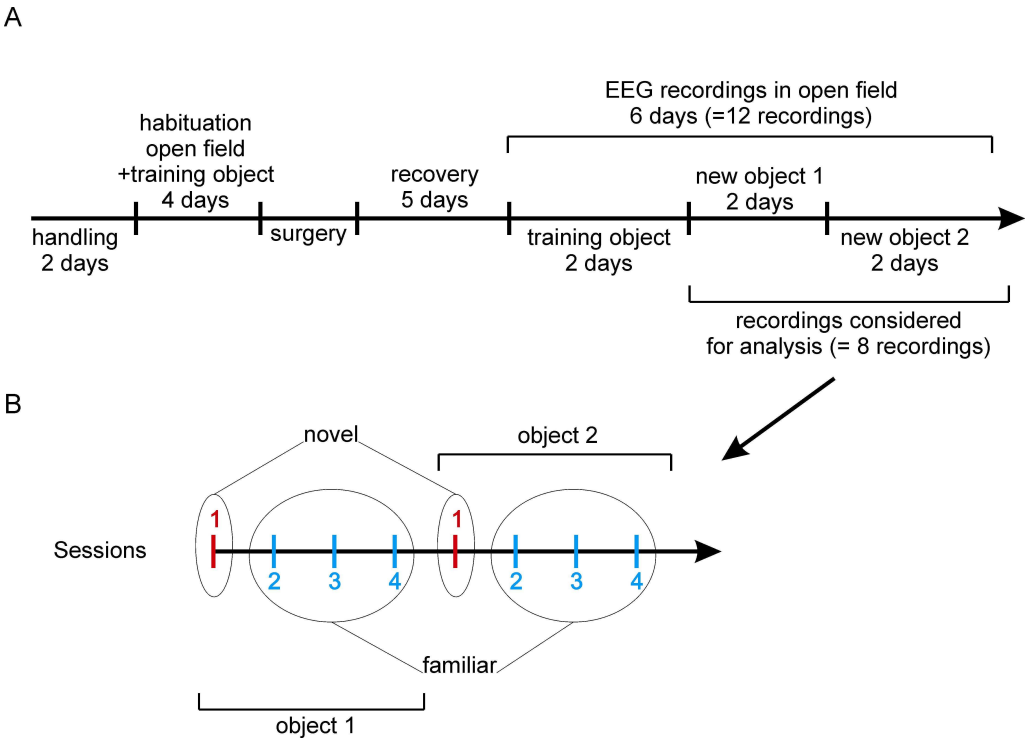


Figure 15: Experimental design. A) After 2 days of handling twice a day the animals were habituated to the recording environment. The surgery was then performed and after 5 recovery days the animals were recorded for 6 days in total, 2 days per object (modified from Lapray *et al., accepted*). B) The first session with each new object are pooled together in the novel condition. The 3 other sessions per object are pooled together in the familiar condition.

Handling procedure were progressive in duration to not induce any aversion between the experimenter and subjects. The rats were habituated to be carried in hands without gloves in order for the animals to associate this action with a non dangerous situation. The handling lab coat was not washed for the complete set of experiments and the same soap was used for hands cleaning to not introduce anything new in the procedure. When no apparent stress could be seen during handling sessions (no escape, cleaned fur, no screams, …) the animals were then transported in their own cage in a close metallic rolling cupboard (see Fig. 16A) twice a day in the recording room.

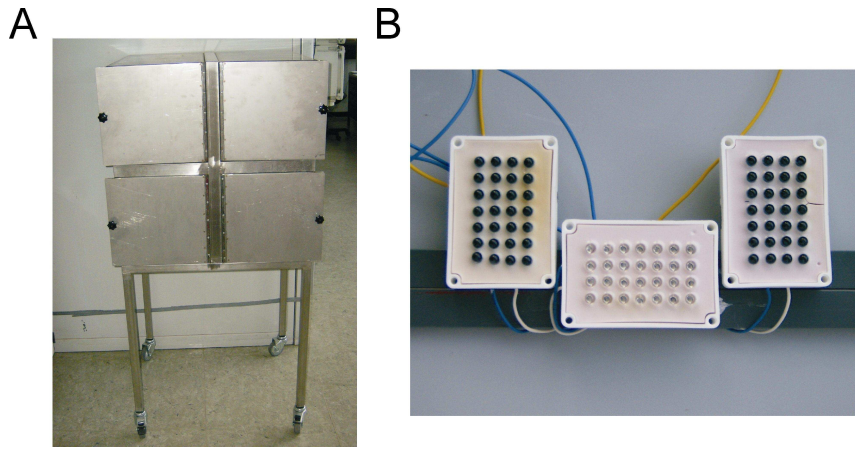


Figure 16: rolling cupboard and IR spotlights. (**A**) The home made rolling cupboard was used to transport the animals from the animal facility to the recording room. This allowed the animal to be separated from the other while staying in their own cage and the metallic structure was a perfect Faraday cage isolating the different telemetric recording systems from the one under recording. (B) These 3 IR spotlights were used to illuminate the object and the whiskers of the animal in the openfield without warming the environment.

When removed from the cupboard, one after the other, they were placed in an isolated metallic chamber for 10 min to allow the animal to relax since the transport and the new environment conditions could be, depending on the animal, a big stress generator. The rat was then carried in an open field placed at the centre of this chamber for 10 min in the dark. In the open field an object

(training object, see Fig. 17) was present always at the same place near the wall with a camera above it and an home made infrared (IR) lighting structure (Fig. 16B). This was composed of 3 IR spotlights, each one composed of 30 IR Light-Emitting Diodes (LEDs), positioned at an optimal angle to highlight the whiskers of the animal when approaching the object. NonLED IR light sources were not used because this kind of equipment produces a lot of heat which would change the condition of recording in the time. The animal would have probably avoided the warmer place e.g. the highlighted object. Between each session the open field and the object were cleaned with 0.1% acetic acid.

3.2.2 Surgical procedure

After the habituation period the animals were operated following the same surgical procedure as described above for the telemetric system validation experiments.

3.2.3 EEG and video recordings

After surgery, a recovery period of 5 days was given to the animals before starting the first recording session. ECoG signal (recorded continuously at a sampling rate of 1000 Hz) and video data (25 frames/s, 720*576 pixels) were collected simultaneously and stored on a personal computer via a CED and Spike2 software (Lapray *et al.*, *accepted*). In order to define the whisker-object contacts a CCD camera with progressive-scan sensor was used with a fixed shutter-speed (1/250 s) to record 25 high-resolution pictures per second. The camera was placed around 30 cm above the object in order to well visualise the area around the object, the object, the animal and his whiskers (Fig. 18). The

rats were placed during 2 days in the open field, twice per day for 10 min with the “training object” at the same place as before (first session always between 09:00 and 11:00 and second between 14:00 and 16:00). In the following 2 days the object was substituted by a “new object” before a new object exchange (Fig. 17). The objects were chosen to be as different as possible to each other and to the objects the animals got to know in the standard animal facility. Each rat was recorded 120 min in total corresponding to 40 min per object.

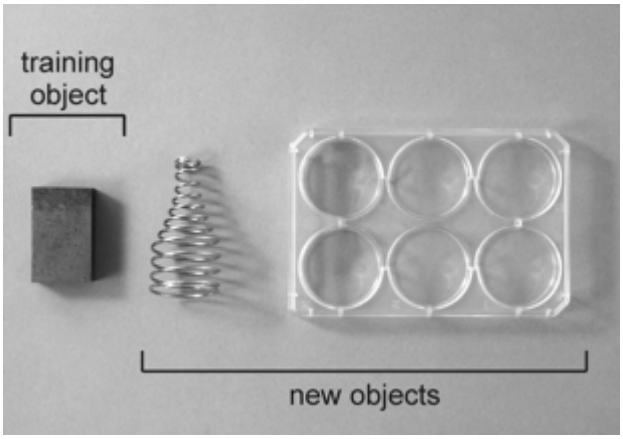


Figure 17: The different objects used in the novelty detection experiments (modified from Lapray *et al.*, *accepted*).

3.2.4 Data analysis

3.2.4.1 Trial definition

First, a video analysis was performed to define 1 s long exploration trials starting every time the animal moved toward the object and touched the object with the whiskers contralateral to the cortical recording site (Fig. 18). The eight recording sessions with the two new objects (4 per object) were analysed, whereas the 4 recording sessions with the “training object”, were not

considered for this study. The 8 sessions with the novel objects were separated into 2 groups: the first session for each new object were averaged, pooled together in the “novel condition” (n=2 sessions) and the 3 remaining sessions for each object in the “familiar condition” (n=6 sessions).

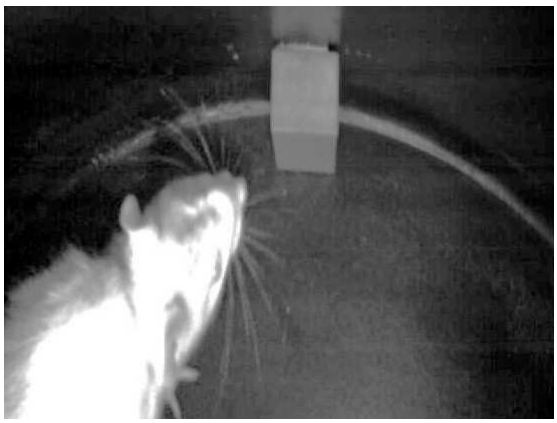


Figure 18: Contact object-whiskers. Under IR light the animal explores one open field where an object, in this case the training object, is placed always at the same place near the wall. Exploration trials are defined by the movement of the animal toward the object and its contact with the whiskers contralateral to the recording site.

3.2.4.2 Signal processing of the novelty detection trials

All trials were first analysed by eye and rejected whenever they presented any ECoG artefacts. The data were imported to MatLab 7 and further data analysis were performed as previously described (Lachaux *et al.*, 2000; Rodriguez *et al.*, 1999). The signal was band-pass filtered (20-80 Hz, Butterworth 3rd order filter, “butter.m”) and a Hilbert transform (“hilbert.m”) was applied on each trial followed by a pseudo-Wigner-Ville transform (Time-Frequency toolbox, http://tftb.nongnu.org, “cwt.m”). This method has been shown to be particularly appropriated for the detection of induced γ activities in non-stationary signals. The resulting time-frequency maps were normalized (using

maximum and minimum peaks for each map) and averaged over trials (n=63 and 156 trials for the novel and familiar conditions, respectively) and animals (n=5). Further statistical analysis were performed in 5 Hz frequency bands that were extracted from these maps with an overlap of 2.5 Hz. *P* values were calculated with parametric unpaired samples t-tests after testing the Gaussian distribution of the time-frequency relative power samples. After analysing all frequencies in the complete gamma band, only the bands showing significant differences between novel and familiar conditions were taken into account and further analysed session by session. *P* values were then calculated with a Dunnett's multiple comparison test after testing the Gaussian distribution of the samples and calculated using one-way ANOVA.

The total time spent on the objects during the first and second session as well as the number of object explorations were calculated as a measurement of novelty detection (Dere *et al.*, 2007) and level of exploratory activity, respectively. *P* values were calculated with paired *t*-test after testing the Gaussian distribution of the samples. Values are given as mean ± s.e.m.

4 Results

4.1 Impact of the implanted telemetric recording system on animal behaviour

The validation of the new wireless unit necessitated to investigate, after the surgical transmitter's implantation (described in Materials & Methods, page 32), the general impact on the animals' behaviour and the quality of the collected data in comparison with known brain activities (Lapray *et al.*, 2008; Lapray *et al.*, *in press*).

4.1.1 Post-surgical animals' behaviour

Wireless technology has been developed to minimise the stress induced by traditional *in vivo* techniques such as the tethered model (see details in the Introduction, page 6) to record freely moving animals. It was then crucial to control several post-surgical behaviours.

4.1.1.1 Animals' welfare

Laboratory rats are known to gain on average 4-5 g a day during the first months of their life (Whishaw, 2004) and any uncomfortable situation results within body weight loss. The six animals used for this study were then daily weighted as a first health indicator. All the implantations were followed by a typical loss of weight and two days after the surgical operation a minimum peak was reached corresponding to a loss of 5.4±0.4% of pre-surgery body

weight. Five days after the implantation the animals started to gain weight and reached the initial value after ten days (Fig. 19). Also no infections or mortality due to the implantation were observed.

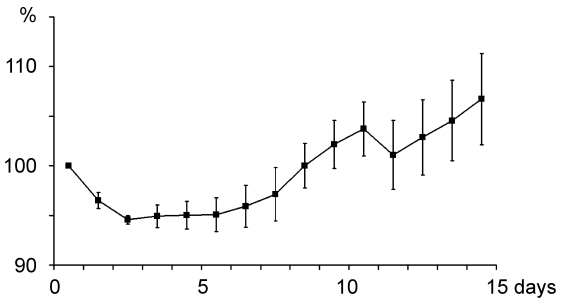


Figure 19: Time course of the post-surgery weight.

Several other qualitative parameters can be easily controlled to detect any discomfort induced by an experimental protocol. The general aspect of the animal is a very good indication of comfort or discomfort. Particularly visible in albinos rats the fur must be clean and no pilo-erection visible. The eyes must be protruded and ears and paws pink coloured as an indication of a good blood circulation. Furthermore rats are curious and must be interested by anything that happened in their environment. The daily visit of the experimenter must therefore induce attention and exploration (Wolfensoh & Lloyd, 1998). All these parameters have been under surveillance and no particular sign of discomfort or pain have been noticed in the implanted animals.

Finally stressors have a prominent effect on sleep in general and it is known that sleep deprivation disrupts vital biological processes that are necessary for cognitive ability and physical health (Everson, 1995). Rodents have a particular sleeping position, they curl up (Morton *et al.*, 2003; Tang *et al.*, 2007) and anything that would disturb it would generate stress and the associated effects. The six animals used in this study were all able to curl up to sleep already few hours after the end of the surgery (Fig. 20).

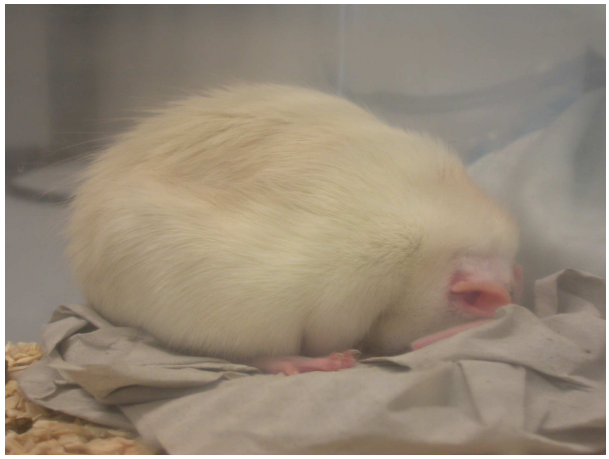


Figure 20: The sleeping posture. Already few hours after the surgery, the animal was able to curl up to sleep, a typical sleeping posture in rodents.

4.1.1.2 Animals' locomotion

To test the impact of the system on the animals' movements, rats were placed for 10 min in an empty open field (60x70 cm^2) and a behavioural recording was performed with EthoVision (details about equipment can be seen in Materials & Methods, pages 23 to 25). Rats as many rodents avoid free spaces (Whishaw, 2004) and then explore their environment following the walls with some rapid and short crossing in the middle of the area. This exploratory behaviour was observed and comparable for both implanted and non-implanted animals in the open field (Fig. 21). In addition, to quantify the impact of the transmitter on the animals' locomotion the total distance covered during the explorative session and the velocity were considered for further analysis. No significant differences were observed for these two parameters (30.3±1.7 m and 5.1±0.3 cm/s for the control animals versus 29.2±1.7 m and 4.9±0.3 cm/s for the implanted animals, each group n=6). It was concluded that the device did not restrict limb movements during locomotion, one prerogative for the development of such a technique.

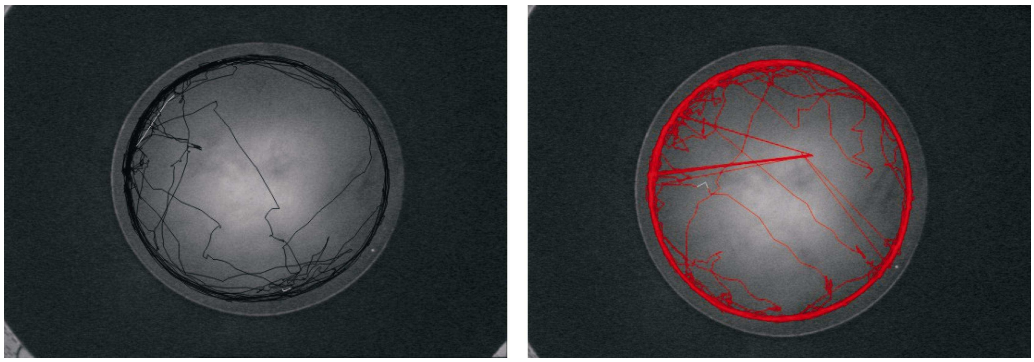


Figure 21: Explorative pattern comparison between control and implanted animals in an empty open field. The left panel shows the exploratory way followed by a non-implanted animal and in the right panel that of an implanted rat in the same conditions.

4.1.2 Quality of the recorded ECoG

The general animals' behaviour was not impaired by the implantation of the transmitter and it was then necessary, in order to complete the validation of the new system, to collect brain electrical data from behaving animals and to compare them with known studies. Animals were then recorded during three distinct states easily separable from the ECoG traces: wakefulness, slow-wave sleep and under isoflurane anaesthesia (Fig. 22). ECoG recordings were measured with a sampling rate of 500 Hz in a normal laboratory setting with no precautions taken to limit any kind of external electromagnetic interferences.
Wakefulness was characterised by a so-called "desynchronised" low-amplitude ECoG with dominant activity in the 4-8 Hz band whereas slow-wave sleep was clearly distinguished by a synchronised ECoG with high-voltage slow-waves in the δ frequency range (1-4 Hz) and some spindles (10-14 Hz) in an immobile animal with closed eyes as reported by many studies (Franken *et al.*, 1998; Gervasoni *et al.*, 2000; Timo-Iaria *et al.*, 1970). Under isoflurane anaesthesia burst-suppression pattern characterised by a depressed background activity

alternating with high voltage activity were observed as described in the literature (Hudetz, 2002).

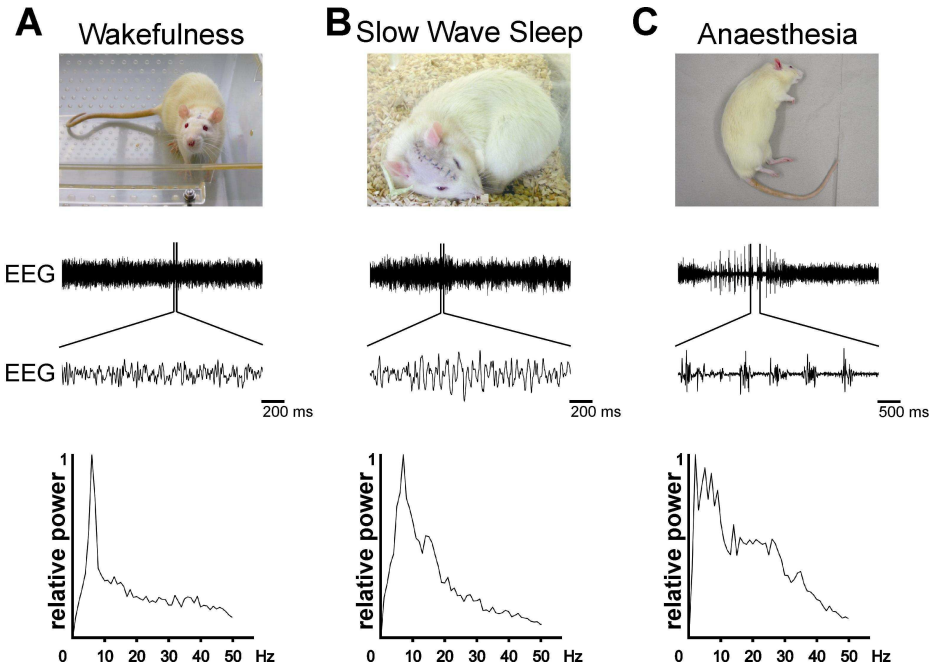


Figure 22: ECoG recordings of distinct brain states. The animals were recorded during A) wakefulness, B) slow wave sleep activity and C) under isoflurane anaesthesia. The recorded ECoG traces were similar to what has been described in the literature (modified from Lapray *et al.*, 2008).

4.1.3 Behaviour-ECoG monitoring in different environments

Wireless technology has also been developed to record behaving animals in a wide quantity of environments therefore the animals were recorded under different environmental conditions. ECoG recordings were then performed in an open field (60x70 cm^2) (Fig. 23A), in a two arms maze (Fig. 23B) and in water, three environments intensively use in traditional behavioural protocols (Whishaw, 2004). These recordings were performed with a distance between

the transmitter and the receiver of 0.2 to 3 m. The signal was devoid of movement artefacts during the recording sessions except for the grooming behaviour where some electrical spikes could be observed especially when the animal was cleaning its belly. These artefacts can be easily identified by the video monitoring and removed off line.

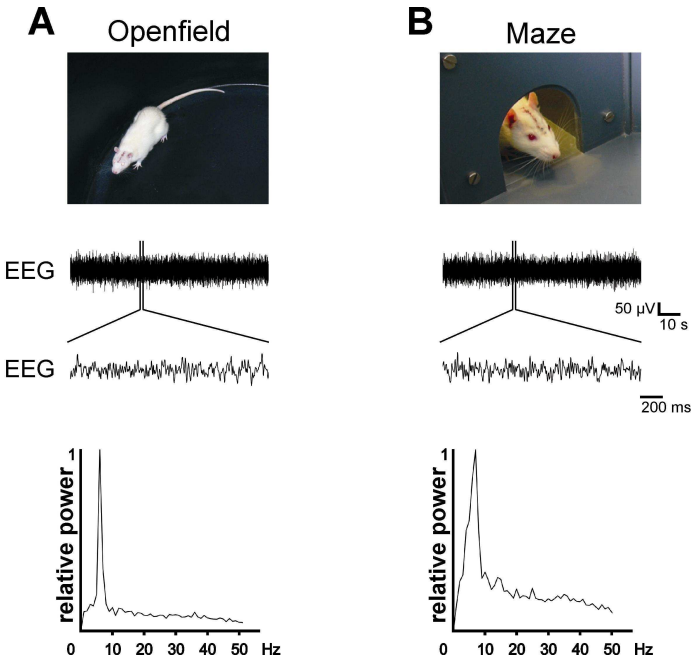


Figure 23: Quality of the signal in different environments. The animal was recorded in A) an openfield and B) a 2 arms maze (modified from Lapray *et al.*, 2008).

The two arms maze corresponded to a circular central platform equipped with guillotine doors and two out of the eight possible arms were opened. This method is traditionally used in learning and memory associated experiments (Whishaw, 2004). The water maze test is also widely used in behavioural experiments with the famous Morris test. We recorded then the animals in an open field full of water (around 150 litres). The signal recorded was devoid of distortions and stable in an area of 30 cm around the antenna when this one was placed beside the open field at a level below the water's surface.

The developed telemetric recording system would then make possible the synchronous recordings of brain electrical activity as well as behaviour in environments where the use of traditional methods were prohibited.

4.2 Novelty Detection results

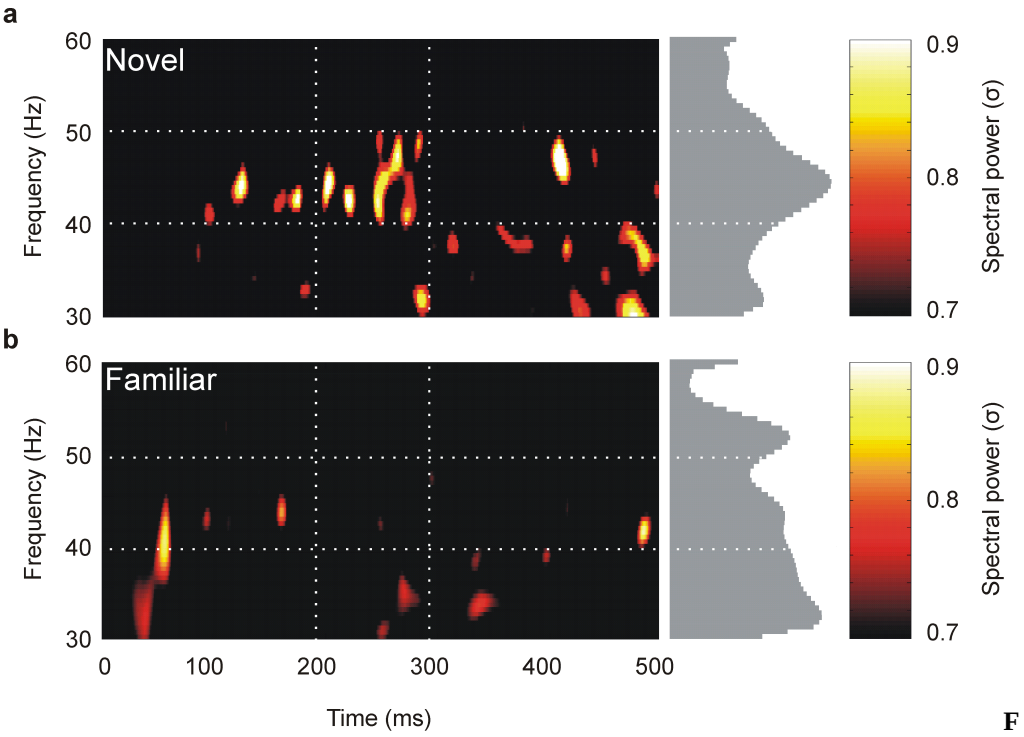
4.2.1 Perception of novelty

The investigation of novelty detection necessitated the use of behavioural parameters highlighting the perception of novelty by the animal. It has been previously documented that rats spend significantly more time in exploring a new object compared to a familiar one (Steckler *et al.*, 1998). Therefore the total time spent by the animals exploring the new objects during the first two experimental sessions were calculated. The exploration time spent during the first session amounted to 51.6 ± 11.2 s (n=10 sessions) and was significantly ($p=0.0314$) longer when compared to the second session recorded later the same day (31.1 ± 4.4 s). This result could not be explained by a reduction of the overall activity during the second session, calculated through the number of contacts made by the animal with the object, since the animals presented a similar exploratory activity during the first (11.7 ± 1 exploration occurrences) and the second (12.6 ± 1.2 exploration occurrences) session. These behavioural data indicate that the animals perceived the object as novel only during the first session which allowed then the comparison of the brain activities for the novel and the familiar conditions.

4.2.2 Novelty related cortical activity

The averaged time-frequency maps (see calculation details in Materials & Methods, page 40) revealed prominent differences in the ECoG activity during exploration of a novel versus a familiar object (Fig. 24 below). One distinct activation period could be distinguished 200 to 300 ms after the initial contact of the whiskers with the novel object when compared to the familiar condition.

The relative power of each frequency in the ECoG during this time window displayed two major differences between exploration of a novel versus a familiar object. The first one is a peak at ~45 Hz specific to the novel condition (Fig. 24a). After analysing in detail the complete 20-80 Hz frequency range it was possible to restrict this activity to a relatively narrow γ frequency band between 40-47 Hz (see Figs. 24 and 25).



F

igure 24: Average time-frequency maps of the ECoG recorded above the barrel cortex of 5 adult rats between 30 and 60 Hz. Pseudo-Wigner-Ville transforms were averaged over all trials and subjects (n=5) for the (a) novel (n=63 trials) and (b) familiar (n=156 trials) conditions. Significant differences in the ECoG γ band activity between novel and familiar conditions could only be detected in the frequency ranges 40-47 Hz and 30-37 Hz between 200 and 300 ms after the whiskers' first contact with the object. The relative power of each frequency between 200 and 300 ms after the first whisker contact is illustrated on the right part of each map (grey traces) (from Lapray et al., *accepted*).

This activity with a maximum frequency of 43.3 ± 0.26 Hz (n=63 contacts) peaked at ~255 ms after the first contact of the whiskers with the novel object and was highly specific to the novel condition (p=0.0084) (Fig. 25). The analysis of this band during the successive sessions of recording revealed that this response slightly, but not significantly, decreased during the second session and significantly declined in the subsequent two sessions (between 220 and 285 ms; Fig. 25b). This induced synchronized activity could be seen in the filtered 500 ms raw signal (Fig. 26) to consist of at least 5 cycles.

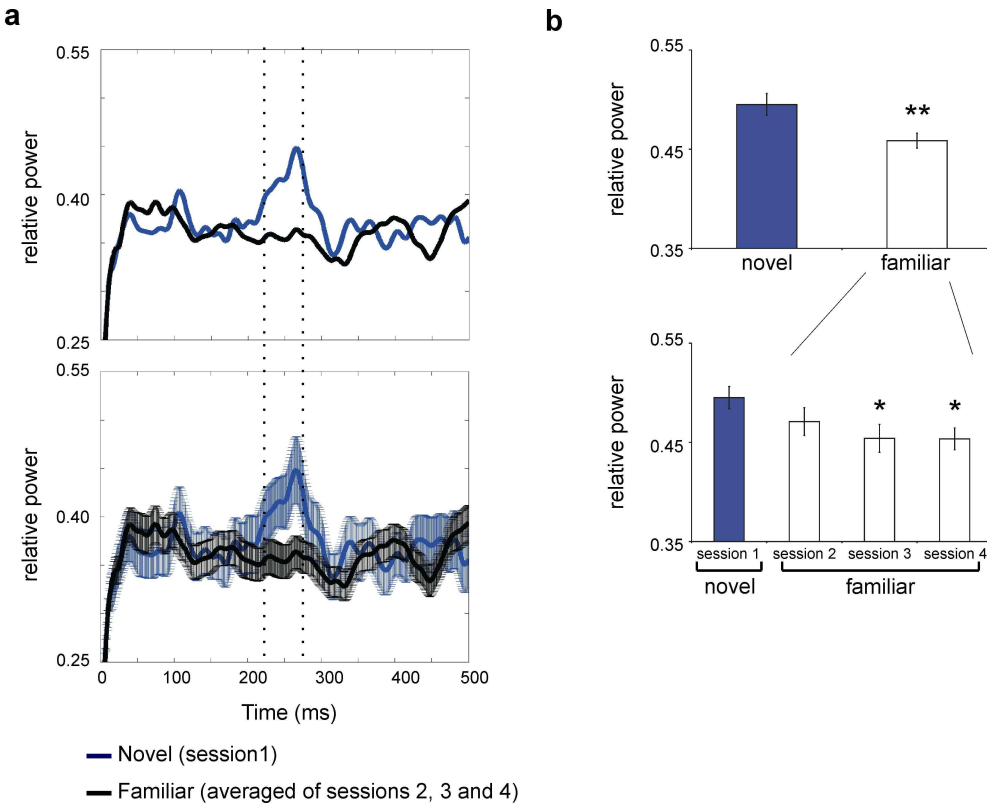


Figure 25: Statistical analyses of the novel object related γ peak in the 40-47 Hz frequency band. This band frequency was one of the only two bands showing significant differences between novel and familiar conditions after testing the broadband γ range. a) The time course of the averaged relative power of the 40-47 Hz band for the novel (n=63 trials) and familiar (n=156 trials) condition is plotted (50 points smooth) without (upper graph) and with s.e.m. for each ms (lower graph). One distinct peak in the novel condition is observed with a significantly higher power between 220-285 ms after the first contact of the whiskers with the

object. b) The relative power of the band frequency is first compared between novel (n=63 trials) and familiar (n=156 trials) conditions using an unpaired t-test (upper histogram). The familiar condition was then decomposed in the different sessions (lower histogram), every bar corresponding to the average of 2 recording sessions (one per object; session 1 = 63, session 2 = 50, session 3 = 55 and session 4 = 51 trials) in 5 recorded animals and P values were calculated with a Dunnett's multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) comparing each session to the first one (novel condition) after calculating a one-way ANOVA. The observed response slightly, but not significantly, decreased during the second session and significantly declined in the subsequent two sessions (modified from Lapray *et al.*, *accepted*).

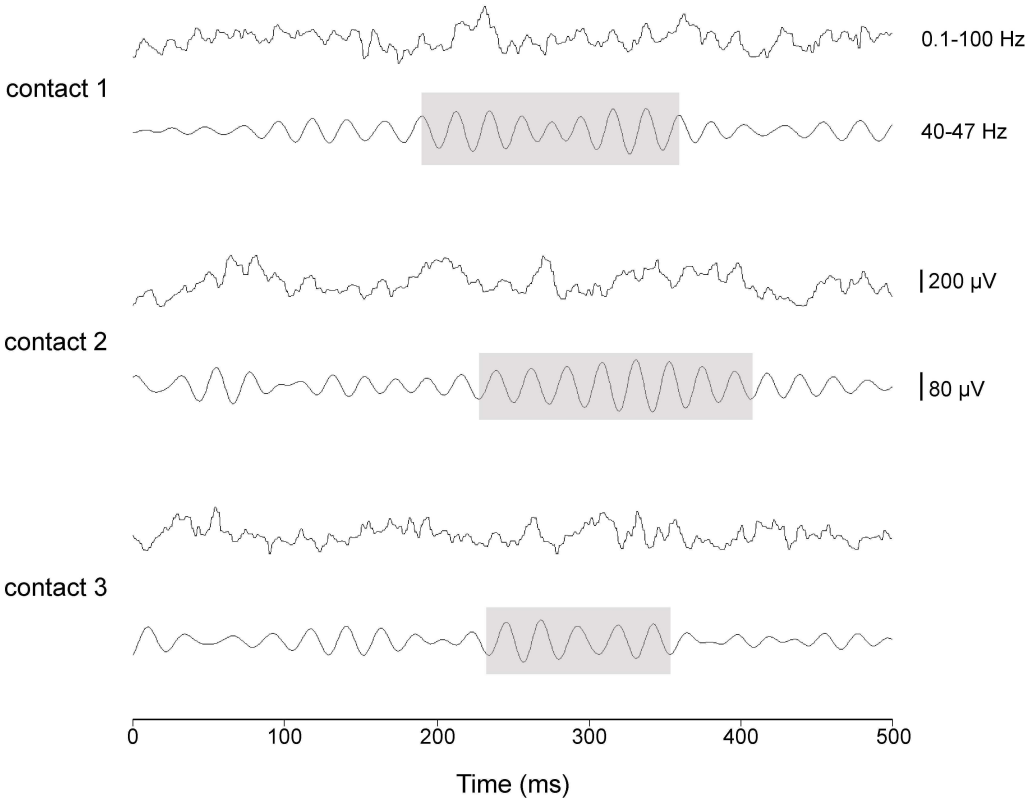


Figure 26: Raw and filtered ECoG traces after contact of the whiskers with a novel object. Three 500 ms ECoG traces are displayed corresponding to 3 successive contacts performed by the same animal during one session with a novel object. For each trial the filtered trace between 40 and 47 Hz using a Butterworth 3rd order filter is shown below. Induced oscillations in the γ range can be observed between 200 and 400 ms after the first contact with the novel object (gray rectangles) (from Lapray *et al.*, *accepted*).

This activity was accompanied by a decrease in the low γ range (Fig. 24) during the first 400 ms. This activity was restricted to the 30-37 Hz band frequency (Fig. 27a) with a robust and significant (p=0.0007) decrease in the relative power between 200 and 290 ms (34.6 ± 0.25 Hz peaked at ~254 ms, n=63 contacts) when compared to the familiar condition (n=156 contacts) (Fig. 27b). This activity significantly increased immediately during the second session and remained stable in the two subsequent sessions.

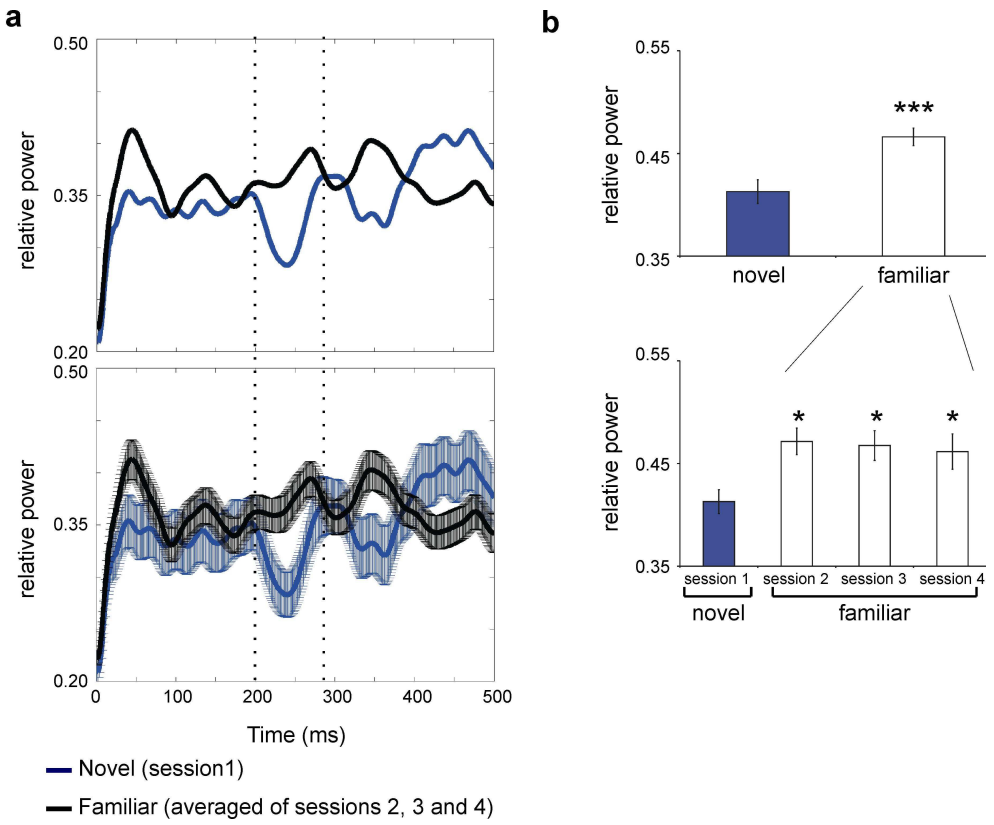


Figure 27: Statistical analyses of decrease in the 30-37 Hz frequency band related to the novel object. This band frequency was the second band showing significant differences between novel and familiar conditions after testing the broadband γ range. a) The time course of the averaged relative power of the 30-37 Hz band for the novel (n=63 trials) and familiar (n=156 trials) condition is plotted (50 points smooth) without (upper graph) and with s.e.m. for each ms (lower graph). One distinct decrease is observed in the same time window as the 40-47 Hz band frequency increase (cf. Figs. 25, 26) with a significantly lower power between 200 290 ms after the first contact of the whiskers with the object. b) The relative power of the

activity band was first compared between the novel (n=63 trials) and familiar (n=156 trials) conditions using an unpaired t-test (upper histogram). The familiar condition was then decomposed in the different sessions (lower histogram), every bar corresponding to an average of 2 recording sessions (one per object; session 1 = 63, session 2 = 50, session 3 = 55 and session 4 = 51 trials) in 5 recorded animals and P values were calculated with a Dunnett's multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) comparing each session to the first one (novel condition) after calculating a one-way ANOVA. This decrease disappeared significantly already after one session and reached a stable level (modified from Lapray *et al.*, *accepted*).

The novel object related increase in the 40-47 Hz and decrease in the 30-37 Hz bands could only be observed for the first session with the new object and were independent of the interval between the 4 successive recording sessions. These data indicate a strong relationship between these two neocortical activity patterns and the detection of a novel object.

4.2.3 Novelty unrelated cortical activities

The 1 s trials analysis showed also late γ activations (Fig. 28) significantly stronger in the novel condition compared to the familiar one. The first late γ response induced by a novel object was centred at 43.9±0.3 Hz (n=63), peaked at ~675 ms, and the second activity pattern occurred ~960 ms after the initial contact and revealed a peak in the power spectrum at 44±0.3 Hz (n=63). In contrast to the γ activity observed at ~255 ms, the latter two γ components were more influenced by the interval between the recording sessions. These late components (>500 ms) could not directly be correlated to the process of exploration and detection of a novel object, since they were not only present during the first session (Fig. 28).

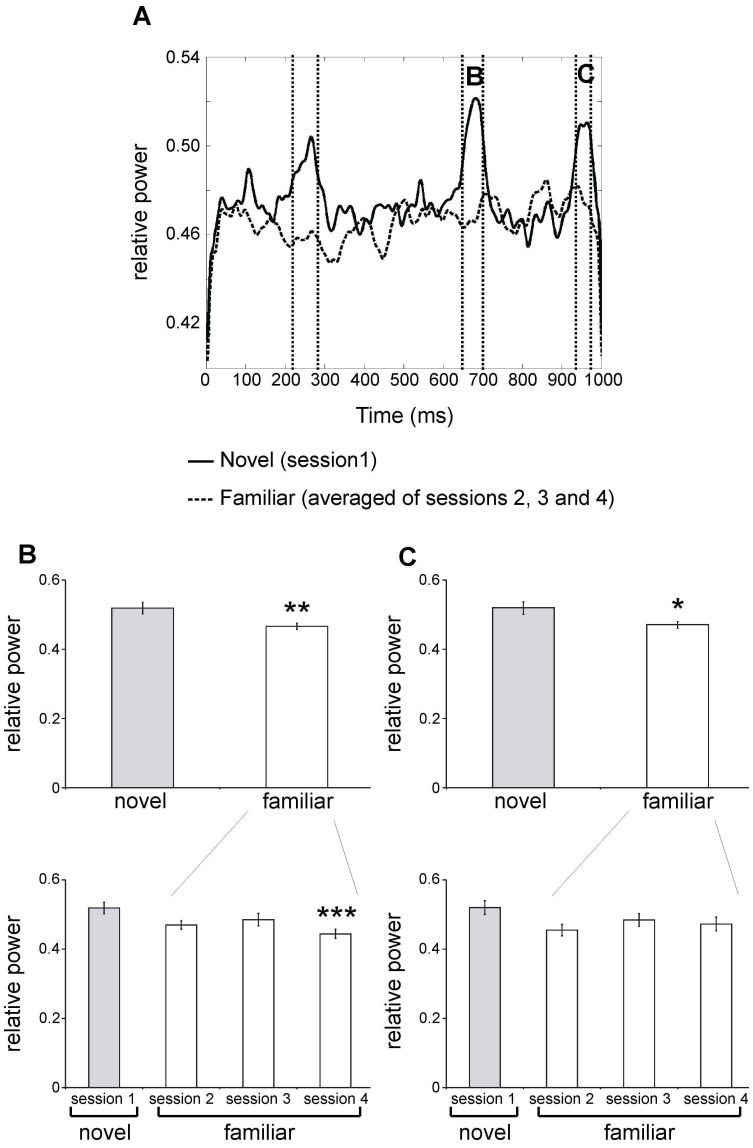


Figure 28: Novelty unrelated γ activities. When compared to the averaged data of the familiar object, the 40-47 Hz band-frequency calculated from the novel object shows 2 distinct peaks (50 points smoothed) in the late part of the analysed 1 s trials (>500 ms) with a significantly higher power at (**B**) 650-700 ms and (**C**) 950-975 ms after the first contact. These 2 activities could not be directly correlated to novelty detection since they were sensitive of the time between the different sessions.

5 DISCUSSION

5.1 Telemetric recording system development

Telemetric recording systems have faced an increased interest from the scientific community during the last two years. The reasons are diverse, but all due to the desire of performing electrophysiological recordings with awake, freely moving animals without the restrictions of a large recording set-up.
The choice of the recording method carries intrinsic limitations that have to be considered to capture the information of interest. Below the different techniques are discussed that can be used for electrophysiological recordings *in vivo* as well as the advantages and disadvantages of wireless technology.

5.1.1 Commonly used *in vivo* recording methods

Three major ways for collecting data *in vivo* are commonly used which are the anaesthetised, head restrained and tethered models.
It is well accepted now that anaesthetised models do not allow the possibility to observe the reality of the acting brain but an artificial state (Bourne, 2003). It has been shown that multi-unit responses in barrel cortex to control deflections of vibrissae in rats were characterised by a large late component in awake in comparison to urethane anaesthetised animals (Simons *et al.*, 1992). Furthermore the multi-unit activity in the auditory cortex induced by subsequent clicks exhibited increases in latency, peak latency and response duration under anaesthesia in contrast to awake state (Rennaker *et al.*, 2007). The choice of the anaesthetic agent is also crucial and may result in different responses of the central nervous system. However this technique allows the

recording of animals without any stress or pain and carries no limitations considering the number of parameters that can be collected simultaneously.
In contrast the head restrained model offers the chance to collect data from awake animals but the behavioural possibilities are limited. The animal reacts more passively to the environment in comparison to an unrestrained situation. This method is commonly used for collecting information during visual tasks (Cecala & Freedman, 2008) and in sleep research where sleep-wake cycles have been shown to be similar, after habituation, to unrestrained animals (Gervasoni *et al.*, 2000). It has also been shown that this method induces a limited stress (Parry & McElligott, 1993), a critical point since the effect of stress on brain activity is mainly unknown.
Finally the tethered method allows the simultaneous recording of brain activity and behaviour in relatively freely moving animals. However it has been demonstrated to be a stress generator (Brockway & Hassler, 1992) with an increase in rodents and non human primates heart rate (Adams *et al.*, 1988; Bohus, 1974) and to induce sleep modifications in B6 mice (Tang & Sanford, 2002).

5.1.2 Stress effects on brain activity

When considering *in vivo* experiments in awake animals stress is an important factor which effects on the working brain are mostly unknown. Stress related changes have been recently observed in REM sleep quantities and sleep patterns in hippocampus and amygdala after acute immobilisation in rodents (Hegde *et al.*, 2008). Furthermore controllable and uncontrollable stress has been shown to impair CA1 long term potentiation (LTP), whereas uncontrollable stress enhanced LTP in the dentate gyrus and increased baseline response in the amygdala (Kavushansky *et al.*, 2006). It is also known that

stress leads to secretion of glucocorticoids resulting in dendritic hypertrophy in the basolateral amygdala inducing general locomotor impairment associated with anxiety (Mitra & Sapolsky, 2008). Finally stress has been associated with atrophy in CA3 area of the hippocampus (Vyas *et al.*, 2002).
Wireless technology has been developed as a solution to these stress associated effects.

5.1.3 The wireless technology

The telemetric recording method offers the big advantage to collect data from totally freely moving animals and has been shown to minimise the stress artefacts unavoidable in traditional electrophysiological recordings (Kramer & Kinter, 2003). Brain activity can be correlated to unrestricted behaviour in theoretically unlimited environments. However the number of parameters that can be collected, e.g. the number of recording electrodes, and the duration in time of the experiments limited by the battery life are the major limitations of this technique. The distance between antenna and transmitter and the cost of the available systems are also reducing the use of this method and were the reasons that motivated the development of a new unit.

5.1.4 A novel telemetric recording system

5.1.4.1 Impact on the animal's behaviour

The developed recording system offers several advantages compared to the currently available ones despite a recent and fast increase of quality on the market. The transmitter has a relatively small size and weight, a critical point

to decrease or better eliminate the impact of the recording set-up on the animals' behaviour. The locomotion after surgical implantation has been shown to be unchanged between implanted and control rats with no significant differences for the total distance covered in an open field during 10 min (Lapray *et al.*, 2008). The average moving speed has also been shown to be unaffected and the pattern of exploration was similar with animals spending more time following the walls and avoiding free spaces. Furthermore the animals were still able to curl-up to sleep already few hours after the surgery indicating the absence of discomfort and pain. Finally the weight increases of the animals 5 days post-surgery was also a good welfare indicator (see Results, pages 43-45).

5.1.4.2 Technical properties

The incorporation of an ADC converter in the transmitter, the homemade software to display and store the data simultaneously with the video signal and the possibility to change wires and the battery easily make this system inexpensive, portable and easy to use and reuse. A transmitting range of the signal of up to 3 m without any loss of data allows to perform behavioural experiments in a wide variety of environments. However the large transmitting range and the impossibility to switch ON one system independently necessitates the isolation of the animal under recording. Except this latter no particular precautions have to be taken in order to limit any kind of external electromagnetic interferences. The large transmitting range and the high sampling rate of the system give rise to higher battery consumption reducing the battery life (around 25 h at 500 Hz sampling rate). Therefore the system can be switched ON and OFF from a distance which permits experiments lasting a few weeks. Finally after sacrificing the implanted animal and

extracting the transmitter, the unit can be reused several times after changing battery and wires.

5.1.4.3 Future developments of the system

Many desirable developments are required and two in particular: the number of recording channels and the battery life, two improvements that unfortunately go against each other. A larger number of recording channels with high sampling rate would mean a higher power consumption and even worse an increase of the transmitter size. The possibility to use an analogue signal to increase battery life is not indicated due to the weak quality of such data.
A further miniaturisation to allow telemetric recordings in small mammals, such as mice, is also an important improvement. Finally the possibility to record several animals at the same time and the stimulation of specific brain areas are also important issues for animal research and are currently in the process of development. Steady progress in electronics can also be expected in the near future which will lead to powerful telemetric systems.

Wireless technology development was not the final goal of this work but to understand the brain mechanisms and in particular, in the perspective of this thesis, to search what kind of large scale neuronal activity in the cortex of a rat could be associated with the detection of new objects.

5.2 Novelty detection experiments

5.2.1 The use of wireless technology

The cortical activity correlated with novelty detection in the rat barrel cortex has never been investigated before this work. Furthermore it is believed that stress induced by environmental changes or any restriction of the animal behaviour has an effect on brain activity and body parameters, as highlighted by many studies (Adams *et al.*, 1988; Bohus, 1974; Brockway & Hassler, 1992; Hegde *et al.*, 2008; Kavushansky *et al.*, 2006). Therefore the choice of telemetry has been motivated by the small impact of the recording set-up on the implanted animal compared to traditional *in vivo* recording methods despite the limited number of parameters that can be recorded simultaneously.

5.2.2 The ECoG recording

It has been shown that ECoG compared to EEG in humans has higher spatial resolution, broader bandwidth (i.e. 0–500 Hz versus 0–50 Hz), higher characteristic amplitude (i.e. 50–100 μV versus 10–20 μV) and far less vulnerability to artefacts (Schalk *et al.*, 2008). Furthermore the use of screws as electrodes increases the stability of the ensemble for a better signal. Finally LFP recordings were prohibited by the lack of information concerning the existence and localisation of a novelty detection signal in the barrel cortex.
The ECoG recording was then considered to be most appropriate method for the detection of the signal of interest.

5.2.3 The experimental design

The experiments were designed to leave the animals completely free in their exploration of the environment (see Materials & Methods, page 38). The task was as simple as possible to minimise the introduction of uncontrollable behavioural parameters. The use of similar objects with small variations such as texture differences was not indicated since the goal of these experiments was to search for the novelty detection related cortical activity and not to define the ability of the animals to distinguish objects on details which has already been investigated (von Heimendahl *et al.*, 2007). Therefore the three objects for this study were chosen to be totally different from each other in shape and size and also not related to anything the animals could have met in the animal facility (see Materials & Methods, Fig. 17, page 39). Furthermore this work has been focused on the whiskers and barrel cortex related activity making it necessary to design experiments in which the use of other senses by the animals would be strongly reduced. The recordings were then performed in the dark under IR lights and in an isolated room. Finally the duration of the recording sessions has been limited to 10 min due to the relatively little environment to explore.

5.2.4 Trials definition

In order to compare the different recordings it was necessary to define the trials that were further analysed. Every trial lasted 1 s starting when the animal contacted the object with one of his whisker pads. Because every contact was different concerning the position of the animal in regard to the object and the side of the whiskers pad making the first contact, the trials were pooled in three categories. The criterion of classification was the whisker pad side, in regard to the recording site, making the first contact with the object. Thus, only the

contacts initiated with the contralateral whiskers pad to the recording site were further analysed. In addition the size of the ECoG screws (0.5 mm) was believed to be large enough to cover an important area of the barrel cortex to visualise huge cortical activations correlated with the activity of a large part of the whiskers' pad. The recordings should then not be largely affected by the specific vibrissae row that started the object exploration.

5.2.5 Novelty detection related γ activity

To our knowledge this is the first report demonstrating that the detection of a novel object during an explorative task can be associated with a neocortical activity pattern. It has been previously shown that γ as well as β oscillatory activity accompany the presentation of a novel auditory stimulus in the human EEG (Haenschel *et al.*, 2000) and that β2 oscillations (23-30 Hz) are transiently and selectively increased during the active exploration of a novel environment in the hippocampus of freely moving mice (Berke *et al.*, 2008). Using c-Fos activation as a marker of neuronal activity it has been possible to provide the first proof of a specific response in the barrel cortex during the exploration of a new environment (Montero, 1997; Staiger *et al.*, 2000). In addition to these observations the results exposed in this work indicate that the detection of a novel object is accompanied by a rapid and transient synchronised increase of a narrow band (40-47 Hz) of the γ range in rat barrel cortex concurrently to a strong decrease of the low γ oscillations (30-37 Hz) (see Results, pages 49-54). It is not possible to know if the increase in activity is associated to changes in local synchronization, changes in the number of activated neurons or the strength of their activation, one limitation of ECoG recordings (Fell *et al.*, 2003). Interestingly this activity appears with the same latency (200-300 ms) as in human auditory cortex following the presentation of

a novel stimulus (Haenschel *et al.*, 2000) and corresponds with what has been shown in human perception tasks (Bauer *et al.*, 2006; Kaiser & Lutzenberger, 2003; Rodriguez *et al.*, 1999; Tallon-Baudry *et al.*, 1996; Tallon-Baudry *et al.*, 1997; Tallon-Baudry & Bertrand, 1999). This latency also coincides with the novelty P3 wave that has been observed over frontal and central areas in different species after the presentation of a novel stimulus in an auditory detection task (Ranganath & Rainer, 2003;Wang *et al.*, 1999;Yamaguchi *et al.*, 1993). It has been stated that this duration was necessary for object recognition (Buzsáki, 2006) allowing the interaction of many cortical areas. It has also been shown in human spatial selective attention tasks that high-frequency γ-band activity (60–95 Hz) in presumed primary somatosensory cortex was enhanced 100-500 ms after the stimulus onset in MEG recordings (Bauer *et al.*, 2006). In this study the increase of activity was correlated with the presentation of attended in comparison to unattended stimulus, whereas α and β band activity were suppressed in these areas. In contrast to these experiments where the attention of the subjects was focused on the “attended” stimulus, e.g. the familiar items, our experimental design made the “unattended” stimulus, e.g. the novel item, relevant for the animal. Therefore a change in the subjects’ attention may participate to the observed increase in γ band after the tactile detection of a novel object. Bauer et al. suggested that γ band oscillations play an important functional role in the early stages of the somatosensory system for processing behaviourally relevant stimuli. Furthermore, they hypothesized that γ band synchronization in primary sensory cortex is instrumental in communicating with higher somatosensory areas.

5.2.6 The late γ activities

In contrast to the first significant activation at 200-300 ms, which corresponds well with previous reports on stimulus-induced γ oscillations (Bauer *et al.*, 2006; Haenschel *et al.*, 2000; Rodriguez *et al.*, 1999; Tallon-Baudry *et al.*, 1996; Tallon-Baudry *et al.*, 1997; Tallon-Baudry & Bertrand, 1999) the late activities (at ~675 ms and ~960 ms) have not been correlated with any distinct cognitive performance so far (see Results, pages 54-55). However induced β activity accompanied by a broadband γ activation has been shown to be enhanced ~680 ms after the presentation of a novel auditory stimulus in human EEG (Haenschel et al., 2000). Late γ activities have also been observed during a visual perception task using "Mooney faces" (Rodriguez et al., 1999) as well as after a tactile spatial attention task (Bauer et al., 2006). In the present study the two late activations (>500 ms) could not directly be correlated to the detection of the novel objects. These late responses may reflect reverberating activity patterns, which are fed back into the barrel cortex from subcortical and other cortical regions associated with the memory process.

5.2.7 Gamma oscillations an artefact?

A recent study has shown that induced γ-band EEG activity in visual perception tasks may well be of ocular rather than of neuronal origin (Yuval-Greenberg *et al.*, 2008). In contrast to these results demonstrating a broadband γ response post-stimulus, the work reported here showed a neocortical response in a rather narrow γ frequency range. The influence of ocular saccades can be excluded since the animals were recorded in the dark and the behavioural task was predominantly related to the movement of the whiskers, active at a frequency lower than 20 Hz (Ahissar *et al.*, 2000). Furthermore, muscle

artefacts would be present in the experimental conditions with the novel as well as familiar object. Our ECoG recordings from freely moving rats clearly indicate that distinct γ activity can be transiently recorded from the barrel cortex when the rat is actively exploring a novel object.

5.2.8 Structures possibly involved in the observed γ activity

The observed synchronised signal collected through the ECoG electrodes most likely corresponds to an incoming input on the barrel cortex which will then be computed and downstream on higher cognitive areas such as S2 and hippocampus.

The mechanical deflexion of the whiskers gives rise, through the thalamocortical projections, to a large wave of depolarisation in the barrel cortex after 15 ms and then spreads briefly thereafter into the motor cortex, as has been shown by voltage-sensitive dye imaging (Ferezou *et al.*, 2007) and EEG studies (Megevand *et al.*, 2008). Due to the long latency preceding the appearance of the novelty related signal other barrel cortex afferences implicated in the treatment of novel stimulus must be involved.

The barrel cortex receives afferents from a restricted number of cortical and subcortical areas and the perirhinal cortex is one of them. This structure is one of the strongest bi-directional connection of the barrel cortex (Jones & Diamond, 1995) and has been shown to be involved in novel object discrimination (Barker *et al.*, 2007). Furthermore the perirhinal cortex - prefrontal cortex interaction has been demonstrated to be crucial in conferring processing advantages to novel items. It has also been shown that prefrontal cortex neurons increase their firing rate by approximately 200 ms after the presentation of a novel stimulus in monkeys performing a visual task (Ranganath & Rainer, 2003). In addition a large potential has been recorded

above the frontal cortex in humans 200-300 ms after a novel auditory stimulus presentation.

The barrel cortex is also strongly innervated, as well as the prefrontal cortex and perirhinal cortex, by cholinergic and noradrenergic fibers coming respectively from the basal forebrain and the locus coeruleus. These two neurotransmitters increase their cortical levels in response to sensing novel objects (Ranganath & Rainer, 2003).

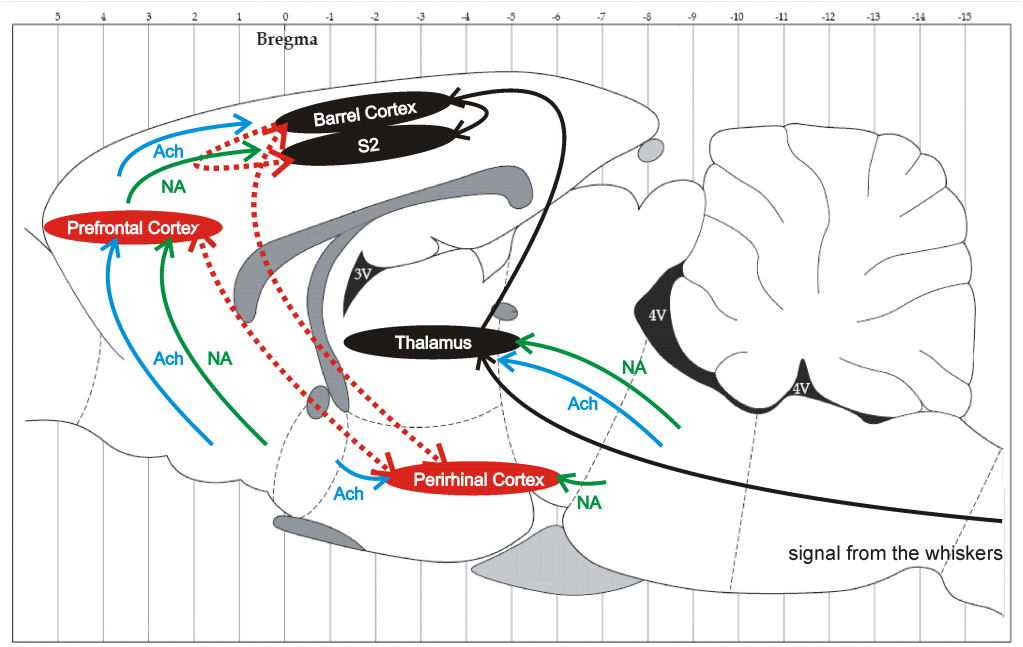


Figure 29: Anatomical connections possibly involved in the observed γ activity. The mechanical deflexions of the whiskers induced by the exploration of the new object is relayed in the brainstem up to the thalamus and finally to the barrel cortex and S2 (black circuit). The novelty related signal may be sent to the perirhinal cortex and after being computed in the perirhinal cortex - prefrontal cortex loop is fed back in the barrel cortex and then in S2 and higher cognitive areas (red circuit). The cortical-subcortical activity is sustained by an increase of the animal's attention provided by the basal forebrain cholinergic (ACh) and locus coeruleus noradrenergic (NA) systems (blue and green circuits respectively).

It hence seems more likely that the 200-300 ms necessary for the appearance of the observed "γ burst" in the barrel cortex is the time needed for the prefrontal and perirhinal cortices to compute and return the new information. The returned signal should be optimal to target higher cognitive structures in order to store the new information under the control of cholinergic and noradrenergic systems (Fig. 29). In addition it has been observed recently that localised γ oscillations in rats' neocortical areas such as the somatosensory cortex were phase biased by the δ hippocampal rhythm (Sirota *et al.*, 2008). The hippocampus could therefore also have a role in the observed γ activity by temporally coordinating spatially widespread neocortical assemblies to transfer the information to associative networks.

5.2.9 Novelty related γ activity and information storage

It is widely accepted that memory formation is dependent on changes in synaptic efficiency that permits strengthening of neuron associations. Activity dependent synaptic plasticity at appropriate synapses, presumably described by the LTP model, is believed to be both necessary and sufficient for storage of information (Lynch, 2004). The critical event leading to induction of LTP appears to be the influx of Ca^{2+} ions into the post-synaptic spine mediated through NMDA receptors and the downstream cellular consequences of this increase. In Hebbian learning theories correlated synaptic input and AP output are associated with increases in synaptic strength. A step change in voltage due to an incoming excitatory input leads to an asymptotic increase response until a steady state is reached. The duration of the voltage change is dependent on the cell type. It has been shown that hippocampal pyramidal neuron membranes need ~25 ms to reach 63 % of the stable response corresponding to their membrane time constant. Therefore the arrival of another excitatory input on

the membrane within this time constant allows the summation of the signals which increases the probability to trigger an AP in the post-synaptic element. Furthermore it has been found that pairing of axonally initiated APs with subthreshold EPSPs within 25 ms range increased dendritic back-propagating AP amplitude and associated Ca^{2+} influx and then promoted synaptic plasticity (Magee & Johnston, 1997). In addition this time window, corresponding to the γ frequency range, has been shown to be the most suitable timescale in cooperative activities for information transmission and storage in cortical circuits (Harris *et al.*, 2003). Moreover it has been observed that despite the wide range of possible single-neurons firing rate (0-400 Hz) inter-neuronal networks can be synchronised by $GABA_A$ synapses preferentially within the γ frequency range (Wang & Buzsaki, 1996). Gamma synchronised oscillations across forebrain structures, in addition to providing the temporal structure for a local network of pyramidal cells, may be the most appropriate means to communicate and bind anatomically distributed populations of neurons (Buzsaki & Chrobak, 1995). Possibly the γ rhythm presents all the characteristics to forward messages in the most effective way from neuronal populations to downstream neurons and then to promote LTP related memory formation. This synchronised neuronal firing may well be also crucial to produce a coherent object representation (Buzsáki, 2006; Singer *et al.*, 1997).

5.2.10 Role of glial cells

During the last decade many observations gave a new role to glial cells in brain information processing together with neurons. It has been recently shown that the myelination by oligodendrocytes, essential for nervous system functions, was promoted by an astrocyte-neuron interaction (Ishibashi *et al.*, 2006). In their study it has been demonstrated that the secretion of adenosine

triphosphate (ATP) resulting from neurons firing APs induced the release of a cytokine (the leukaemia inhibitory factor, LIF) by astrocytes, thereby enhancing myelination. Astrocytes have also been involved in extrasynaptic NMDA receptor modulation on pyramidal cells and interneurons and synaptic transmission optimisation (Nadkarni & Jung, 2007; Nadkarni *et al.*, 2008). Furthermore it has been recently demonstrated *in vivo* that increased neuronal discharge was associated with increased astrocytic activity and a high correlation of Ca^{2+} signals in neighbouring astrocytes (Hirase *et al.*, 2004). In addition it has been shown that astrocytic glutamate released that activates extrasynaptic NMDA receptors mediated synchronised oscillations in CA1 groups of neurons of hippocampal slices preparation (Fellin *et al.*, 2004).

Glial cells and more precisely astrocytes, by their effects on NMDA receptors and on myelination process, may play an important role in controlling the dynamics of the neuronal networks and then to participate in the observed γ burst novelty detection related activity.

6 Outlook

The developed telemetric recording system will necessitate some improvements to have a chance to be widely used. An increase of the number of channels as well as a longer battery life will be the most important aspects for the next generation of units. Furthermore a smaller transmitter's size, the possibility to stimulate specific brain areas and to record several animals at the same time have also to be considered in the future.

The novelty detection cortical related activity study is preliminary and would then necessitate further investigations. The structures responsible for this activity and the effects on downstream neurons are the main questions that have to be answered. Recording the barrel cortex simultaneously with the thalamus or the prefrontal-perirhinal cortices are important issues that have also to be addressed. Finally the type of cells responsible for the detection and storage of novel information are of importance to be known.

7 Summary

During this thesis a new telemetric recording system has been developed allowing ECoG/EEG recordings in freely behaving rodents (Lapray *et al.*, 2008; Lapray *et al., in press*). This unit has been shown to not generate any discomfort in the implanted animals and to allow recordings in a wide range of environments.

In the second part of this work the developed technique has been used to investigate what cortical activity was related to the process of novelty detection in rats' barrel cortex. We showed that the detection of a novel object is accompanied in the barrel cortex by a transient burst of activity in the γ frequency range (40-47 Hz) around 200 ms after the whiskers contact with the object (Lapray *et al., accepted*). This activity was associated to a decrease in the lower range of γ frequencies (30-37 Hz). This network activity may represent the optimal oscillatory pattern for the propagation and storage of new information in memory related structures. The frequency as well as the timing of appearance correspond well with other studies concerning novelty detection related burst of activity in other sensory systems (Barcelo *et al.*, 2006; Haenschel *et al.*, 2000; Ranganath & Rainer, 2003). Here, the burst of activity is well suited to induce plastic and long-lasting modifications in neuronal circuits (Harris *et al.*, 2003).

The debate is still open whether synchronised activity in the brain is a part of information processing or an epiphenomenon (Shadlen & Movshon, 1999; Singer, 1999). The present work provides further evidence that neuronal network activity in the γ frequency range plays an important role in the neocortical processing of sensory stimuli and in higher cognitive functions.

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Oral Communications

Development of a new telemetric recording system, Sino-GermanWorshop on sleep Research, Invited
Talk, April 2008, Harbin, China.

Collaborations

Dr. Alfonso Represa, Institut de Neurobiologie de la Méditerranée (INMED), Marseille, France.

Poster presentations

Lapray, **D.**, Bergeler, J. and Luhmann, H.-J., **Neuronal signature of novelty detection in the barrel cortex of freely moving rats**, *IAK meeting*, Novembre 2008, Mainz, Germany.

Lapray, **D.**, Bergeler, J. and Luhmann, H.-J., **Neuronal signature of novelty detection in the barrel cortex of freely moving rats**, *6th FENS meeting*, July 2008, Geneva, Switzerland.

Sapin*, E., **Lapray***, **D.**, Luppi, P.-H., Goutagny, R., Berod, A., Ravassard, P., Hanriot, L., Clément, O., Fort, P. and Léger, L., **Evidence that multiple populations of GABAergic neurons are involved in paradoxical (REM) sleep control**, *6th FENS meeting*, July 2008, Geneva, Switzerland.

Lapray, **D.**, Bergeler, J., Dupont, E., Thews, O. and Luhmann, H.-J., **A new wireless device for EEG recording in freely moving rats**, *87th Annual Meeting of the Deutsche Physiologische Gesellschaft*, March 2008, Cologne, Germany.

Lapray, **D.**, Bergeler, J., Dupont, E., Thews, O. and Luhmann, H.-J., **A new wireless device for EEG recording in freely moving rats**, *EPICURE Second Annual Meeting*, January 2008, Malta.

Goutagny, R., Fort, P., **Lapray**, **D.** and Luppi, P.-H., **Role of dorsal paragigantocellular nucleus in paradoxical sleep regulation: a study combining electrophysiology and pharmacology across vigilance states in the rat**. *Society For Neurosciences (SFN) 35th Annual Meeting*, Novembre 2005, Washington, USA.

Luppi, P.-H., Goutagny, R., Verret, L., Gervasoni, D., Boissard, R., **Lapray**, **D.**, Salvert, D., Léger, L., Peyron, C. and Fort, P. **Anatomy of GABAergic machinery in REM sleep control**, a communication in the symposium "Evolving concept of REM sleep - GABAergic Inhibition, disinhibition or both". *World Federation of Sleep Research Societies (WFSRS)*, 2005, New Delhi, India.

Léger, L., Boissard, R., Goutagny, R.,Verret, L., **Lapray**, **D.**, Sapin, E., Peyron, C. and Luppi, P.-H. **Pontine and hypothalamic pathways and transmitters involved in the regulation of paradoxical sleep**. *3rd International Workshop: Sleep as a window to the waking world*, 2005, Rostov-on-Don, Russia.

Courses

- 24-26 November 2008: **Licensee Training Course for Animal Research** (Modules 1-3 validated), Wolfson Institute, London, UK.

- 10-13 January 2008: **EPICURE mini course** on "Study of the phenotype in experimental animal model of epilepsy" and "Study of the phenotype in epilepsy patients ", University of Malta, Malta.

- 01-05 October 2007: **BCCN/NWG course** on "Analysis and Models in Neurophysiology", Institute of Biology, Albert-Ludwigs University, Freiburg, Germany.