Synopsis

Research in this laboratory concentrates on the neural mechanisms of perception and object recognition. Although our basic research revolves around vision, a number of independent collaborators are also investigating the relationship between neural activity and perception using other sensory modalities. I firmly believe that such scientific questions require a multimodal methodological approach that integrates information obtained from single units with that derived from mass action potentials as well as from a number of activity-related, surrogate signals such as those monitored during noninvasive neuroimaging experiments. Parallel to our ongoing neuroscientific research, therefore, we are also working to develop methodologies that will permit us to the study neural networks in the context of behavioral paradigms. We have already designed and implemented two high-field magnetic resonance imaging systems for functional, anatomical and spectroscopic imaging. The systems are endowed with all the necessary hard and software to conduct simultaneous imaging and recordings, and they are being used to study the function, connectivity, and neurochemistry of the non-human primate brain. Furthermore, while continuing to exploit traditional neuroimaging in our experiments, we are also investigating the relationship of neural activity to the MR-measurable hemodynamic responses and experimenting with methods that do not rely on hemodynamic responses at all. In the context of the last-named project, a group of synthetic and coordination chemists in my laboratory are attempting to synthesize and evaluate MR-detectable smart probes that change magnetic properties as a function of the concentration of ions and molecules involved in neural signaling. Smart contrast agents, if designed and tested appropriately, promise to revolutionize invasive neuroimaging and would represent a quantum leap forward in signal-to-noise ratio, spatial detail and specificity, while affording unprecedented temporal resolution.

Selected Current Research Directions & Perspectives

Conscious Visual Perception – Psychophysics, Physiology & fMRI Studies
This research group investigates the mechanisms of color and motion processing and of multistable perception. A representative example of the research in the group is the study of binocular rivalry (BR), the stochastic perceptual alternations experienced when viewing dichoptic stimuli. References to the extensive bibliography on binocular rivalry can be found in our reviews (Logothetis, 1998b; Logothetis, 1999; Blake and Logothetis, 2002; Logothetis, 2006).

Binocular Rivalry – An Example of Multistable Perception
Initial theories on the mechanisms underlying binocular rivalry suggested that the competition between percepts is resolved in the primary visual cortex (striate cortex or V1) through inhibitory interactions between monocular neurons. Past and present research in our laboratory, however, showed that rivalry is due to a series of processes, each of which is implemented by neural mechanisms at different levels of the visual hierarchy. In fact,
dominating perception can comprise local visual features that are distributed throughout the visual field, and for that matter, contained in both the left and right eyes’ views. In our lab, human psychophysical work on binocular rivalry continues with or without concurrent neuroimaging or EEG data collection. At the same time, possible neural correlates of the perceptual alternations are studied in monkeys trained to report precisely what they perceive.

Over the last two decades we have recorded from many different visual cortical areas while the subject performs a simple categorization task, e.g. pulling and holding one lever for as long as an animate object is perceived, and another lever when a geometrical pattern is seen (Leopold and Logothetis, 1996; Sheinberg and Logothetis, 1997). We have also carried out experiments with a variant of rivalry known as flash suppression, in which one stimulus is presented first, followed by a second one. The second stimulus is then perceived for a period of a couple of seconds, followed by regular rivalry. Flash suppression can occur with or without a spatial conflict. In the case of binocular flash suppression (BFS), dissimilar patterns are presented in corresponding portions of the two eyes (Wolfe, 1984; Sheinberg and Logothetis, 1997). In so-called generalized flash suppression (GFS), the sudden presentation of a surrounding pattern after the subject views a salient target for several hundred milliseconds results in the target’s immediate and sustained disappearance (Wilke et al., 2003). The advantage of the flash suppression paradigm is that the time of occurrence of the first perceptual transition is precisely defined and permits a detailed investigation of the neural responses. Monkeys can master the rivalry or flash suppression tasks and they typically yield psychometric functions that are similar to those obtained from human subjects. While the animals report perceptual alternations, single- and multiple-unit activity (SUA and MUA) as well as local field potentials (LFP) are recorded in striate cortex, as well as in many different extrastriate visual areas.

**Neurophysiological Correlates of Perceptual Dominance & Suppression**

The results of all experiments so far indicate that the majority of stimulus-selective neurons are not affected by perceptual suppression, but continue responding to their preferred stimulus whether the latter is perceived or not. Surprisingly, only a fraction of the brain’s neurons’ activity is related with what the animals perceive at any given time. This subset of neurons is distributed over the entire visual pathway rather than being located in a single area in the brain. The percentage of perceptually modulated neurons is small in area V1 (about 10-15%) and increases in the early extrastriate cortices (20-40%) to reach a fraction about 70% and 90% in the prefrontal and inferior temporal cortices (ITC), respectively (Logothetis and Schall, 1989; Leopold and Logothetis, 1996; Sheinberg and Logothetis, 1997). In other words, we are unaware of a great deal of the activity going on in our brains. We have, of course, long known that we are mostly unaware of the activity in the brain that maintains the body in a stable state— one of its evolutionarily most ancient tasks. Our experiments show that we are also unaware of much of the stimulus- or task-related neural activity that could in principle generate—at least in part—our conscious experiences.

Furthermore, in many cortical areas, even those cells whose responses depend on the conscious perception of the stimulus show only modest activity changes whenever their preferred stimulus dominates perception. It should be noted here that the alternation of perception during rivalry is almost indistinguishable from that experienced during the physical alternation
of the visual patterns. In both cases, perceptual suppression is strong, with the dominant pattern appearing as if it were being presented by itself. Nonetheless, the modulation of MUA in the early extrastriate cortex is only a small fraction of that observed during the physical alternation of the stimuli. This small population modulation appears to be sufficient to cause the robust alternating patterns of excitation and inhibition of cells in ITC during the perceptual dominance and suppression of a pattern, respectively. In fact, in ITC most neurons respond vigorously when their preferred pattern is perceived, and usually no differences are found between the response to a physical change of stimulus and a perceptual change. Finally, about one-third of the response-modulating cells fired more strongly when their preferred stimulus was suppressed. The neurons whose activity seems to be in anti-correlation with the animals' perception of the driving stimulus might be part of an inhibitory mechanism that is separate from, and to some extent independent of, the mechanisms of perception. Such an independent mechanism was predicted by William Levelt in 1965 in his seminal study on binocular rivalry; he was the first to notice - in a series of elegant psychophysical experiments - the differential effects of stimulus-strength on dominance and suppression.

Our ongoing studies suggest that prefrontal cortex (PFS) activity also correlates strongly with perception. In PFC the firing rate of almost 70% of visually selective neurons closely followed the induced visual percept (project by T. Panagiotaropoulos). The vast majority (95%) of these perceptually modulated neurons fired more when their preferred stimulus was perceptually dominant. Multi-unit activity also reliably reflected perceptual suppression.

Over the last few years, a number of human fMRI studies have questioned the small contribution of the primary visual cortex in the strong perceptual alternations during rivalry. In fact, fMRI studies of perceptual suppression have revealed a robust drop in V1 activity when the stimulus was subjectively invisible. In our opinion this “controversy” ignores an important characteristic of hemodynamic responses: their extreme sensitivity to neuromodulation. The BOLD signal is primarily affected by changes in excitation–inhibition balance, and this balance may be controlled by neuromodulation more than by the changes in spiking rate of a small set of neurons. Because they both involve modulation from diffuse ascending systems and cortical feedback, attention or “conscious perception” are likely to induce large changes in the fMRI signal by proportionally changing the excitatory and inhibitory conductances – and thus the overall excitability - of areas like the primary visual cortex. This hypothesis received support from our latest experiments on flash suppression; we found that the LFP signals in V1 show a clear and statistically highly significant modulation in the α–band frequency range but not in multiple-unit activity (MUA) or single-cell spiking (Wilke et al., 2006). LFP modulation was also apparent in extrastriate cortex. Thus, perceptual suppression appears to be reflected in a widespread decrease of low-frequency LFP power combined with a locally restricted decrease in the MUA of higher stages of visual processing. Further support for the above notion comes from a very recent study combining physiology and fMRI in behaving monkeys (Maier et al., 2008). These investigators found that both neurophysiological and hemodynamic signals were in close agreement during conventional stimulus presentation, showing strong visual modulation to presentation and removal of a stimulus. During perceptual suppression, however, only the BOLD response and the low-frequency local field potential (LFP) power showed decreases, whereas the spiking and high-frequency LFP power were unaffected.
Future Plans & Perspectives

All in all, the studies above show that a widespread inter-areal network of neurons reflects - at least to a certain extent - the changes perceived while viewing ambiguous, multistable stimuli. Higher visual association areas have larger perception-related neural populations which show all-or-none responses during dominance and suppression. Changes in activity in lower areas are more subtle, accompanied by strong modulatory effects.

An important observation, but one which complicates matters, is the fact that perceptual modulation of neural activity is context-dependent. We recently measured the responses of MT (V5) neurons and examined the degree to which the percept-related responses of individual neurons depend upon the specific sensory input. Surprisingly, we found that even small differences in the stimuli led to significant changes in the signaling of the perceptual state by single neurons. It is thus possible that nearly all feature-responsive neurons in this area, rather than a select subset, can contribute to the resolution of sensory conflicts, and that the role of individual cells in signaling the perceptual outcome is tightly linked to the fine details of the stimuli involved (Maier et al., 2007).

An important (and unavoidable) question arising from these studies is: Are we ever going to learn something about the actual mechanisms of such complex phenomena by continuing with the same experimental approach? What exactly does the modulatory input do? How do areas interact? Can we detect causal relationships or information transfer between different areas? What is the functional role of feedback?

As mentioned above (see Philosophy, Aims & Outlook), the conventional neurophysiological approach is unlikely to answer such questions. A first step towards a better understanding of mechanisms is our newly planned multi-electrode and multi-site recordings, aided by neuroimaging experiments in the behaving animal. The success of such an experimental approach, however, strongly depends on increasing our level of understanding about neural signals and their interactions. It will also depend on insightful inputs from theory. Terms like “neural representation”, “system complexity” or “dynamic interactions” seem to be void of an experimentally testable pragmatic meaning. Brain-like complex systems need theoretical descriptions that are instigated by the brain’s structural and functional data rather than imported from theoretical physics or theoretical economics. We see the success of our further experimentation partly depending on successful communication between the experimental and the abstract levels of our understanding of systems and between analytical extraction of detail and synthetic abstraction of organizational principles.

Object Recognition - Psychophysics & Physiology Studies

The aim of this project is to understand the role that extrastriate cortex plays in visual object recognition. Many of the ideas and experiments have been described in some detail in previous reviews (Logothetis and Sheinberg, 1996; Logothetis, 1998a; Logothetis, 2000; Hoffman and Logothetis, 2008; Gauthier and Logothetis, 2000).

Encoding of Holistic Information

We started our research on recognition by first examining in great detail the performance of macaques in categorization and identification tasks. Following extensive training, the animals
learned to discriminate individual objects from a set of highly similar distracters, a task not unlike the problem of identifying a specific face or a particular bird species (so-called “expert behavior”). Their ability to generalize from known to novel views was found to be almost identical to that of expert humans. Subsequent physiological recordings from individual neurons in the inferior temporal lobe, near the anterior medial temporal sulcus (AMTS), revealed a subpopulation of cells that were actually activated selectively by views of learned objects which had been unfamiliar before training. The cells were most active when the target was presented from one particular view and became less and less active as the object was rotated in depth. Objects that a monkey consistently failed to recognize never elicited a selective response, and no selectivity was found in animals which were not experienced with these object classes (Logothetis et al., 1994; Logothetis and Pauls, 1995; Logothetis et al., 1995).

In a continuation of this line of investigation, we tested how different theoretical models of object recognition account for expert behavior in both humans and monkeys and what happens in the monkey brain during the process of training and familiarization (Sigala and Logothetis, 2002; Sigala et al., 2002). The results indicated – once again – important similarities between human and monkey recognition strategies. Neither species compared the new stimuli to class prototypes or based their decisions on conditional probabilities along stimulus dimensions. Instead, they classified each object according to its similarity to familiar members of the alternative categories, or with respect to its proximity to a linear boundary between the learned categories.

**Encoding of Diagnostic Features**

The first series of experiments on categorization and identification used object classes in which identification relied on processing of holistic information. In our examination of the role of ITC in feature encoding, we also conducted experiments in monkeys trained to categorize parameterized stimuli, for instance, line drawings of faces or fish. Each schematic stimulus consisted of an outline and four varying features, e.g. eye height, eye separation, nose length and mouth height for the face drawings. Each feature could take three discrete values and the categories were separable along two of the four dimensions of the stimuli. In the face drawings, for instance, diagnostic dimensions were eye height and separation. Electrophysiological recordings showed that neurons in the ITC did indeed react selectively to diagnostic features (Sigala and Logothetis, 2002).

Obviously, such a priori parameterization cannot be done easily when the stimuli are complex objects or entire scenes. Natural images contain structure on many spatial scales distributed nonhomogenously across the image and are thus good examples of complex, redundant visual forms. In another series of studies, we therefore identified diagnostic fragments by using a method developed by Phillip Schyns and his colleagues. With this technique the stimuli are presented behind occluders, which consist of a mid-gray mask punctured by a number of randomly located windows (“bubbles”) through which the occluded image is visible. The monkeys continued to perform their discrimination task on the partially visible images. Whether they could identify the partially visible stimuli depended on whether the occluder uncovered image parts critical for task performance. Diagnostic feature selectivity was found in both the MUA and the LFP responses, suggesting again that such features are explicitly encoded in the
response of neurons (Nielsen et al., 2006b). However, an interesting difference existed between MUA and LFP responses. While MUA responses were homogenously distributed across the tested portion of ITC, the LFP responses were selective only in the anteriorly located recording sites, a finding suggesting that diagnosticity is first encoded in the posterior IT cortex. This result also demonstrates the power of combined analysis of field potentials and spiking activity for mapping structure to computational function in the brain.

**Detecting Objects in Scenes: Effects on Neural Responses**
As described above in the studies of multistable perception, IT cortex neurons tend to respond when a visual pattern is perceived. This property was also revealed in experiments on object and scene recognition. Recordings were carried out while monkeys looked for and identified familiar targets embedded in natural scenes. Initial results showed a group of visual neurons that exhibit stimulus-selective neuronal bursts just prior to the monkey’s response. During exploration, neuronal activation often began shortly before effective targets were fixated, but only if the target was the goal of the next fixation. The magnitude of this early activation was found to vary inversely with reaction time, indicating that perceptual information is integrated across fixations to facilitate recognition (Sheinberg and Logothetis, 2001).

**Mapping Object-Selective Areas with fMRI**
The representation of shape and objects was also studied in fMRI experiments with anesthetized or behaving monkeys. In one series of experiments, computer-generated 3-D objects defined by shading, random dots, structure elements or silhouettes were presented either statically or dynamically (rotating). Our findings suggest that 3-D shape representations are highly localized although widely distributed in occipital, temporal, parietal and frontal cortices, and may involve common brain regions regardless of shape cue (Sereno et al., 2002). Ongoing work examines the specificity of this network across different experimental subjects. Finally, a number of researchers in the lab are interested in plasticity and learning. Learning about the world through our senses obviously constrains our ability to recognize our surroundings. How learning-induced changes in recognition manifest in neural populations is one question being pursued by members of the recognition group (Rainer et al., 2004b;Rainer et al., 2004a;Lee et al., 2005;Liebe et al., 2006;Nielsen et al., 2006a;Nielsen et al., 2006b;Sigala et al., 2006;Dahl et al., 2007;Hoffman et al., 2007;Nielsen et al., 2008).

**Future Directions**
Just as in the case of multistable perception, physiological investigators of object categorization and identification suffer from the lack of integrative methodologies. Our attempts to optimize the combined physiology and fMRI experiments in the behaving animal serve the aim of studying recognition by analyzing the response of networks rather than of isolated cells or recording sites.

**Neuronal Basis of Sensory Integration**
The perception of our environment depends on information acquired by different sensory organs. To improve perception and to obtain a coherent and faithful ‘picture’ of our sensory environment, the brain has to merge the information obtained from different senses. In cases where proper sensory integration fails, we experience an illusory percept. For example, when we hear a ventriloquist we attribute the voice to the moving mouth of the dummy.
How the brain merges information from different senses and how information from one sense aids the analysis of sensory information from another is a challenging question in systems neuroscience. A first step is to understand when this integration occurs during sensory processing. According to one hypothesis, integration occurs as one of the last steps and only after each sensory system has thoroughly processed its information. According to a second hypothesis, integration already occurs at very early stages of sensory processing, in order to bias the processing of each sense to be in accordance with the processing in other senses.

We address this question using the auditory system as a model and exploiting the latest functional magnetic resonance imaging (fMRI) technology. Our experiments combine different sounds with simultaneous touch and visual stimulation and search for regions in the brain where the combination of these stimuli leads to significant enhancements of activity. Such response enhancement is a classical characteristic of sensory integration, resulting from the synergistic combination of sensory information.

Our recent results demonstrate that integration of touch- and sound-related activity occurs very early during auditory processing. By segregating different functional fields in the auditory cortex (Petkov et al., 2006), we could show that this merging of sensory information occurs in the secondary auditory cortex. Hence, it occurs not as early as possible, but still very early during sensory processing and in areas which are classically regarded as purely unisensory. In addition, our data suggest that the touch-related information comes as feed-forward input to auditory cortex and hence does not depend on higher association areas in the brain and is not part of a cognitive modulatory feedback. These results demonstrate that those regions of the brain uniquely devoted to the processing of a single sense are rarer than classically thought. Instead, most of the brain is concerned with merging information across senses and creating a coherent percept. As a next step we will address exactly how the information from one sense modulates or biases the processing in another sense (Kayser and Logothetis, 2007; Kayser et al., 2007; Lippert et al., 2007; Kayser et al., 2008).

**Evolutionary Basis of Communication Group**

Monkeys, like humans, rely on members of their own species for social interactions and survival. Vocal communication facilitates interaction, warns the animals of danger and keeps the group together during group movement. Behaviorally we have known that monkeys are exquisitely sensitive to vocalizations from members of their own species and that they can vocally recognize individuals. What was not clear is whether there are dedicated neuronal regions in the primate brain for processing species-specific vocalizations. In humans, a patch sensitive to voice has been seen in the anterior temporal lobe, but little is known about possible brain sites that are involved in voice recognition in monkeys.

In an attempt to localize voice-sensitive areas in monkeys, we used fMRI to map cortical regions that might respond to macaque vocalizations, including coos and grunts, as well as the calls of other animals and natural noises such as thunder and running water. A small region of the monkeys' temporal lobes did indeed become active in response to monkey voices but not to other sounds (Petkov et al., 2008). The voice-sensitive area was found to distinguish the voices
of individual monkeys. Responses diminished when one monkey’s voice was played repeatedly, but perked up again when a new voice was played.

Figure 1: Auditory cortex regions preferring species-specific vocalizations in two alert monkeys (#1 & #2). Combined and coregistered data from six of the experiments with each animal. The color code from orange to red indicates voxels with a clear and significant preference for macaque vocalizations. The cyan-to-blue color code identifies voxels with no preference for vocalizations. Slice orientation and position are shown in the lower inset. The left panel shows some of the sound categories used. A1, field in primary auditory cortex; Tpt, temporoparietal; Pro, proisocortex of the temporal pole.

This study challenges the idea that there is little to be learned from other animals about human communication and it has important implications for how human language evolved. The human-voice region appears to have repositioned to a different anatomical position in the brain than where the monkey region was observed. This means that the comparative study of the voice regions in both humans and monkeys could disclose not only how evolution affected the voice regions but also how the neighboring human speech and language regions were affected by the brain differentiation that gave rise to human language and cognitive abilities. Second, the observation of clear evidence for vocal-recognition regions in the brains of living nonhuman primates supports the notion that human language evolved gradually. It now seems more likely that the vocal-recognition systems of nonhuman primates served as a neuronal basis upon which human verbal recognition and language evolved, rather than a striking evolutionary event which occurred recently within our human lineage and cannot be studied in extant nonhuman primates (Petkov et al., 2008) and Petkov et al 2008, in press.
The study on the monkey voice area was selected as the scientific highlight of the year by the Federation of European Neuroscience Societies (http://fens.mdc-berlin.de/shoty/?action=show&id=9)

**The Neurophysiology of the fMRI Signal**

LFP signals reflect the input and local processing in cortex, while MUA and SUA mirror the activity of projection neurons, i.e. the output of the examined site. Electrophysiological studies examining the individual contributions of different LFP frequency bands, multiple-unit activity, and spiking of individual neurons are probably our only realistic chance of gaining insights into the neural mechanisms of hemodynamic responses and their meaning in the context of different cognitive tasks.

The relationship of neocortical LFPs and spiking activity to the BOLD signal itself was examined directly in concurrent electrophysiology and fMRI experiments in the visual system of anesthetized and alert monkeys. These studies found that the BOLD responses reflect input and intracortical processing rather than pyramidal cell output activity. Initially, both LFPs and spiking seemed to be correlated with the BOLD response, although quantitative analysis indicated that LFPs are better predictors of the BOLD response than multiple-unit or single-unit spiking. The decisive finding leading to the papers’ conclusions, however, was not the degree of correlation between the neural and the fMRI responses or the differential contribution of any type of signal into the BOLD responses, but rather the striking, undiminished hemodynamic responses in cases where spiking was entirely absent despite a clear and strong stimulus-induced modulation of the field. Similar dissociations between spikes and CBF had been demonstrated earlier and again very recently in a number of studies using other techniques.

**Structural Neurovascular Coupling**

A number of researchers in the laboratory continue the study of neurovascular coupling by investigating both its structural and its functional components. To study the organization of the vascular system we combine corrosion casts, immunohistochemistry, and cytochrome oxidase (COX) staining. Our detailed measurements of regional vascular length density, volume fraction, and surface density revealed a similar vascularization in different visual areas. Interestingly, the highest degree of correlation was found between vascular density and steady-state metabolic demand as measured by COX activity, rather than between vascular parameters and density of neuro-glia elements. This observation suggests that although the number of neurons and synapses determines an upper bound on an area’s integrative capacity, its vascularization reflects the neural activity of those subpopulations that represent a “default” mode of brain steady state. A further attempt is currently underway to precisely map the vascular system of the monkey cortex using scan electrode microscopy (SEM) with large-range stage motorization that permits the scanning of large samples. In addition, a framework is being developed that can model the flow of blood through the cerebral vasculature based on simple fluid-dynamical principles. Three-dimensional advective transport, vasculature-tissue exchange and diffusion within the tissue make it possible to simulate oxygen transport, drug delivery and alike. The framework provides a means to compute blood pressure, flow and scalar transport.
**Functional Neurovascular Coupling**

In our research into functional neurovascular coupling, we recently used esfMRI. Electrostimulation induces a strong decoupling of MUA from LFP by shutting down the output of an area as a result of strong feedforward inhibition elicited by the simultaneous activation of the excitatory and inhibitory recurrent circuits of neocortex. The results show – once again - that activation of an area can occur even when the output of that area is entirely blocked. Input-output dissociations like the ones just described are not rare phenomena that can only be induced experimentally.

*Figure 2: Vascular casts showing the details of the cortical vascular system of monkeys.*

When cortical areas are “feedforward” stimulated with simple sensory stimuli, then field potentials and spiking are correlated, barring certain stimulation conditions like those described above. Activations due to cognitive processing, on the other hand, might be dominated by top-down and neuromodulatory signals that might increase the excitation-inhibition balance of cortical microcircuits without necessarily being associated with a concomitant increase in the spiking of task-selective neurons. The effects of neuromodulation on large populations can be studied in a number of ways, including investigation of spontaneous cortical activity (The influence of specific neural events, oscillations and synchrony on the fMRI signal within and between visual areas of the macaque brain). Finally, the study of functional neurovascular coupling greatly may profit from attempts to calibrate the BOLD signal, in order for the latter to reflect as close as possible the actual energy metabolism.

**Philosophy, Aims & Outlook**

**Study of Global Neural Networks**

**Functional MRI guided Multisite – Multielectrode Recordings**

Studies of the neural mechanisms of cognitive capacities can only go beyond microphrenology (reporting the position and frequency of gnostic units) if recordings are carried out with multiple electrodes (E-I circuit information) at multiple sites (interaction and integration information). Such undertakings are usually hampered by practical considerations, including the feasibility of multiple chamber implantations, exact positioning of electrodes, quality and stability of chronic implants, and last but not least theory-driven analysis of data. We believe that the combination of fMRI with MR-based in vivo connectivity (see below), combined with miniaturization of implants and MR-guided surgery will greatly help to employ this approach in cognitive experiments with trained animals.
Parallel to technical progress in data acquisition, there is an obvious need for new computational tools to analyze and interpret the resulting large data flow from experiments and simulations. Brains are characterized by every property that engineers and computer scientists would detest and would rather avoid altogether. They are chaotic, unstable, nonlinear, nonstationary, non-Gaussian, asynchronous, noisy, and very often unpredictable in fine grain. Advances in fields such as information theory, analysis of dynamics systems, and synchronization between chaotic systems lead to increases in the use of nonlinear multivariate techniques. Generalized synchronization and phase synchronization of chaotic oscillators are examples of concepts studied with multivariate nonlinear time series methods. Mathematical techniques, such as various dimensionality reduction techniques, mean field theory, pattern classifiers and dynamic coupled models are also likely to help us gain insights into multidimensional point processes (e.g. spikes) and continuous analog signals (e.g. field potentials), yet the success of their application, in our opinion, depends on a sound, data-driven conceptual framework and theory rather than the implementation of strategies developed in physics and engineering. The philosophy just discussed here drives our intensive collaboration with theoreticians, modelers and analysis experiments, to whom we provide data from a large number of projects involving different sensory and cognitive systems.

**In vivo Connectivity with Microstimulation and fMRI**

Microstimulation during fMRI provides a unique opportunity to study certain aspects of networks. To be more specific, concurrent microstimulation and fMRI experiments – among others – provide information about projective fields, networks underlying electrostimulation-induced behaviors, anterograde connectivity, neuromodulatory systems, as well as about the functional neurovascular coupling. The combination of microstimulation with ultra-high resolution (e.g. 125x125x600 μm³) enabling the visualization of activation of individual laminae may also help separate feedforward and feedback influences at a given cortical site. We recently developed and optimized this technology, and we are currently using it in combination with electrophysiology and fMRI in anesthetized and alert monkeys to study cortico-cortical signal propagation. Stimulation of cortical afferents, for example, appears to disrupt the propagation of signals, most likely because of the simultaneous activity of the recurrent excitatory and inhibitory networks.

Stimulation of hippocampus in rats reveals the effects of regional synaptic plasticity on the effective connectivity of subcortical and cortical areas. Specifically, the induction of LTP through the stimulation of the perforant path leads to network-level modification of connectivity, with recruitment of contralateral sites, and most importantly of many different cortical and subcortical sites that are related to memory, learning, and habituation. The existing anatomical connectivity by no means reduces the value of the fMRI-detectable effective connectivity, because the very same sites are not excited before LTP is induced or when NMDA blockers are used.

**In vivo Connectivity with Paramagnetic Tracers**

The application of MRI-visible paramagnetic tracers to reveal in vivo connectivity can also provide important subject-specific information for multi-site, multi-electrode intracortical recordings in combined behavioral and physiology experiments. To establish the use of such
tracers in the non-human primate, we recently compared the specificity of the anterograde tracer Mn2+ with that of wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) in experiments tracing the neuronal connections of the basal ganglia of the monkey. It was shown that Mn2+ and WGA-HRP yield the same projection patterns and that the former tracer crosses at least two synapses, for it could be found in thalamus following injections into the striatum.

In a recent publication we provide additional evidence that Mn2+ reaches the cortex following striatum injections and thus is transferred even further than previously shown. In other words, when used as a paramagnetic MRI tracer Mn2+ can permit the visualization of neural networks covering at least 4 processing stages. Moreover, unilateral intravitreal injections show that Mn2+ is sufficiently synapse-specific to permit visualization of the lamina of the dorsal lateral geniculate nucleus (dLGN). Interestingly, the transfer rate of the substance reflected the well-known axonal size differences between the parvocellular and magnocellular layers of dLGN. After intravitreal injections we were able to demonstrate transfer of Mn2+ into several subcortical and cortical areas, including the anterior inferotemporal cortex. The specificity of the trans-synaptic transfer of manganese that we report here indicates the value of this tracer for chronic studies of development and plasticity, as well as for studies of brain pathology.

Oscillations

Oscillations might be useful for structuring distributed representations in time and space, because their synchronization can coordinate processes across different cortical areas. Their spatiotemporal pattern can modulate synaptic integration such that signals may be routed through cortical pathways such as those required to solve a behavioral task. The timing of oscillatory local field potentials recorded from pairs of micro wires implanted in multiple cortical areas is so precise that latencies can be determined and compared across areas.

One research line in our laboratory examines whether interactions between remote cortical areas can be identified, and if so, if the spatiotemporal structure of these interactions provides evidence about the direction of information flow. In the past, cortical timing was generally studied with neuronal response latencies assuming feed-forward processing. Yet, cortico-cortical connectivity is dominated by lateral and feedback rather than feed-forward connections. Potential differences in the timing between different types of connections might be studied by a careful examination of the amplitude and phase of oscillatory activity. Monkeys are trained to perform demanding behavioral tasks that require cortical processing rather than highly over-trained stereotypes. They perform memory and sensorimotor tasks during which the animals can be challenged by dynamically increasing memory load or the similarity of stimuli that need to be compared or by increasing the required precision of sensorimotor integration. Recordings are carried out with arrays of tetrodes. The future aim of this group (M. Munk) is to exploit this technology as well as its analysis methods for multiple-tetrode – multiple-site recordings.
Technical Developments

Overview

A number of investigators and support engineers in the laboratory concentrate on the development and further improvement of technologies that would permit the implementation of an integrative approach to systems neuroscience questions. Completed, in progress and future projects can be roughly grouped as shown in the list below. The names of individual laboratory members are not included for the sake of legibility; they can be found by consulting the individual reports for each research group.

- **Interpretation of Neural Signals**: Studies of volume conduction, information-theory-based band separation, burst-spiking prediction from field potentials and vice versa (in collaboration with W. Maas, Graz, Austria, S. Panzeri, Genoa, Italy, and G. Kreiman, Cambridge, USA), causality studies (in collaboration with B. Schölkopf, MPIK), spatial dependence of neurovascular signals. Combined EEG and electrophysiology studies aiming to investigate whether the EEG signal contains information about neuronal spiking from a population of neurons, and if so, to estimate the components of the comprehensive EEG signal that can be used to model the underlying neuronal activity.

- **Developments in monkey MRI & fMRI**: High-resolution imaging w/ implanted coils, diffusion and perfusion imaging, diffusion tensor imaging (DTI, in collaboration with G. Parker, Manchester, UK), parallel imaging, single-voxel spectroscopy & CSI, behavior monitoring in high-field scanners. The aim is to develop methods that combine fast scanning with high spatial resolution imaging. These series of studies will greatly benefit from the expected interactions with the NMR center of the MPIK.

- **Structural Neurovascular Coupling**: Vascular casts, immunohistochemistry, micro X-ray tomography, models of CBF, CBV & scalar transport (in collaboration with B. Weber, ETH Zürich). The aim of the project is to build realistic models of area-dependent hemodynamic responses.

- **Functional Neurovascular Coupling**: Combined electrophysiology & fMRI experiments, neuropharmacology-electrophysiology-fMRI, hypercapnia & BOLD calibration. This research line examines the relationship of the BOLD and perfusion fMRI signal to various components of the mean extracellular field potential, in particular under certain informative conditions that induce dissociation between subthreshold and spiking cortical activity.

- **In Vivo Connectivity**: Manganese-enhanced MRI (MEMRI), microstimulation & fMRI. The importance of this project cannot be overestimated. In future investigations of perception and cognition, our hope is to combine multi-tetrode recordings simultaneously at different cortical (or subcortical, e.g. pulvinar) sites. The multi-electrode & multi-site measurements will be primarily guided by in vivo connectivity studies.
• **Neuromodulation:** Capillary microsampling (LC-MS/MS) & fMRI, recording & neuromodulator monitoring, microstimulation & neuromodulator sampling (in collaboration with G. Rainer, Friburg). Correct interpretation of neuronal activity in the context of different cognitive tasks will eventually require us to face the problem of separating feedforward stimulus-induced processing from changes in activity due to neuromodulation and top-down feedback. Simultaneous electrophysiology and neurotransmitter/neuromodulator monitoring is one strategy for separating and analyzing different types of signals.

• **Molecular Imaging:** Ca2+ sensitive Gd-based contrast agents (CA), amino-acid sensitive Gd-based CA, biocytin-based contrast agents (in collaboration with the H. Mayer, M. Maier & L. Wesemann of chemistry department of the Tübingen University, E. Jakab Toth, Orleans, France and D. Parker, Durham, UK). The aim of the project is to develop fMRI modalities that report actual neural activity rather than hemodynamic responses.

The rest of this section offers a few examples of completed or on-going methodological work and discusses its perspectives for the next seven-year time period.

**MR Methodologies**

**FMRI in Alert Monkeys**
Functional imaging in alert trained animals is a more difficult undertaking than one might think. In particular, the application of fMRI at high fields can only be successful if the challenges presented by the highly increased sensitivity to image artifacts are systematically and rigorously addressed. Over the last six years we have been testing and improving a number of different methodologies that can ensure artifact-free acquisition of data from the entire brain of monkeys. A selection of these methods is briefly described below.

The magnetic field throughout the sample is position-dependent, resulting in inhomogeneities that are much greater at high fields. Sample motion can lead to changes in the local magnetic field, which in turn lead to geometric distortions of the EPI images and local nonlinear warping of the images. These distortions are especially severe when gradient-echo echo-planar imaging (GE-EPI) is used. The head motion of the monkey can be minimized by head-fixating devices, but body and jaw movements may also introduce strong main field (B0) fluctuations and considerably change the shimming of the field. The only remedy to the problem is appropriate training, although this is aided by precise monitoring of any head, jaw and body movement. We have recently developed both the hardware and the software required to track such movements (Kelliris et al., 2007). We have showed that body and jaw movements do indeed introduce major MRI image artifacts even when the animal’s head is immobilized. A novel behavioral paradigm for the training and scanning of the monkeys is presented which takes into account the necessity for controlled movement behavior. Our findings and techniques now permit successful alert monkey fMRI at high magnetic fields. In addition, we have developed a variant of the well known search-coil eye-tracking system (Oeltermann et al., 2007) that can be used within the magnet to measure eye movement with high resolution (in min of arc).
On the data-analysis side, navigator-echo based techniques (corrections of dynamic off-resonance k-space distortions) were developed that minimize the image-to-image variability and enhance traditional motion-correction procedures (Pfeuffer et al., 2007). Moreover, pattern recognition methods such as correlation analysis, support-vector machines (SVM), linear discriminant analysis (LDA) and Gaussian naïve Bayes (GNB) were evaluated using data collected at high field (7 Tesla) with higher resolution than conventional fMRI studies (Ku et al., 2008). The performance of the various algorithms was found to depend on the nature of the brain activity being categorized: for several tasks, many of the methods work well, whereas for others, no method performs above chance level. An important factor in overall classification performance is – once again – careful preprocessing of the data, including dimensionality reduction, voxel selection and outlier elimination.

Figure 3: The left panel shows the activation of temporal lobe. The right panel compares the functional activation obtained with GE-EPI and SE-EPI with an 8 cm surface coil over the monkey’s ear. (A) Anatomical images, FLASH, FOV 9.6×9.6 cm, matrix 256×256, TE/TR 10/750 ms. (B) Functional maps acquired with a 2-segment SE-EPI sequence with window of 9.7 ms and 104 Hz/pixel show functional activation along the entire lower temporal lobe. FOV 9.6x9.6 cm, matrix 64x48, TE/TR 40/1000 ms. (C) Functional maps acquired using a 2-segment GE-EPI show strong functional activation in early visual areas and STS, but none in the anterior temporal lobe or more posterior near the ear canal. FOV 9.6x9.6 cm, matrix 64x48, TE/ TR 19/1000 ms. The same areas that show activation in the GE-EPI are seen in the SE-EPI activation map. a comparison of the activation maps with anatomy shows that the activation in the
Quite a few years ago we established high-resolution fMRI and chemical shift imaging of the primary visual cortex and early extrastriate areas in the alert monkey at 4.7T (Logothetis et al., 1999; Pfeuffer et al., 2004b; Juchem et al., 2005; Goense and Logothetis, 2006; Goense et al., 2007) and later at 7T (Pfeuffer et al., 2004a; Pfeuffer et al., 2007); both setups can now be used for combined fMRI, electrophysiology and microstimulation experiments (Goense and Logothetis, 2008). Frontal and temporal areas, however, remain a challenge. A clear disadvantage of high fields is that susceptibility artifacts from air-filled cavities like the ear canal and nasal cavity cause signal loss and distortion. To allow fMRI of temporal areas, we use an optimized SE-EPI which recovers signal lost with GE-EPI, and we correct for susceptibility-induced image distortion (Goense et al., 2008). SE-EPI has the added advantage that, in contrast to GE-EPI, where the functional signal derives to a large extent from veins, the SE-EPI signal arises from the microvasculature and hence better represents the neural activation. Using this protocol at 7T we were able to show fMRI of the entire visual pathway in the awake primate with robust and widespread activation in all temporal and ventral areas of the brain, including areas adjacent to the ear canal.

**Surface Coils and High-Resolution fMRI**

A comprehension of the computations carried out by the neocortical microcircuits, among others, relies on the study of the interlaminar connectivity patterns and the intralaminar physiological processes in vivo. High-resolution functional neuroimaging, enabling the visualization of activity in individual cortical laminae or columns, may greatly contribute to such studies. Yet the BOLD effect as measured with the commonly used GE-EPI contains contributions from both macroscopic venous blood vessels and capillaries. The low density of the cortical veins limits the effective spatial specificity of the fMRI signal and yields maps that are weighted toward the macrovasculature and thus can deviate significantly from the actual site of increased neuronal activity. Spin-echo (SE) sequences yielding apparent T2-weighted BOLD images have been shown to improve spatial specificity by increasing the sensitivity of the signal to spins of the parenchyma, particularly at high magnetic fields.

![Figure 4: (A) GE-BOLD activation map; resolution=333x250 micrometer square. For GE-BOLD fMRI, the activation is maximal at the surface (vessels), while the SE-BOLD map (resolution=333x326 micrometer square) shows maximal activation in Layer IV.](image)

We have implemented SE-fMRI at 4.7T to examine the specificity and resolution of functional maps in the primary visual cortex of monkeys (Goense and Logothetis, 2006; Goense et al., 2007). Cortical layers could be clearly visualized, and functional activity was predominantly localized in cortical layer IV/Duvernoy layer 3. The choice of sequence parameters influences the fMRI signal, as the SE-EPI is by nature sensitive to T2* in addition to its T2 dependency. Using parameters that limit T2* effects yielded higher specificity and better visualization of the cortical laminae.
At high resolution, exact functional-to-structural registration is of critical importance, because even small differences in geometry that arise when different sequences are used for functional and anatomical scans can lead to wrong localization of activation and erroneous interpretation of data. When we used SE-EPI for functional and anatomical reference scans, we noticed a precise spatial colocalization of the largest fractional changes with the Gennari line, suggesting peak activity in Layer IV. Interestingly, this very same layer coincided with the largest relaxivity changes as observed in steady-state cerebral blood volume measurements using the intravascular agent monocrystalline iron oxide nanoparticles (MION).

A Combined MRI and Histology Atlas of the Rhesus Monkey Brain

Rationale
In biology, structure and function are closely linked everywhere one looks. This principle is spectacularly evident in the central nervous system, where function reflects the input, local organization and output of structurally distinct regions. The tight structure-function relationships are evident on many organizational levels ranging from the relation between conformation of membrane proteins and cell electrodynamics to the coupling of functional specialization of, say, a cortical area to its cyto- and myelo-architectonics. At the systems level, we first need an accurate map of the architectonic areas with reference to MR images in the same animal before we can begin to understand the anatomical localization of functional activity in different cortical areas.

The aim of this project was to map the detailed architectonic subdivisions of the cortical and subcortical areas in the rhesus monkey using high-resolution MR images and the corresponding histological sections in the same individual. The map of cortical and subcortical areas was derived from both horizontal and coronal sections using a number of different staining methods. Five parallel series of sections were stained for Nissl substance or with antibodies against parvalbumin, calbindin, calretinin, and a non-phosphorylated epitope of a neurofilament protein, SMI-32 (Kadharbatcha S.Saleem and Logothetis, 2007).

Acceptance & Future Plans
The Atlas was well received by primate researchers worldwide; what follows are representative comments: “This superb and richly illustrated atlas of the Macaque brain is a neuroanatomist’s dream. It combines brain slices stained with several different methods with corresponding structural MRI slices, stereotaxic coordinates and detailed drawings demarcating landmarks, cytoarchitectonic and functional regions. It should be considered the Gold Standard for anyone working with the Macaque brain”. By B. Miller. “This much needed atlas is meticulous and clear, and a brilliant exposition of the basic anatomical relationships of the macaque brain. It will be a
treasured reference and guide for both experts and beginners over a wide range of research areas." By K.S. Rockland. “This atlas of the macaque brain will serve as a valuable resource to the neuroscience community because it combines detailed architectonic maps with high-resolution structural MRI that preserves information about spatial coordinates." By D.C. Van Essen.

The work will be extended by adding sagittal series as well as a detailed map of the monkey vascular system in collaboration with Bruno Weber (ETH) and Anna Lena Keller (MPIK, Tübingen).

MASS SPECTROMETRY-BASED NEUROCHEMICAL ANALYSIS

Rationale and Definitions
The main characteristic of cortical microcircuitry is strong excitatory and inhibitory recurrence (see also “Philosophy, Aims & Outlook”). Activation of the cortex sets in motion a sequence of excitation and inhibition in every neuron, and the time evolution of E-I interactions is far longer than the synaptic delays of the circuits involved. In other words, from an electrophysiological point of view cortical excitation and inhibition are practically inseparable events. In addition, electrical measurements of the extracellular field potential, relying on local geometry might have strong limitations in revealing the role and magnitude of neuromodulatory input, which induces proportional changes in excitatory and inhibitory conductances. Neuromodulation not only alters synaptic transmission, but it is critically important for network behavior such as self-organization and associative memory.

In our efforts to develop and apply integrative approaches that go beyond a regional analysis of the spiking of a few neurons, we have started implementing mass spectrometry (MS), particularly in conjunction with capillary hydrophilic interaction chromatography (HILIC), to measure the dynamic changes in the concentration of multiple neurochemicals in the anesthetized and alert monkey, combined with physiology and/or fMRI (Zhang et al., 2008).

MS is an established analytical technique that identifies the chemical composition of a compound or sample based on the mass-to-charge ratio of charged particles. It is capable of fast, structure-specific detection and can provide characteristic information, including molecular mass and fragmentation ions, thereby allowing the identification and quantification of a target compound. In principle, it can be used to detect any neurochemical substance from the brain. Neurochemicals in the brain are of significant structural diversity and their concentrations span several orders of magnitude. MS-based neurochemical analysis approaches provide great opportunities for high-sensitivity measurements of different classes of neurochemicals, including but not limited to amino acids, monoamines and neuropeptides.

Although MS has become an indispensable tool for neuropeptide analysis, with enormous potential for the discovery of novel signaling peptides and biomarkers, until recently only a limited number of studies had reported on the analysis of small neurochemicals such as classical neurotransmitters, in part due to the difficulties in separating polar molecules with current chromatography techniques with MS-compatible conditions.
Development and First Results
Three postdoctoral fellows recently implemented HILIC-MS/MS in our laboratory. We were able to demonstrate the feasibility of simultaneously determining the concentrations of six neurotransmitters (acetylcholine, serotonin, dopamine, gamma-aminobutyric acid (GABA), glutamate and aspartate) in the extracellular brain fluid (EBF).

We used HILIC-MS/MS to analyze the EBF from the monkey brain. A push-pull sampling method was used to collect EBF from the prefrontal cortex (PFC) of conscious monkeys at flow rates in the range of low nl/min. The detection limits of acetylcholine, serotonin, dopamine, GABA, glutamate and aspartate were 0.015, 0.15, 0.3, 1.2, 6 and 15 femtomoles, respectively, allowing us to quantitatively determine the concentrations of these six neurotransmitters simultaneously from 200 nl in vivo samples (Zhang et al., 2007).

We further studied the effects of an intracortical application of cholinergic agents in the cortex on the concentration of these chemicals in the EBF. We observed significant increases in extracellular neurochemicals like glutamate and glutamine after the application of ACh or nicotinic agonists. During the ACh application experiments, the profile of exogenously applied ACh and its product choline were monitored, showing that ACh itself remained in the low nM range 30 min after the injection of a 1 mM solution due to the fast cholinergic metabolism in the brain.

Future Plans
Currently this method can be used in combination with electrophysiological measurements. Experiments planned for the near future will use the combined approach to study both the principles of microcircuits, for example during electrical microstimulation of cortical afferents, the effects of diffuse ascending systems, which reflect the animal’s internal state, on cortical processing, and the balance between neurotransmission and energy supply. Within the next couple of years we expect the method to be fully implemented for simultaneous neurochemical measurements and fMRI.

SMART Contrast Agents

Rationale
Many functional neuroimaging techniques, including optical imaging of intrinsic signals (OIS) and fMRI, rely on the regional hemodynamic responses elicited during brain activation. Such techniques have relatively low spatial resolution, and are slow (on the scale of seconds) compared to actual neural activity (which takes place in milliseconds). Consequently the development of imaging modalities that do not depend on hemodynamics has long been considered of great importance, and a number of techniques have been already developed and are being used for the optical imaging of neural activity. They include techniques relying on membrane potential, pH, and ion (Ca²⁺, Na⁺, K⁺, Cl⁻, Mn²⁺) indicators, as well as protein and neurotransmitter markers. Unfortunately, no such markers currently exist which would be suitable for functional MR imaging.

Functional MRI with smart contrast agents (SCA) that act as event or molecule markers could combine the specificity of cellular neural recording techniques with noninvasive whole-brain
coverage. At present only a handful of laboratories worldwide are working on the synthesis of agents that could be used to implement ion or neurotransmitter imaging in MR scanners, and a marker that has been proved to work in vivo has yet to be synthesized. For example, the first SCA designed for real-time calcium imaging was developed about a decade ago (Li et al., 1999), but delivery of the sensor to neurons in quantities that would be sufficient for functional imaging in vivo has not yet been reported, and a closer inspection of the SCA structure in our laboratory actually suggests that the structure of that molecule cannot, in principle, justify its anticipated functional properties.

About five years ago, encouraged by the successful development of techniques that permit electrophysiological recordings, injections, and microstimulation in combination with MR imaging, we started a research line in our laboratory that focuses on the synthesis and characterization of SCA. The project is being carried out by postdocs and graduate students of synthetic and coordination chemistry in tight collaboration with the chemistry department of the University of Tübingen as well as with two well-established physical chemistry labs in the UK and France. Our efforts were financially supported by a prize (€400,000) from the Jeantet Foundation, a grant (€600,000) from the Hertie Foundation, and with running costs covered by the Max Planck Society. Aided by this funding, our group has now established excellent technical conditions for chemistry research. They include state-of-the-art chemistry labs with standard equipment (8 working hoods), ESI mass spectrometer, fluorescence spectrometer, preparative HPLC, and a 300 MHz (7T) NMR spectrometer that can be used, among other things, for relaxometry studies. The chemistry group is now an active member of the COST D38 Action entitled “Metal-Based Systems for Molecular Imaging Applications”, a network consisting of about 30 European research groups.

Despite the expected initial difficulties and the fact that chemistry is not the primary focus of our department, we were able to synthesize and characterize more than 30 potential SCA using gadolinium or europium as paramagnetic ions (see below). A few of the synthesized SCA were sensitive to pH and others exhibited remarkable sensitivity towards Ca$^{2+}$; the physicochemical characteristics of the latter were considerably better than any other currently available Ca$^{2+}$-sensitive MR probe. All results have now been published in 6 papers in peer-reviewed chemistry journals. What follows briefly explains the essence of this research line, the basic findings, and our future plans.

**What is a Smart Contrast Agent?**

To generate an MR image, say an anatomical image of the brain, one needs a contrast mechanism that can separate different brain parts from one another (e.g. gray from white matter, or cerebrospinal fluids), and a mechanism by virtue of which that contrast can be calculated for each voxel. MRI contrast can be generated from a number of different quantities, including regional spin density, water diffusion, or so-called relaxation times. The latter term refers to the exponential decay of magnetization following a radiofrequency pulse.

There are different kinds of relaxation processes known as T1 (due to spin-lattice interactions), T2 (due to spin-spin interactions) or T2* (susceptibility effects), each reflecting different interactions of the spins with their environment or with other spins. Most MR images in biomedicine rely on the clever selection of acquisition parameters that make the image
differentially sensitive (also called weighted) to a relaxation process. For example, the usual anatomical images of the brain are most often T1-weighted images, exploiting the differences in the T1 of gray and white matter; most functional MR images, on the other hand, are T2- or T2*-weighted. Just as relaxation-rate differences at different sites might be used to generate anatomical contrast, changes over time in one or more relaxation rates, at a single site, might be exploited to image changes in the physical-chemical state. This very simple principle underlies the functional contrast in fMRI, which in fact detects changes in relaxation times between test and control epochs.

In conventional fMRI the relaxation-rate changes fortuitously reflect the natural physical and chemical events accompanying variations in neuronal activity. Spin motion due to blood flow, diffusion, or changes in field homogeneity because of alterations in the concentration of deoxyhemoglobin all affect the relaxation rates and can yield a measurable fMRI signal. Yet the specificity and spatiotemporal resolution of this signal as well as its functional contrast-to-noise ratio are significantly limited by the architecture, density and dynamics of the vascular system.

SCA are simply molecules whose relaxivity is a function of the concentration of a useful ion or molecule, usually called the target. The term “relaxivity” denotes the ability of a contrast agent to shorten the relaxation time of nearby water protons. The higher the relaxivity is, the shorter the proton relaxation time.

Typically, an SCA, just like a conventional anatomical contrast agent, is a paramagnetic ion complexed with organic molecules. As a result of their unpaired electrons, paramagnetic metal
ions act as potent MRI contrast agents (Merbach and Toth, 2001). Some of them shorten T1 without causing substantial T2 effects (e.g. gadolinium, Gd\(^{3+}\)), whereas others induce line broadening that masks the signal intensity (e.g. superparamagnetic iron oxide nanoparticles). Gadolinium has the greatest effect on T1 and hence is more commonly used than other ions. Yet, like other lanthanides, Gd in its native form is highly toxic, and it is therefore always used after complexation with various ligands. An example is the well-established clinical intravascular contrast agent Magnevist; a gadolinium chelate (Gd-DTPA) that, when injected, is confined to the intravascular space and increases image contrast.

In contrast to conventional anatomical agents, SCA molecules are synthesized in such a way that a change in the concentration of a target molecule affects the three-dimensional configuration of the complex, which in turn changes at least one of the three fundamental physical properties of paramagnetic complexes: (a) the number of water molecules coordinated to the paramagnetic ion; (b) the lifetime of a water molecule bound to the paramagnetic ion, and (c) the rotational correlation time of the complex. Parameter variations lead to relaxivity changes. Figure 6 illustrates the functional principle of an SCA. The complex can be thought of as a crab with alkylphosphonate pendant arms (Fig 5, left panel). For low Ca\(^{2+}\) concentrations the contracted arms minimize the access of water to the paramagnetic ion; an increase in Ca\(^{2+}\) concentration changes the coordination of the “arm” molecule (Fig 5, right panel), and the latter increases, for instance, hydration (the first parameter).

**Synthesis and Evaluation of SCA in our Laboratory – Completed Work**

We have synthesized a number of complexes containing phosphonate groups at variable distances from the macrocyclic (MR-responsive) moiety. They exhibited changes in the MR signal when the pH of the medium was changed from physiological to weakly acidic. Extensive physicochemical studies indicated that changes in coordination between the phosphonate group and the lanthanide metal do indeed occur at different pH values. Optimization of such substances is in progress (Mamedov et al., 2007).

Contrast agents were also synthesized that exhibit remarkable sensitivity towards calcium ions. Common calcium chelators were synthetically modified in order to tune their affinity towards extracellular concentrations of calcium. A monomacrocyclic agent containing the low-affinity calcium chelator APTRA was synthesized and analyzed; the agent doubled its T1 relaxivity in the presence of Ca\(^{2+}\) compared to a calcium-free solution (Dhingra et al., 2008b).

Several other bismacrocyclic MR agents were prepared that were coupled to synthetically modified calcium chelators (BAPTA, EDTA, DTPA) in order to target extracellular calcium concentrations (Dhingra et al., 2008a; Mishra et al., 2008). Further improvements in the chelators led to a modified EGTA-containing agent whose physicochemical characteristics were found to be much better than those of any other currently available calcium-sensitive SCA. In addition, the relaxivity response of these agents in biologically relevant environments such as cerebrospinal fluid (CSF) and the extracellular matrix (ECM) suggests that these complexes might be appropriate SCA for the detection of neural activity (Angelovski et al., 2008). In vivo testing is currently in progress.
On-Going Work and Future Plans
Currently our chemistry groups follows two research-and-development pathways: (a) further novel design, synthesis, and in vitro testing of gadolinium-containing and DO3A-based complexes that are sensitive to changes in calcium flux, in pH, or in concentrations of neurotransmitters and neuromodulators. Although our group is strongly application-oriented and remains primarily focused on synthesis and testing, e.g. relaxometry studies, we - together with our colleagues from the University of Tübingen – are also tackling emerging chemistry questions regarding the design and synthesis of new ligands, coordination chemistry, and physicochemical characterization the synthesized molecules. (b) Following characterization of the molecules in simple solutions, cerebrospinal fluid (CSF), and extracellular matrix (ECM), we use the rat model to directly image possible changes in relaxivity - and thus in the fMRI signal – during brain activation. To this aim, we use a number of different experimental designs and protocols, including sensory and electrical stimulation, and terminal depolarization. To map SCA-signaled activity we use combinations of T2-, T2*- and T1-weighted echo planar imaging (EPI). A complete analysis package for image preprocessing, registration of functional and anatomical images, and analysis of functional data (based on the general linear model, GLM as well as spatial and temporal ICA) has been already developed in our laboratory and is currently in routine use. Our future research lines include:

Enhancing Sensitivity with Polymetallic Complexes and Improved Stoichiometry
As mentioned above, the relaxivity of a paramagnetic ion denotes its efficacy per concentration unit of the ion. A relaxivity change of approximately 100%, 50% or 33% in tissues perfused by a mono-, bis- and tris-macrocycle, respectively, will thus yield the same signal change in a T1- weighted image for the very same concentration of the target molecule, e.g. Ca\(^{2+}\). Alternatively, increasing the number of paramagnetic molecules will also increase the SCA’s efficacy. One potential research strategy is thus to synthesize polymetallic complexes (i.e. those with more than one Gd\(^{3+}\) ion) which amplify the effects of a single target calcium ion. The efficiency of this approach can be considerably enhanced by also improving the complex-to-Ca\(^{2+}\) stoichiometry. Finding novel Ca\(^{2+}\) chelators and modes of interaction between Ca\(^{2+}\) and the moiety affecting the MR signal, e.g. Gd-DO3A, could possibly increase the SCA/Gd\(^{3+}\) : Ca\(^{2+}\) ratio and thus increase the sensitivity of the agent and the MRI signal.

Enhancing Signal by Changing the Chelate for Gd\(^{3+}\)
Recently, novel types of Gd chelates have been reported that have similar complex stability but much higher relaxivity values compared to DO3A-type systems. The increased number of coordinated water molecules and resistance to reversible anion binding (which reduces the T1 signal in DO3A chelates) might bring about even higher relaxivity changes of the SCA upon interaction with Ca\(^{2+}\). The aim of this research line would therefore be the synthesis of the current SCA analogs where DO3A units (MR reporters) are replaced by the aforementioned novel chelates.

Elimination of SCA Concentration Effects on Ca\(^{2+}\) Measurements (International Collaboration)
One limitation of our current probes is the difficulty in distinguishing alterations in the MRI signal due to SCA-calcium interactions from signal changes due to the concentrations of the SCA itself. This confound is more pronounced when using our molecules, because they are small and their concentration changes quickly after injection, i.e. their steady-state period is brief. To
resolve this problem we have begun collaboration with Prof. Alan Jasanoff at the Massachusetts Institute of Technology (MIT). The aim of the joint effort is to synthesize ratiometric probes that produce both strong T1 and strong T2 relaxation, but for which the ratio between T1 and T2 relaxivity (T1/T2) varies as a function of calcium concentration. Because the latter ratio is independent of probe concentration, the probes are likely to report only the concentration changes of calcium.

**In-Text Citations**


