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The structure and function of intrinsic brain activity

Christopher Lewis
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The research presented in this thesis was carried out at the Institute for Advanced Biomedical Technologies at the University of Chieti G. d’Annunzio, Chieti, Italy, the Ernst Strüngmann Institute (ESI) for Neuroscience in Cooperation with Max Planck Society, Frankfurt, Germany and the Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Netherlands. It was made possible by financial support from EU FP7 200728 (Brain-Synch) and the Smart Mix Programme of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Culture and Science (BrainGain).

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Christopher M. Lewis, 2014

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The structure and function of intrinsic brain activity

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Chapter 1

General introduction and outline
Background

Day to day experience has likely given you the impression that your brain is neither static nor quiet. Far from the sterile, inert passivity of a digital computer, your brain hums with perpetual energy, an internal life of its own. This cerebral restlessness informs even our most subdued moments. During sleep, while our bodies assume a near total cessation of activity, our brains march on, giving rise to the intricate and oddly coherent tapestry of dream. Likewise, while sitting quietly on a bench in an empty park or running through a crowded shopping center, our brains are equally at work, churning out the stream of thoughts, feelings and bodily commands which we know as our selves.

Despite this obvious fact, the vast majority of research into brain function has ignored the brain’s internally generated activity in favor of understanding the brain’s response to external stimulation or overt behavior. As a result, a great deal of effort has gone into analyzing the behavior of the brain as it appears on average. That is, the brain response when many identical stimuli are presented or when many identical trials of a particular task are performed. Averaging brain responses in this manner affords a number of desirable features. First, because brain responses are often small and difficult to measure, repetitions provide a robust estimate of the most reliable pattern of activity produced by a given experimental event. Additionally, many variables affect brain responses and parametrically controlling as many variables as possible over repeated trials, allows the experimenter to disentangle unique components from complex mixtures. Finally, all physical measurements include a degree of noise. Analyzing average values, as opposed to individual realizations, insures that noise components that are uncorrelated between measurements are reduced.
For these reasons, neuroscience research has largely relied on the analysis of average brain responses when investigating and constructing conceptual models of brain function (Gawne and Richmond, 1993; Gerstein and Kiang, 1960). This averaging approach has provided an immense body of empirical fact, relating a wide variety of brain responses to many experimental manipulations of physical or cognitive variables. We have gained insight into how brain responses are modulated by the parameters of stimulation and the potential role of different brain regions in sensory processing, cognition and behavior. Additionally, by combining the averaging approach with anatomical studies, simple circuits have been constructed which have been able to reproduce the observed pattern of responses in early sensory areas. Such circuits have enabled the complex biology to be reduced to straightforward, mathematical models. The success of this approach and of the derived phenomenological models has led to the widespread adoption of conceptual models that posit response averaging as a potential operational scheme for neural computation (Shadlen and Newsome, 1994). These models propose that neurons function by computing averages over the noisy input of their individual pre-synaptic partners. The jump from variable measurements to the postulation of neuronal noise, has the implication that the brain operates in a stochastic manner and suggests that average brain responses represent “signal” in the brain, and deviations from the mean response reflect “noise” (Shadlen and Newsome, 1995). Viewing the brain in this manner has had a major influence on neuroscience research and debates of brain function, neural representation and computation.

However, the transformation of signal averaging from a useful analytical strategy into a theory of brain operation has enforced a narrowness of vision when it comes to the brain’s internal dynamics and its role in brain function. Due to the fact that the brain’s intrinsic activity is de facto inaccessible to experimental control or monitoring, it necessarily contributes to variability in brain signals which are not reflected in the average response. Intrinsic activity is therefore posited to be a source of neuronal noise. Under this conceptual milieu, the reification of trial-
variability as systemic noise in the brain has led to a large family of brain theories that may throw the baby out with the bathwater. Further, the perspective on brain function derived from this stance has severe limitations that necessitate a re-evaluation of intrinsic activity and the operational mode of the brain.

Although a great preponderance of brain is devoted to accurately representing the external world, some simple anatomy starkly highlights the fact that the brain is largely concerned with its own, internally generated activity. The information available to us about the external world is constrained to modality specific afferents that convey sensory signals from the peripheral sensory epithelia to the central nervous system. Our interaction with the external world by means of volitional motor output operates through a similar bottleneck. Even in sensory portions of the thalamus or the input layers to primary sensory cortex, only approximately 10% of connections arise from the sensory organs (Sillito and Jones, 2002). A similar situation holds for the primary motor cortex and motor thalamus, the locus of the brain’s volitional muscle output (Porter, 1985; Rivara et al., 2003). In both areas, ostensibly specialized to represent features of sensory stimulation and precise motor plans, the vast majority of connectivity is devoted to synapses arising from cells with no direct sensory or motor projection (Sanes and Donoghue, 2000). These recurrent connections consist of dense connectivity with local cells, lateral connections between distinct local groups and feedback connections that descend from higher order cortical areas to lower order areas (Douglas et al., 1989). Activity arising from these recurrent connections, likely to be the dominant source of input for many cortical cells, contributes to intrinsically arising variance in neural responses. The situation is only exaggerated when considering higher-order association areas for which there appear to be only very provisional, context-specific dependencies on stimulus or experimentally controllable parameters.

The over-all impression given by neuroanatomy is reinforced by the physiological properties of intrinsic activity. The magnitude of a stimulus response is often
measured in terms of a change from pre-stimulus (baseline) activity. Due to the fact that the estimation of a stimulus response is triggered on the occurrence of an event fixed in time, this activity often appears larger than baseline activity, which is not aligned with respect to the external stimulus event. However, when comparing stimulus activity as a ratio with baseline activity on a single trial basis, many measures of neural activity exhibit stimulus related responses of the same magnitude as the ongoing, intrinsic activity of the brain (Arieli et al., 1996; Raichle and Mintun, 2006). The same is true when the average response is compared to the magnitude of variability across identical realizations of a stimulus or event (Scobey and Gabor, 1989; Snowden et al., 1991; Vogels and Orban, 1990). Both, pre-stimulus activity and variance in evoked responses are likely to be the result of intrinsic activity. In both cases, intrinsic activity, either prior to, or in the course of stimulation, contributes to brain responses to a similar degree as stimulus parameters. In the traditional framework, this variability is viewed as a signature of stochasticity on the part of networks or individual cells (Shadlen and Newsome, 1995).

The imbalance between intrinsic and stimulus related activity is amplified when considering the energetic requirements of brain activity. While the brain accounts for only 2% of the body’s mass, it uses over 20% of its total energy (Raichle and Gusnard, 2002; Raichle and Mintun, 2006). This expenditure is in large part a result of the brain’s ongoing, intrinsic dynamics. The additional amount required for even the most mind-bending tasks or excessive stimulation is a tiny proportion (<5%) of that used in the day-to-day, nonstop activity of the brain itself (Raichle and Mintun, 2006; Sokoloff et al., 1955). From this perspective, in particular, the average responses evoked by sensory stimuli look woefully small and it is tempting to wonder if all that energy is being exerted to generate meaningless noise.

Together, these features of brain structure and of the character of brain responses seem to demand a closer investigation of what function intrinsic activity may serve
in brain operation. However, additional facts also highlight the need for new theories of brain operation and lend support to the idea that intrinsic activity may serve an essential role. Our understanding of the response properties and function in higher order brain areas, especially in association cortex, are currently severely limited. Although increased investigation is starting to delineate the response properties of circumscribed portions of association cortex, to date, most formulations of operation in these areas suggest dynamic regimes far from the simple feed-forward, signal averaging models that have sufficed in early sensory areas (Harvey et al., 2012; Mante et al., 2013; Rigotti et al., 2013). It seems likely that as the proportion of synapses arising from sources without a direct connection to primary sensory or motor neurons increases, intrinsic brain dynamics are responsible for a greater proportion of regional operation.

The situation in association cortex is mirrored and exaggerated by the need to move beyond brain responses in delimited, local regions and begin to develop an understanding of brain function across distributed networks. Although much of our current knowledge of brain function has come from in depth studies of a few neurons or local populations in single cortical areas, an understanding of the brain’s operational principles will require simultaneous recording of the many areas whose concerted efforts are involved in even the most basic tasks. While simple feed-forward circuits have sufficed in early sensory areas operating in isolation, top-down factors of task and state heavily influence even the simple sensory responses in early sensory areas (Gilbert and Li, 2013). Intrinsic activity is likely to provide an important marker for the state of brain operation, which, far from being noise, may constrain, modulate and elaborate patterns of evoked activity.

As evidence regarding the reliable structure and possible function of intrinsic brain dynamics increases, it is important to begin to conceptualize new operational schemes for brain circuits and networks (Buonomano and Maass, 2009; Laurent, 2002; Maass et al., 2002; Singer, 2013). The conflation of noise arising from
incomplete and imprecise measurement with the existence of noise in the brain must be reevaluated and examined with measurements from increasing portions of relevant brain networks. Such measurements may provide important diagnostics on the state of the brain and what we once considered noise may be found to provide key computational capabilities, such as context. As we once constrained sources of variability by parametric stimulation protocols, we may now constrain evoked brain responses during single trials by including intrinsic variables in our models. Continued investigation will need to determine the effective scope, impact and reach of the brain’s intrinsic dynamics.

Outline

This thesis presents a body of work that attempts to push forward our understanding of this critical boundary between the brain’s intrinsic dynamics and the activity imposed on the brain from external sources. I argue that, far from noise, this intrinsic activity has robust structure, reflects experience, influences behavior, and shapes perception. I also promote a view for studying brain function that takes into account variables beyond our experimental control and try to suggest ways in which we might incorporate them into our working models of brain function.

In chapter 2 I outline previous work that has led to a conceptual switch from average- to trial-based analysis and that highlights the role, which intrinsic activity has in modulating brain responses. I then briefly explain the experimental methods used and the analytic approach applied throughout the rest of the thesis. Chapter 3 presents a study of learning and attempts to link changes in intrinsic activity with changes in behavioral performance that occur as a result of intensive training on a new perceptual task. Chapter 4 looks at the state of intrinsic activity within the brain prior to learning a new perceptual task and uses that activity to predict individual measures of competence and acquisition of the task. Chapter 5 investigates rhythmic synchronization phenomena between visual areas that reflect
stimulus properties during visual stimulation and reoccur in the absence of stimulation. Chapter 6 uses a single trial approach based on methods in machine learning to compare the stimulus selectivity for stimulus-induced patterns of brain activity with more traditional, trial average approaches. Finally, in chapter 7, I present some conclusions that can be drawn from this body of work and look forward to future questions that must be addressed and new directions of inquiry that may contribute important insight to this new conceptualization.
Chapter 2

Concept, methods and analytic approach
Concept

The reductionist approach in science has as a central tenet the rigorous explanation of complex systems through the systematic separation of piecewise components. The goal is the explanation of integrated bodies through the individual operation of isolated units. In this framework, the parts of a system must be studied separately, so that a basic function can be ascribed and the combination of individual units leads to the unified behavior of the system. Analysis of system behavior thus operates with the logic of engineering, in which one attempts to reduce variability to a minimum so that basic input-output functions can be defined. Operational units can thus be given a concise, encapsulated form and the combination of individual units leads to a tractable synthesis. However, unlike engineered systems, which can easily be reduced to circumscribed components, the real world is a tangled web of dependencies, and contingencies that preclude the trivial segmentation of parts from wholes. In the wild, variability and exception are the rule and components overlap, mix and counter-act. There is considerable debate concerning the appropriate level of analysis for biological systems and little consensus that a reductionist approach is tenable. This is perhaps nowhere more true than in biology. Life seems to thrive on diversity and offers a proliferation of vague boundaries, border cases and variation. Across the domain of biology, variability seems to play an essential role in the operation of life. From providing genetic diversity as a fuel for natural selection, to the role of differential expression in development and homeostasis, variation provides a crucial element in defining and maintaining the structure and function of biological organisms.
The traditional view of brain function relies on the logic of stimulus-response mapping based on a reductionist, or engineering, perspective on brain function. (A) Descartes illustration of the pathway from physical stimulus to the brain and interaction with the mind. (B) Illustration of Pavlovian conditioning experiment in which behavioral responses to stimuli are monitored and associations are formed.

In neuroscience, variability has often been associated with noise and the dominant view suggests that the brain operates by averaging brain responses across neuronal populations in order to distill the reliable signal from fickle, individual responses (Shadlen and Newsome, 1994; 1998). However, an alternative perspective, that variability may be a signature of the brain’s internal state and that it may play a critical role in brain function, has received increasing evidence and attention (Arieli et al., 1996; Engel et al., 2001; Fox and Raichle, 2007; Katz and Crowley, 2002).

These two ideas have stirred controversy in the study of brain function since its beginning. The view that the brain operates largely as an elaborate collection of stimulus-response mappings has a long tradition that arguably arose with the formation of rationalism and empiricism. Its most explicit formulation in modern thought came from the work of Pavlov and gave rise to the behaviorist approach to psychology (Pavlov, 1928; Skinner, 1938). The logic of stimulus-response mapping imbued the young field of neurophysiology as embodied by the reflex arc
theory of Sherrington (Sherrington, 1929). Others, such as John Dewey, William James and Thomas Brown, initially disputed these ideas and promoted a more active, integrated stance with respect to psychological and neural function (Brown, 1914; Dewey, 1896; James, 2011). According to James:

Whilst part of what we perceive comes through our senses from the object before us, another part (and it may be the larger part) always comes out of our own head. William James (1890)

![Figure 2 The Berger Rhythm.](image)

Differences in electrical potentials recorded from the surface of the human brain in the dark. (A) Illustration of surface potential with eyes open or shut. (B and C) Two separate recordings when a human subject tried to look for an object in the dark. Actively looking suppressed the Berger rhythm. These early results suggested distinctly different patterns of activity correlated with behavioral state. (From Adrian and Matthews, 1934).

Early evidence for an active role of intrinsic brain activity in shaping action and perception was presented by Brown, who found that decorticated cats, in the absence of sensory stimulation, were able to spontaneously produce patterns of activity reminiscent of walking and running. Likewise, in his pioneering work recording electrical potentials from the brains of human subjects through scalp electrodes, Berger found large amplitude, rhythmic variation of electrical activity in the absence of stimulation (Figure 2, Adrian and Matthews, 1934; Berger,
In fact, as Berger noted, he was impressed by the minor effects on activity observed during mental work:

Of course, one should not at first entertain too high hopes with regard to this, because mental work, as I explained elsewhere, adds only a small increment to the cortical work which is going on continuously and not only in the waking state'. Hans Berger (1905)

However, this thread of work and the associated perspective largely lay dormant for the ensuing century, during which time the focus on stimulus-evoked brain responses produced a great deal of important insight into brain function and organization. Much of this focus might be attributed to the difficulty in assessing and quantifying the intrinsic state of the brain, compared with the relative ease of systematically relating brain responses to controlled sensory stimuli, motor actions and well-designed cognitive tasks.

Near the turn of the millennia, the importance of intrinsic activity was brought to the forefront when high degrees of structure, reliability and organization were found across experimental systems, methodologies and scales. In each case, innovative technologies allowed new perspectives on the organization of neural activity and suggested that intrinsic activity may play an important role in brain function.

Figure 3 Spontaneous activity in a population of retinal ganglion cells. Sequential frames illustrating the firing rate across a population of retinal ganglion cells in the absence of visual stimulation. The well-ordered pattern of activity originating in the lower right portion of the population spreads systematically to the top left. These patterns are responsible for ocular segregation and retinotopy throughout the brain suggesting an active role for the brain’s spontaneous activity. (From Meister et al., 1991).
First, using novel multi-electrode arrays that allowed the simultaneous recording of dense populations of retinal cells, it was shown that spontaneous activity, prior to visual experience, was highly organized and critical for the ordered arrangement of retinal projections to the thalamus (Galli and Maffei, 1988; Meister et al., 1991). Figure 3 depicts the well-ordered propagation of retinal ganglion cell firing during a period of spontaneous activity. Such coordinated activity was hypothesized to play a critical role in the development of sensory topographies. These surprising results confirmed theoretical work, which suggested that self-organized patterns of activity could explain the formation of topographically ordered projections in the nervous system (Willshaw and Malsburg, 1976) and led to intense interest in the role of intrinsically generated activity in development (Huberman et al., 2006; Shatz, 1996; Thompson, 1997). It was found that spontaneous activity, prior to sensory experience, led to topographically ordered sensory maps at multiple points in the sensory hierarchy (Ackman et al., 2013; McLaughlin et al., 2003). Further, similar patterns of activity were found during development in other parts of the brain, such as the hippocampus, spinal cord, and cortex (Buonomano and Merzenich, 1998; Katz and Shatz, 1996).

Shortly afterward, using newly developed voltage sensitive dyes that enabled the recording of membrane voltage across large areas of cortex, Arieli and colleagues published a sequence of studies which unequivocally showed that intrinsic activity in local cortical populations was highly structured and that intrinsically generated activity in this signal could explain large proportions of evoked brain responses (Figure 4, Arieli et al., 1995). They showed that intrinsically driven variability effected measures of local population activity, as well as the responses of single cells (Arieli et al., 1996; Tsodyks et al., 1999), and that patterns of intrinsically generated activity reflected the exquisite functional and anatomical organization of primary visual cortex (Kenet et al., 2003). In the absence of visual stimulation, primary visual cortex was shown to spontaneously reproduce patterns of activity exhibited during visual stimulation.
Concept, methods and analytic approach

Figure 4 Spontaneous activity in primary visual cortex of the cat.
(A) Optical imaging from a continuous patch of anesthetized cat visual cortex. Light indicates increased local activity and dark depicts reduced activity. From left to right are shown: a map of the functional topography of primary visual cortex, an instantaneous frame of spontaneous activity that resembles this functional map, and an instantaneous frame of an evoked response to visual stimulation with an oriented grating. (B) Comparison of orientation dominance columns computed from stimulus evoked activity and intrinsic activity (from Kenet et al., 2003).

In the same period, investigators using functional neuro-imaging in humans, found that homologous regions of motor and sensory cortex were correlated between the cerebral hemispheres, despite the fact that subjects were not engaged in any behavior or task (Biswal et al., 1995). This surprising finding suggested that intrinsic activity might be coordinated to reflect functional and anatomical organization at the scale of the entire brain. Subsequently, this was shown to be the case for a collection of brain areas typically suppressed during the execution of a wide class of common psychological tasks (Greicius et al., 2003; Raichle et al., 2001; Shulman et al., 1997). The finding of widespread networks of intrinsically generated activity instigated a boom in interest in the structure of intrinsic activity in the human brain.
Figure 5 Intrinsic activity in monkey compared to evoked activity and anatomy.
(A) Map of intrinsic connectivity from the fMRI BOLD signal reflects a functional network involved in attention. (B) A pattern of evoked activity during a spatial attention task. (C) The pattern of anatomical connectivity from a node (right lateral intraparietal) of the network. From (Vincent et al., 2007).

Following the initial reports, there appeared a proliferation of intrinsically generated networks whose topography closely matched the anatomic connectivity of distributed regions, as well as patterns of activation commonly seen during cognitive, perceptual or motor tasks (Fox and Raichle, 2007). Figure 5 depicts the correspondence of intrinsic activity within one well-characterized network involved in the control of attention with both the pattern of activity seen during the execution of a task requiring attention, and the distribution of anatomical connectivity from a node of that network. Deviations from typical patterns of intrinsic connectivity were demonstrated to relate to a large number of neurological and psychiatric disorders, suggesting important relevance of these intrinsic networks for brain function (Zhang and Raichle, 2010). These studies were followed by investigation of the electrical correlates of this activity and behavioral consequences of intrinsic activity (Laufs et al., 2003a; 2003b; Leopold and Logothetis, 2003).
Figure 6 Intrinsic activity affects behavior.  
(A) Correlation of trial-to-trial variability in firing rate versus perceptual decision in the awake monkey. Monkeys were presented with an ambiguously moving stimulus and were required to make a decision regarding the dominant direction of motion. Recordings were obtained from area MT+; an extra-striate area critical for motion perception. Firing rates were determined per trial and correlated to the behavioral report in that trial. The figure shows that variations in behavioral report across trials could be partly predicted by intrinsically generated (sensory stimulation was constant) fluctuations in neuronal firing rates in across trials (Britten et al., 1996). (B and C) Intrinsic activity in specific sensory areas reflects perceptual categorization of a bi-stable visual stimulus (Hesselmann et al., 2008b). (B) Human subjects were briefly presented with a bi-stable visual stimulus and had to make a decision about the object represented. (C) Pre-stimulus activity within visual areas selective for different stimuli classes was indicative of the later perceptual decision.  

Investigation of intrinsic variability of single cells had earlier been related to perceptual decisions on a single trial basis. In a similar vein, activity within sensory or motor areas was used to predict the amplitude of evoked responses, the strength of motor output or differing perceptual decisions to identical stimuli. Figure 6 depicts two examples, one in monkeys and one in humans, of intrinsically generated variability biasing perceptual decisions. In each case, pre-stimulus or pre-decision variability in neural responses was able to predict variability in behavioral measures (Bair et al., 2001; Britten et al., 1996; Fox et al., 2007; Hesselmann et al., 2008b; 2008a; Sapir et al., 2005).  

The structure and functional relevance of intrinsic brain activity has been established across a wide range of methodologies and scales. In the work presented here, I attempt to present a coherent picture of intrinsic activity across spatial and temporal scales. I connect measures of intrinsic activity with behavioral performance, learning and also with potential electrophysiological underpinnings.
In doing so, I hope to demonstrate the robustness of intrinsic activity, as well as its generality across the many levels at which the brain must be investigated in order to begin to understand its intricate function. I hope that interest in intrinsic brain dynamics continues to grow and that with the addition of new experimental techniques and analytic methods, the role of intrinsic activity in the function, maintenance and formation of distributed brain networks is elaborated and embraced.

**Method**

As I stated in the previous section, technological advancement has contributed to the appreciation of intrinsic brain dynamics at each stage. First, the method of electroencephalography, pioneered by Berger, enabled him and later Adrian and others to appreciate the degree of rhythmic structure in spontaneous brain activity. Later, the advent of multi-electrode arrays, voltage sensitive dyes and *in vivo* imaging techniques allowed the observation of brain activity from dense populations and large swaths of cortex not previously achievable with traditional electrophysiological methods. Finally, neuroimaging opened up a period of intense investigation into the organization and functional relevance of intrinsic dynamics in the human brain that continues today.

No portion of the brain functions in isolation. Rather, neurons, local circuits, and whole brain networks participate in coordinated activity patterns which bridge temporal and spatial scales. How the brain operates within and across scales and exploits this richness in order to support adaptive behavior is a critical open question in neuroscience. Brain activity must produce robust patterns of activity which bridge the temporal scales at which it exists. Likewise, brain activity is distributed across space at multiple scales, from single cells, firing action potentials, to large local populations and whole networks. I have taken advantage of a number of these techniques in order to bridge temporal and spatial scales in the
study of intrinsic brain activity. I will briefly explain the methods used and the logic behind their application in this section.

Functional magnetic resonance imaging is a powerful tool for non-invasively recording activity in the brain in regions on the scale of millimeters and with a temporal resolution of seconds. These regions contain tens of millions of cells with myriad morphologies and physiology, however, because it allows imaging of the entire brain, understanding the aggregate behavior at this coarse spatial resolution has proved exceedingly informative of how whole brain networks engage in perception and cognition. Further, because it is non-invasive, it can be used to map brain activity in human subjects and clinical populations, allowing the investigation of human-specific abilities and deficits. I have used fMRI in order to investigate intrinsic activity in relation to learning and the performance of perceptual tasks. It has enabled whole brain networks of activity to be revealed that are intimately related to specific behaviors and reflect the recent history of subjects. However, understanding how the networks observed at this level relate to the underlying activity of neuronal populations, or circuits is a tall order. For this reason, I added the investigation of intrinsic dynamics from more direct measures of neuronal activity in order to begin to bridge this explanatory gap from phenomenon to mechanism.

Electro-corticography (ECoG) allows the simultaneous measurement of electrical potentials from the surface of the brain on scales ranging from millimeters up to centimeters. Crucially, ECoG arrays can be designed to span whole cortical lobes or multiple cortical areas and therefore enable the spatial coverage necessary to explore intrinsic dynamics on the scale of the distributed networks observed with fMRI. The utilization of custom ECoG arrays can thus begin to bridge the level of the macroscopic signals observed with brain imaging to the mesoscopic level of local neuronal populations. In the data, I analyzed as part of this thesis, micro-machined ECoG arrays were used to cover the external surface of large parts of the
left cortical hemisphere in two awake, behaving monkeys. This configuration provided a rich data set in which to investigate the organization of intrinsic activity across cortical areas and link it to neuronal dynamics on the time scale of milliseconds. I used ECoG in order to investigate the coordination of intrinsic dynamics between visual areas, to relate them to area topography and to investigate their role in sensory processing.

Recording from single cells and small populations of cells is the gold standard technique for neurophysiology and understanding of brain function at the neuronal level. I have used selective recordings of neurons from the primary visual cortex of awake, behaving monkeys in order to connect the signals recorded at the surface of the brain with ECoG to the activity of small groups of neurons. I investigated stimulus related information on a single trial basis from small neuronal groups and the activity in specific frequency bands. By comparing single trial responses across multiple features and classes of visual stimulation, I was able to demonstrate that activity in specific frequency bands was selectively involved in sensory coding. These results suggest that stimulus-evoked responses interact with recurrent cortical dynamics in the coding of sensory stimuli.

**Analytic approach**

While technological advancement has enabled increased appreciation of intrinsic brain activity, this must be accompanied by new analytic techniques that can assess individual trials, particularly in cases where there is no external alignment event. A large portion of current work on intrinsic activity focuses on the determination of ‘functional connectivity’ by assessing the degree to which different regions exhibit correlated activity in the absence of stimulation or a task. The analysis of these intrinsically defined networks has enabled the use of measures of network and graph connectivity in order to describe the structure and topology of correlated brain areas. Further, the described functional networks can be related to anatomical connectivity and associated with behavioral or pathological features. Additionally,
in this framework, individual differences in intrinsically defined networks can be used to relate changes in functional connectivity to individual measures of performance. These correlations enable one to connect measures of intrinsic activity with behavior and to link variation in intrinsic activity to behavioral variation.

Lastly, individual trials of sensory stimulation or task performance can be investigated to understand the role of intrinsic activity in trial-to-trial variability. One approach applied here is to use methods from machine learning in order to quantify the stimulus-related information in single-trial activity. In this manner, decoding can be used as a surrogate down-stream brain area in order to estimate parameters of stimulation or cognition that are encoded by the single trial response profile. Techniques like these promise to contribute important insight into how the brain actively represents stimuli and task goals through neural populations. Finally, in order to understand the interplay between stimulus-evoked patterns and intrinsic dynamics, new conceptual models are needed. These models may include features of earlier feed-forward, stimulus-response models, but they must take into account intrinsic variables. Likewise, effort towards understanding dynamics in recurrent networks, especially those with asymmetric connectivity, similar to that found in local and distributed brain circuits, and for which no analysis methods currently exist, will be key in advancing our understanding of brain operation.
Chapter 3

Learning sculpts the spontaneous activity of the resting human brain

Published in:


(*) denotes equal contribution
Abstract

The brain is not a passive 'sensory-motor' analyzer driven by environmental stimuli, but actively maintains ongoing representations that may be involved in the coding of expected sensory stimuli, prospective motor responses, and prior experience. Spontaneous cortical activity has been proposed to play an important part in maintaining these ongoing, internal representations although its functional role is not well understood. One spontaneous signal being intensely investigated in the human brain is the inter-regional temporal correlation of the blood-oxygen-level-dependent (BOLD) signal recorded at rest by functional magnetic resonance imaging (functional connectivity-by-MRI, fcMRI, or BOLD connectivity). This signal is intrinsic and coherent within a number of distributed networks whose topography closely resembles that of functional networks recruited during tasks. While it is apparent that fcMRI networks reflect anatomical connectivity, it is less clear whether they have any dynamic functional importance. Here we demonstrate that visual perceptual learning, an example of adult neural plasticity, modifies the resting covariance structure of spontaneous activity between networks engaged by the task. Specifically, after intense training on a shape identification task constrained to one visual quadrant, resting BOLD functional connectivity and directed mutual interaction between trained visual cortex and frontal-parietal areas involved in the control of spatial attention were significantly modified. Critically, these changes correlated with the degree of perceptual learning. We conclude that functional connectivity serves a dynamic role in brain function, supporting the consolidation of previous experience.
Introduction

Spontaneous neural activity utilizes the majority of the brain's energy budget, but its function remains mysterious (Balduzzi et al., 2008; Engel et al., 2001; Hampson et al., 2006; Kenet et al., 2003; Llinás et al., 1998; Raichle and Gusnard, 2002; Raichle and Mintun, 2006; Varela et al., 2001). At the level of single neurons, embedded in the local circuitry of a cortical area, spontaneous activity has been shown to emulate the pattern of activity evoked by the neuron’s optimal stimulus, suggesting that at least at this level of description, spontaneous activity is likely to reflect the history of co-activation within local networks (Tsodyks et al., 1999). At the level of distributed cortical systems, spontaneous activity measured by BOLD-fMRI exhibits covariance structures (or functional connectivity) at ultra-slow frequencies (<0.1 Hz) that are stable across a wide range of behavioral states (anesthesia, task performance, resting wakefulness and sleep) (Boly et al., 2008; Vincent et al., 2007). The topography of BOLD functional connectivity is compatible with both the underlying structural connectivity of the cortex and the functional anatomy of systems engaged by a broad range of tasks (Biswal et al., 1995; De Luca et al., 2005; Fox et al., 2005; Greicius et al., 2003; Hagmann et al., 2008).

It has been suggested that BOLD functional connectivity is largely a physiological marker of anatomical connections or a correlate of intrinsic vascular dynamics without functional or behavioral significance (Drew et al., 2008). This hypothesis is consistent with the stability of functional connectivity independent of behavioral state, as well as the maturation of stable networks from childhood to adulthood in parallel with the myelination of the brain's white matter (Fair et al., 2008). However, other evidence indicates that the strength of correlation within a network can be related to individual differences in cognitive function (Boly et al., 2008; Kelly et al., 2008; Seeley et al., 2007), that spontaneous activity can account for the variability of task-evoked responses (Fox et al., 2007), and that the disruption
of BOLD connectivity in the absence of structural damage is associated to neurological deficits (He et al., 2007). These findings suggest that spontaneous functional connectivity may encode or support the encoding of behaviorally relevant information.

Based on this evidence, we sought to demonstrate that prior experience, i.e. the history of network activation, changes resting functional connectivity in a behaviorally specific manner. We acquired resting fMRI before and after intense training on a difficult shape discrimination task at a specific location in the visual field. If functional connectivity is related to the neuronal changes associated with perceptual learning, then it should exhibit training-specific plasticity.

**Results**

**Perceptual Learning Training**

For several days, 14 healthy subjects were trained to attend to the left lower visual quadrant and report the presence/absence of a target shape (an inverted T) in a circular array of distracters (differently oriented Ts). Stimuli were presented rapidly (150 ms) to prevent eye movements. Eye movements were recorded and monitored to ensure fixation (See supporting information (SI) Fig. S1). The target shape appeared randomly in one of three locations in the left lower (trained) visual quadrant while the distracters appeared in the other three (non-trained) quadrants (Fig. 1A). An average of 5,600 practice trials were necessary to reach a threshold of 80% accuracy in at least 10 consecutive blocks of trials (Fig. 1B for a representative psychophysical curve; see SI Table S1 for individual results). Perceptual learning was specific for the trained shape and trained quadrant, and did not generalize to other quadrants and orientations (Fig. 1C). These behavioral data confirm the well-known retinotopic and orientation specificity of perceptual learning (Crist et al., 1997; Gibson, 1963).
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Figure 1 Behavioral Training and Psychophysics Results.
(A) Illustration of timeline for two trials. On each trial subjects fixated a central spot for 200 ms (Fixation) after which the target shape (an inverted T) was presented at the center of the screen for 2000 ms (Target Presentation), finally an array of 12 stimuli, differently oriented Ts (distracters) with or without an inverted T (target) was briefly flashed for 150 ms (Array Presentation). Subjects attended to the lower left visual quadrant (red square) and indicated the presence or absence of the target shape (red circle) in that visual quadrant (red square and circle were not present in the display; see Supplemental Online Material for more details). (B) Example of a single subject’s learning curve. Each block contains 45 trials. Red line indicates learning threshold of 80% accuracy in 10 consecutive trial blocks. (C) Psychophysical comparison of accuracy in all quadrants. In a control session at the end of training, subjects were asked to discriminate between trained and novel shape orientations in all visual quadrants. A repeated-measure analysis of variance (ANOVA), with Shape (trained, untrained) and Quadrant (trained lower left, lower right, upper left, upper right) as factors, showed a significant main effect of Quadrant (F(3,21) = 3.52, P < 0.05), and a significant interaction of Shape by Quadrant (F(3,21) = 8.49, P < 0.001 ). Post-hoc contrasts (Newman-Keuls test) showed that performance in the trained condition (trained shape in the trained visual quadrant) was better with respect to any other condition. (n = 6; Error bars, ± s.e.m; * P < 0.05).
Retinotopic localizer

Before any exposure to the task, we identified the regions of visual cortex that differentially responded to the stimulus array presented in the four visual quadrants during a passive stimulation condition (Fig. S2A). This was done to avoid any influence of training on the baseline responsiveness of different portions of visual cortex to the localizer stimuli.

Task-evoked modulation in visual cortex

Upon completion of the behavioral training, we measured the effect of learning on responses in visual cortex by comparing blocks of trials in which subjects discriminated stimulus arrays containing either the trained target shape or an untrained target shape (right/left tilted T). The target stimuli were always presented in the trained visual quadrant (Fig. S3). As in the behavioral training sessions, performance was higher for trained than untrained targets (Fig. S3).

The effect of learning, i.e. a differential response to trained versus untrained targets, was measured in the regions of interest in visual cortex defined during the localizer scans. The response to untrained shapes was attenuated (as compared to trained ones) in right dorsal cortex corresponding to the attended left lower quadrant, but not in ventral visual cortex corresponding to the non-attended upper visual quadrants (Fig. 2). This pattern indicates topographic and shape specificity of the learning-dependent modulation. There was also a significant difference between trained and untrained shapes in left dorsal cortex corresponding to the homologous (non-trained) right lower quadrant (Fig. 2) [Condition (trained, untrained) by Quadrant (lower left, lower right, upper left, upper right) interaction [F(3,33)=7.75; P=0.0005; post-hoc (Newman-Keuls test) for lower left, P < 0.0002; for lower right P < 0.001]. The modulation localized to right dorsal V1-V3 and V3A-LO, and left V1-V3 on the basis of a population atlas of human visual areas (Van Essen, 2005) (Fig. 2, Table S2). In addition, the response to trained
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shapes was stronger in the contra-lateral (trained) than ipsi-lateral (untrained) dorsal cortex ($P < 0.0002$) consistent with a shape specific modulation.

![Figure 2 Task-evoked modulation of visual cortex after perceptual learning.](image)

Center: stimulus array with colored squares (not present in real display) indicating 4 visual quadrants. Flat maps: visual cortex ROIs obtained from passive localizer scans by stimulating one quadrant at a time (Fig. S2). ROIs are projected onto a flattened representation of posterior occipital cortex using the PALS atlas (Van Essen, 2005). Bar plots: % signal change of BOLD in each quadrant when attending to the lower left quadrant and discriminating trained or untrained targets. Note that all four quadrants of visual cortex were stimulated by the stimulus array, but only the trained visual quadrant in right dorsal, and the homologous area in left dorsal visual cortex show a shape-specific modulation. (Post-hoc Newman-Keuls test, $n = 12$; Error bars, ± s.e.m.; * $P < 0.05$).

A separate whole brain voxel-wise analysis provided further support for topographic and shape specificity. In this analysis, the only significant portion of visual cortex showing preferential activity for trained vs. untrained shape blocks was localized in right dorsal cortex (Fig. 3A) in two regions (V2/V3 and LO) that were contralateral to the attended left lower quadrant, and within the borders of the regions responding to the retinotopic stimulus (Fig. S2C).
These findings are in line with previous studies showing that orientation-specific learning changes the tuning properties of neurons in early visual cortex, and increases the fMRI signal to trained vs. untrained shapes (Gilbert and Sigman, 2007). Here, we show that the modulation in visual cortex is topographically and shape-specific and mainly consists of the filtering of sensory evoked responses to novel (untrained) shapes in the attended quadrant, which presumably leads to a more specific response to the trained shape. The presence of training related modulation in visual cortex homologous to the representation of the trained quadrant is not surprising in light of several recent fMRI studies (Sestieri et al., 2008; Sylvester et al., 2007). These studies suggest that modulations in visual cortex are not restricted to attended locations as previously believed, but extend to unattended locations, especially those in the opposite hemisphere homologous to the attended ones. This pattern reflects a specific computational mechanism for coding the locus of attention in a cortical map based on activity difference between attended and unattended locations (Bisley and Goldberg, 2003; Sylvester et al., 2007).

Task evoked activity outside occipital cortex

A number of parietal and frontal regions responded more strongly during untrained as compared to trained shape blocks (Blue regions in Fig. 3A; Table S2). These regions correspond to the dorsal fronto-parietal attention network (Corbetta and Shulman, 2002), the source of spatially selective attention biases to visual cortex (Bressler et al., 2008), as well as portions of a core control network (Dosenbach et al., 2006) involved in task set maintenance and error tracking. The stronger activation for novel shape orientation likely reflects a higher degree of attention engagement, similar to what occurs during early training, as opposed to when the shape orientation is familiar. Finally, a separate set of cortical regions was more strongly deactivated during untrained as opposed to trained shape blocks (Orange regions in Fig. 3A). These regions correspond to the default mode network.
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Figure 3 Whole brain task-evoked modulation, and spontaneous functional connectivity.
(A) Whole-brain voxel-wise z-map of trained minus untrained shape conditions, corrected for multiple comparisons (Monte-Carlo, $P < 0.05$) and projected onto an inflated representation of the PALS atlas. Central inset shows activation in right dorsal visual cortex projected onto a flattened representation of the occipital lobe. Blue regions: untrained > trained; Orange regions: response for trained > untrained. $n = 12$. (B) Pre- and Post-learning spontaneous fcMRI. Color bar indicates $z$-transformed correlation values for each region pair, positive for red cells and negative for blue cells. Note stability of correlation matrix across sessions (separated by more than 1 week) indicating that within-network functional connectivity is very stable over time ($n = 14$). (C) Post-minus-Pre learning changes in spontaneous fcMRI. Correlation matrix (Fisher $z$-transformed Pearson coefficient) of all possible ROIs pairs in Visual Cortex, Dorsal Attention Network (DAN) and Default Mode Network (DMN). Color bar indicates post-minus-pre learning $z$-transformed correlation values (rest 2 - rest 1 scans) for each region pair. Blue cells: significant correlation difference between dorsal attention and trained visual cortex ROIs ($t$-test, $P < 0.03$, Corrected for Multiple Comparisons): pre-learning > post-learning; Red cells: significant correlation difference between default network and untrained visual cortex ROIs, post-learning > pre-learning; $n = 14$.

(Raichle et al., 2001; Shulman et al., 1997), in which previous studies have also reported deactivations that are attention-load dependent (Shulman et al., 2007; Weissman et al., 2006).

In summary, learning induced specific modulation of task-evoked activity in visual cortex and higher-order 'control' regions of the attention, core and default networks, consistent with the essential role of visuo-spatial attention in perceptual learning (Crist et al., 1997; Gibson, 1963), the retinotopic organization of its top-down influence on visual areas (Tootell et al., 1998), and its role in filtering distracters (Reynolds and Chelazzi, 2004).
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**Functional connectivity changes between networks**

Next, we examined the effect of perceptual learning on the patterns of resting functional connectivity in the brain networks recruited by the task (dorsal attention, default, and visual). Two sets of fMRI resting state scans, in which subjects simply maintained visual fixation, were acquired before and after behavioral training to measure the spontaneous coherence of BOLD signal fluctuations between task regions. The pre-learning scans were obtained before any exposure to the task, while the post-learning scans were obtained after measuring the effects of learning on task-evoked activity. BOLD signal time courses were extracted from all task-specific and localizer regions, and temporal correlation was computed pair-wise during each rest period (functional connectivity or fcMRI).

Functional connectivity was stronger within networks than across networks and the overall topography of correlations was consistent over time ([Damoiseaux et al., 2006; Vincent et al., 2007](#)) (Fig. 3B). Notably, significant correlation differences post-pre learning were detected between networks, but not within network. After learning, the functional connectivity between trained visual cortex and dorsal attention regions (left and right frontal eye field, FEF; right superior parietal lobule, SPL) became more negatively correlated (Fig. 3C; Fig. 4A). Additionally, functional connectivity between untrained visual cortex and several default regions (left medial prefrontal, mPFC; right angular gyrus, AngG; right and left posterior cingulate, pCing) became less negatively correlated (Fig. 3C; Fig. 4B). Finally, in left dorsal visual cortex, i.e. corresponding to the lower right visual quadrant homologous to the trained quadrant and which showed learning-related evoked BOLD change, both modulations occurred, i.e. an increased negative correlation with dorsal attention regions and a decreased negative correlation with default regions (Fig. S4A).
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**Figure 4 Modulation of spontaneous functional connectivity after perceptual learning.**
Flattened brain representation with ROIs in trained visual cortex and dorsal attention network (A) and in untrained visual cortex and default network (B). Bar graphs report correlation values (r) between trained visual cortex and dorsal attention ROIs and untrained visual cortices and default network ROIs before (black) and after (gray) perceptual learning. n = 14. r, Pearson correlation coefficient; Student’s t test, two tails, P < 0.05; error bars ± s.e.m.

A number of control analyses (See Supporting Information Methods) showed that: a) functional connectivity changes were specific to the visual system and did not extend to the auditory system (Fig. S5A); b) other networks recruited during visual perceptual training (motor, core) also showed topographically-specific learning-dependent changes in functional connectivity (Fig. S5B); and, c) functional connectivity changes could not be explained by covert task rehearsal (Fig. S5C).
These findings clearly demonstrate that perceptual learning altered the spontaneous functional connectivity *between* visual cortex and task networks.

**Changes in directed mutual information**

Not only did the total amount of temporal correlation change with learning between visual, attention and default networks, but so did the degree and pattern of directed interaction, as expressed by Granger Causality [*Granger, 1969*]. Two regions are said to display Granger Causality (GC) when the autoregressive temporal model for one region’s time series is improved by including previous time points from the other region. This measure can be calculated in a top-down direction (e.g. from dorsal attention to visual regions) and in a bottom-up direction (e.g. from visual to dorsal attention regions). Comparing pre- to post-learning spontaneous GC, we observed a relative increase in the bottom-up direction from intermediate level visual areas (V3A-LO) to frontal eye field (FEF), but a relative decrease from low-level visual areas (V1d-V3) to FEF bilaterally (Fig. 5A, Fig. S6). Conversely, in the top-down direction, we measured a relative decrease from right FEF to V3A-LO, but a relative increase from right FEF to V1d-V3 (Fig. 5C, Fig. S6).

That learning-dependent changes in top-down and bottom-up GC match across visual areas is significant because the analysis was performed independently for different region pairs and directions. The pattern suggests that interaction between regions in the dorsal attention network is shifting and differentially moving across the visual hierarchy before and after learning.
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Figure 5 Modulation of Granger Causality after perceptual learning.
Changes in Granger Causality (GC) directional modulation after learning that correlate with behavioral performance. (A) Bottom-up GC change. Scatter plots show the positive correlation between bottom-up GC modulation measured as F-statistic score (y-axis) and behavioral improvement measured as trained minus untrained shape accuracy score (x-axis). \( r = 0.68, P = 0.0074 \) for right V1/V2d/V3 -> right FEF; \( r = 0.557, P = 0.037 \) for V3A/LO -> right FEF). (B) Top-down GC Change. Scatter plots show the positive correlation between top-down GC modulation measured as F-statistic score (y-axis) and behavioral improvement measured as trained minus untrained shape accuracy score (green) or untrained minus trained shape RTs score (x-axis). \( r = 0.687, P = 0.0065 \) for right FEF -> right V1/V2d/V3; \( r = 0.55, P = 0.0409 \) for right FEF -> right LO). Green arrows and outlines indicate increased GC (Post-learning > Pre-learning), red dashed arrows and outlines indicate decreased GC (Pre-learning > Post-learning). \( n = 14 \).

Behavioral correlations

Finally, we observed a number of interesting correlations between learning-dependent changes in task-evoked activity, functional connectivity, and granger causality and behavioral performance. First, subjects with higher sensitivity for trained shapes (trained-untrained accuracy) also showed stronger task-evoked modulation in the trained quadrant (Fig. S7) \( r = 0.7, P = 0.006 \) for lower left; \( P > 0.05 \) for all other quadrants). Second, subjects who responded more quickly to familiar shapes (larger untrained-trained RTs) developed more negative correlation post-learning between trained visual cortex and dorsal attention regions \( r = 0.72, P = 0.0038 \) for right FEF-trained visual cortex; \( r = 0.51, P = 0.059 \) for left FEF-
trained visual cortex; \( P > 0.05 \) for all other quadrants) (Fig. S4C). Lastly, the degree of Granger Causality modulation between trained visual cortex and dorsal attention network also correlated with behavior. Subjects with greater changes in GC between the two networks, in both top-down and bottom-up directions, did not perform as well as subjects in whom GC modifications were less pronounced (Fig. 5 A-D). This suggests two non-exclusive interpretations. First, performance could be better when minimal changes to directional network dynamics occur as a result of learning. For example, subjects that try harder may alter their functional architecture to a greater extent, while not necessarily achieving improved performance. Alternatively, performance may be better when, prior to learning, the brain already has a pattern of directional interaction between visual cortex and attention regions that is conducive to better performance.

**Discussion**

The preceding descriptions of learning-induced changes in resting functional connectivity powerfully demonstrate the dynamic nature of spontaneous BOLD coherence and suggest a role in brain function beyond an inert recapitulation of gross anatomy or intrinsic vascular dynamics. We show that prior experience in the form of visual perceptual learning can change the pattern of spontaneous cortical activity between different brain networks in specific ways. The networks that are recruited in the course of training show robust and specific learning-related modulation in resting BOLD connectivity. This provides strong support for the hypothesis that the coordinated activation of cortical networks during behavior shapes the organized pattern of correlated spontaneous activity at rest. By extension, functional networks observed in the adult brain are likely to reflect the patterned history of regional co-activation in the course of development and individual experience. Moreover, the co-localization of learning-dependent functional connectivity changes with task-evoked modulations, and their correlation with learning suggests that patterns of spontaneous activity influence
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the task-dependent recruitment of the same cortical circuits. We discuss first the
interpretation and possible neural bases of observed changes in functional
connectivity. Next, we consider their functional significance, and relationship with
task-driven activation.

The visual perceptual task employed in this study was akin to an entirely new
experience for our subjects. The acquisition of expertise was slow (thousands of
trials) and required the development of an entirely new set of stimulus-response
associations. In addition, subjects had to recruit/execute a number of operations
during training including shifts and maintenance of spatial attention to the left
lower quadrant, development of a perceptual template for the target, and filtering of
unattended information from the distractors. With time, subjects report to see the
target shape effortlessly (as if it 'pops-out' from the background). Prior
neuroimaging studies have established that this task involves an interaction of
visual cortex with fronto-parietal regions, with development of expertise being
associated with sharpening of orientation tuning curves and fMRI response
enhancement in visual cortex, and less pronounced activation of higher-order
control areas (Bisley and Goldberg, 2003; Corbetta and Shulman, 2002;
Gilbert and Sigman, 2007; Sestieri et al., 2008; Sylvester et al., 2007).

Here, we show that perceptual learning also entrains novel patterns of spontaneous
activity in the same neural networks recruited by the task. It is as if learning sculpts
the connectivity of existing functional networks. Specifically, trained visual cortex
and fronto-parietal attention areas that were independent before training (i.e.
resting correlation near zero, Fig. 4A) became negatively correlated after learning.
Across subjects, more negative correlation corresponded with improved perceptual
learning. While the precise definition of the underlying neural mechanisms will
require more invasive recording, we interpret the negative correlation as reflecting
the active (i.e. metabolically demanding) decoupling of fronto-parietal attention
and occipital visual areas. This interpretation is consistent with the experience of
perceptual learning: early in training subjects detect the target only by paying close
Learning sculpts spontaneous activity attention; whereas after learning the development of a target template in visual cortex allows for more automatic and effortless discrimination that requires less on-line top-down control. This trained behavioral state corresponds to a state of anti-correlation between visual and attention regions. Anti-correlation in spontaneous BOLD activity may be related to anti-phase changes in slow cortical potentials and gamma power (He et al., 2008; Miller et al., 2009b; Nir et al., 2008), which may prevent the two systems from interfering with each other under task conditions. Analysis of large-scale neural network models indicates that spontaneous network anti-correlation is an efficient computational state to facilitate independent task recruitment and switching (Deco et al., 2009). This interpretation is also supported by the GC analysis. The increase in bottom-up drive from intermediate visual areas (V3A/LO), which are specialized in object and orientation discrimination (Grill-Spector et al., 2000), to higher-order visual areas (FEF), involved in decision-making, may reflect the adjustment of inter-network information flow facilitating more automatic detection of the target shape. Conversely, the increase in top-down drive from FEF to early visual areas (V1-V3) may correspond to the establishment of filtering mechanisms for unattended information in the trained quadrant. Similarly, the decrease in negative correlation between untrained portions of visual cortex and regions of the DMN can be interpreted in this framework as the attenuation of a 'filter' mechanism for unattended sensory information in the non-trained quadrants, which becomes less important as training progresses. Topographically specific negative correlations between visual cortex and the default network have never been reported before, and this finding links more directly than any previous study (Shulman et al., 2007; Weissman et al., 2006) activity in this network to perceptual operations in contrast to prevalent views of its involvement in self-referential functions (theory of mind, episodic memory, internal thoughts, etc) (Buckner et al., 2008).

Learning-dependent changes in spontaneous coherence also seem to have an impact on how the same circuits fire during the visual perceptual task. In the same
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quadrant of visual cortex in which we observed behaviorally significant post-
learning modulation of task-evoked activity, we also measured behaviorally
significant changes of resting state fcMRI and directional interaction. The idea that
the pattern of spontaneous activity constrains task-driven responses and behavioral
output is consistent with other observations. For instance, the phase of ultra-slow
EEG oscillations correlates with behavioral performance suggesting a relationship
between slow oscillations, task-driven responses and behavioral output (Monto et
al., 2008).

We conclude that spontaneous BOLD connectivity does not simply reflect the
structure of the underlying anatomical circuitry, as has been well documented
(Ghosh et al., 2008; Hagmann et al., 2008; Honey et al., 2007), but underlies
functional links acting as a form of ‘system memory’ that recapitulates the history
of experience driven co-activation on cortical circuitries. Our results in the
perceptual domain closely match a recent report by Albert et al who demonstrated
increased functional connectivity in the motor system only when subjects learn a
novel task, but not when they repeat a familiar task with the same intensity (Albert
et al., 2009). This is a fundamental distinction because it suggests that functional
connectivity encodes novel patterns of co-activation possibly through the
recruitment of specific synaptic mechanisms. A recent study, for instance, reported
widespread enhancement of fMRI activity in hippocampus and connected cortical
regions during micro-stimulation protocols that induce long-term potentiation
(Canals et al., 2009). Perceptual learning may induce similar changes at the
cortical level that are then manifested in modulation of both resting functional
connectivity and task-evoked activity.

Finally, our results provide a new perspective on the functional role of spontaneous
coherence in cortical networks as identified by fMRI. This signal does not reflect a
simple physiological marker of anatomical pathways, but has clear functional
significance as it predicts cognitive performance (Hampson et al., 2006; Kelly et
al., 2008), behavioral deficits (He et al., 2007), and changes with learning (Canals et al., 2009). That the most relevant changes in our study occur between rather than within networks (although more subtle changes did occur within the visual system, personal communication) indicates that this signal may be especially important in linking large-scale cortical networks. This is also consistent with the observation that BOLD coherence is related to low frequency fluctuations of neuronal activity (Leopold et al., 2003; Nir et al., 2008) which are deemed more important for long distance cortical communication (Buzsáki and Draguhn, 2004). One hypothesis we favor is that spontaneously correlated networks represent preferential channels through which neuronal populations in different areas can communicate. The slow fluctuation of coherent activity can be thought of as a temporal scaffold that implements selection mechanisms similar to those described in the communication-through-coherence (CTC) hypothesis (Fries, 2005). Accordingly, communication between distant cortical regions is enhanced by increasing the coherence of spontaneous oscillatory activity, which in turn facilitates the transfer of information coded in spike rates. Originally envisioned to explain synchronization in the gamma band, this hypothesis could be extended to account for the coupling of high frequencies to slow rhythms described in several recent studies (Canolty et al., 2006; Vanhatalo et al., 2004), and their relationship to behavior (Monto et al., 2008).

**Methods**

**Subjects**

Fourteen healthy right-handed volunteers (7 females, aged 20-30) with no psychiatric or neurological disorders, and normal or corrected-to-normal vision participated in the study, after providing written informed consent. The University “G. D’Annunzio” of Chieti’s institutional ethics committee approved the experimental protocol.
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Visual stimuli

The stimulus array comprised 12 Ts arranged in an annulus of 5 degrees radius and displayed across the four visual quadrants. The inverted T was the trained target shape, while differently oriented Ts were the distracters. Presentation timing was triggered by the acquisition of fMRI frames.

Behavioral training

Subjects were trained with daily sessions to attend to the lower left visual quadrant and find the target shape among the distracters while maintaining central fixation. Training lasted from 2 to 9 days and continued until the level of performance was above 80% in at least ten consecutive blocks (Sigman et al., 2005).

fMRI procedure and scanning

Before behavioral training, imaging data were acquired in a scanning session consisting of six runs of resting state, in which subjects were instructed to fixate a small cross under low level illumination and to remain passive (free from pursuing focused thought), and six runs of a functional retinotopic localizer to identify voxels preferentially responding to each visual quadrant. When subjects reached the learning threshold, a second functional session was acquired with six resting state runs and six runs on the trained shape identification task, each consisting of five trained and five untrained blocks alternated with five fixation blocks. More detailed information on MRI acquisition and processing parameters is in SI.

Behavioral analysis

For each block, we recorded the number of positive responses (p) and the reaction time (RT). Detailed discussion of behavioral measures we used is in SI.

Localizer and shape identification task data processing

The BOLD time course at each voxel, for each subject, was subjected to a general linear model with an assumed response function using in-house software. Analysis
details are in SI.

**Regions of Interest Creation**

Regions of Interest (ROIs) were functionally defined for localizer and task data using an in-house clustering algorithm. ROIs were initially defined as 15 mm spheres centered on peaks (threshold between $z$-score 3 and -3), peaks within 15 mm of each other were consolidated into a single ROI.

**fcMRI data analysis**

For each subject and for each resting state period (before and after training), BOLD time courses were extracted from localizer and task ROIs, the correlation matrix was calculated, and $z$-values were obtained using the Fisher transform. A t-test across subjects was then used to threshold the mean correlation matrix ($P < 0.03$, Monte-Carlo corrected for multiple comparisons). We then computed the difference between the first and second rest session correlation matrices for each subject, and the paired t-test between rest periods ($P < 0.03$, Monte-Carlo corrected for multiple comparisons) was used to threshold the mean-difference matrix. Information on preprocessing specific to FC analysis is in SI.

**Granger analysis**

For pairs of ROIs that showed a significant change in correlation between rest periods, we computed Granger-Causality ([Granger, 1969](#)) F-statistics for each subject and each rest period using the Granger Test implementation in the MarkovSwitching Bayesian Vector Autoregression Models Package (MSBVAR [http://cran.r-project.org/web/packages/MSBVAR/](http://cran.r-project.org/web/packages/MSBVAR/)) of the R statistical computing environment ([http://www.R-project.org](http://www.R-project.org)). We computed Order-1 bivariate Granger Tests for each ROI pair and obtained an F-statistic & p-score in each direction for time lag lengths between 1 and 10 MR frames. To investigate Granger Directionality changes between rest periods, we computed the difference between F-statistics in the pre- and post-learning rest periods.
Behavior and brain activity correlation

For each subject, we calculated measures of behavioral improvement (RT & PC, as described above) and then the percent BOLD change related to trained vs. untrained shape in the ROIs. We also computed for each subject and for each pair of ROIs significant differences of fcMRI and Granger Causality between resting states. Using Pearson correlation coefficient we calculated the correlation between behavioral improvement and brain activity modulation (task evoked, spontaneous fcMRI and GC).

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Individual variability in functional connectivity predicts performance of a perceptual task

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Individual variability in functional connectivity predicts performance of a perceptual task.

(*) denotes equal contribution
Abstract

People differ in their ability to perform novel perceptual tasks, both during initial exposure and in the rate of improvement with practice. It is also known that regions of the brain recruited by particular tasks change their activity during learning. Here we investigate neural signals predictive of individual variability in performance. We used resting state functional magnetic resonance imaging to assess functional connectivity prior to training on a novel visual discrimination task. Subsequent task performance was related to functional connectivity measures within portions of visual cortex and between visual cortex and prefrontal association areas. Our results indicate that individual differences in the acquisition of novel perceptual skills can be related to individual differences in spontaneous cortical activity.
Introduction

Healthy observers differ in their ability to perform a variety of visual task (Halpern et al., 1999). Individuals also differ in their ability to improve with training (Fahle and Henke-Fahle, 1996; Fahle et al., 1995; Fahle, 2004; Mukai et al., 2007; Schmitt et al., 2002) i.e. perceptual learning (Gilbert et al., 2001; Sasaki et al., 2010). Initial performance and rate of learning tend to be inversely related (Fahle and Henke-Fahle, 1996; Sasaki et al., 2010). Thus, individuals who perform better initially tend to exhibit slower improvement. Though the physiological correlates of perceptual learning have been well documented at the level of neurons (Gilbert and Sigman, 2007; Raiguel et al., 2006; Schoups et al., 2001), and large-scale networks (Lewis et al., 2009; Li et al., 2004; Mukai et al., 2007; Schwartz et al., 2002; Sigman et al., 2005), it is largely unknown whether the state of the brain prior to training influences future performance or the rate of acquisition of a novel task. Here we investigate the extent to which performance of a novel perceptual task can be predicted on the basis of physiological measures evaluated prior to training. Our measure of performance takes into account both early and late features of the psychophysical learning curve.

Intrinsic neural activity is temporally correlated within widely distributed networks that recapitulate the topography of task-related functional responses (Biswal et al., 1995; Fox et al., 2005; 2006; Greicius et al., 2003; Vincent et al., 2006). Hence, resting-state functional connectivity offers a plausible neural correlate of behavioral predisposition to perform a novel task. Moreover, resting state measures have been correlated with individual performance variability in several cognitive domains (Hampson et al., 2006; Koyama et al., 2011; Seeley et al., 2007; van den Heuvel et al., 2009; Zhu et al., 2011). However, no study to date has shown that functional connectivity, measured prior to training, within cortical circuits later recruited by a novel task, is predictive of future performance.
In previous work (Lewis et al., 2009), we showed that resting state blood oxygenation level dependent (BOLD) signal functional connectivity (FC) changes in task-relevant cortical networks after extensive practice on a novel orientation discrimination task. Critically, post-learning modulations in functional connectivity correlated with individual measures of improvement. Here, we analyze the same dataset to test the hypothesis that functional connectivity in task-relevant circuits, measured prior to training, is predictive of subsequent performance.

**Results**

**Behavior**

Healthy observers (n=14, 7 males) were trained to report the presence/absence of a target shape (an inverted letter T) (Sigman and Gilbert, 2000; Sigman et al., 2005) always presented in the lower left visual quadrant (15) (Fig. 1A, Methods and SI). Targets and distractors (letter Ts of different orientation) were presented together in a circular array at 5 degrees of eccentricity. The criterion for successful acquisition of the task was a normalized accuracy equal to or greater than 80% over 10 consecutive blocks of trials (Normalized Accuracy = (%Hits + %Correct Rejections)-%False Alarms/(1-%False Alarms), each block=45 trials) (Lewis et al., 2009; Sigman and Gilbert, 2000; Sigman et al., 2005). On average, observers took about 5,600 trials or 118 blocks (~4 days of 2-3 hours practice per day) to reach criterion (Fig. 1B). We observed a high degree of individual variability at the beginning of training. Accuracy on the first 10 blocks, the minimum number of blocks performed on the first day, was 41% with large inter-individual variability (range: 13-69%). Psychophysical performance curves were fit using an empirical two-parameter expression: \( a = a_0 + s \times \log(k) \), where \( a \) is accuracy, \( k \) indexes block, \( a_0 \) is initial accuracy on the first block, and \( s \) is a scaling parameter numerically
equal to the initial slope. Fits of the analytic expression to the individual performance data were expressed in terms of variance explained ($r^2$): median $r^2 = 0.68$, range: 0.29-0.93 (Figs. 1B and S1). In addition to $a_0$ and $s$, we evaluated the number blocks needed to achieve criterion (performance $\geq 80\%$) ($k_c$). The three measures were correlated (see SI, Fig. S2) in a manner consistent with prior studies of perceptual learning (Fahle, 1997; Fahle and Henke-Fahle, 1996). Thus, subjects with high initial accuracy learned the task in fewer blocks but at a lower rate of improvement. Conversely, subjects with lower initial accuracy took longer to reach criterion but their rate of improvement was higher. Because of these

Figure 1 Behavioral Training, Psychophysics, Visuotopic Localizer and Regions of Interest.
(A) Experimental paradigm. (B) Accuracy over blocks. Black dots display the average performance, solid red line indicates the fit of the psychophysical model with prediction bounds at 95% of confidence level (dotted lines). (C) Design of visuotopic localizer. Squares of different colors (not shown in experiment) indicate a visual quadrant. (D) Visual ROIs. Eight visual regions defined on the basis of the visuotopic localizer are displayed on the flattened representation of posterior occipital cortex using the PALS atlas (Van Essen, 2005). Blue lines are approximate borders between retinotopic visual areas based on the atlas.
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relations and the relatively small size of the study group, it was not possible to derive independent measures of initial performance and rate of learning. To obtain individual quantitative indices of performance, $a_0$, $s$, and $k_c$ were entered into a principal component analysis (Fig. S3). The first component (PC1) explained 75% of the variance. The second component accounted for 15% of the variance, but its eigenvalue was less than 1 (scree plot Fig. S3) and it was therefore not further considered (Kaiser, 1960). Accordingly, PC1 was used to compute individual measures of performance, which we here define as task fitness ($f$), using the expression, $f = [a_0 s k_c]$ where $w$ is the vector of factor weights (see SI). In the rest of the analysis, we use task fitness to examine the relationship between performance and pre-training resting-state functional connectivity.

**Pre-training functional connectivity in visual cortex and task fitness**

Resting state fMRI and visuotopic localizer fMRI were acquired 24-48 hours prior first exposure to the task (Methods and SI). During the visuotopic localizer scans, subjects were asked to maintain central fixation while quarter-field stimulus arrays were passively presented in a blocked design (Fig. 1C). Regions of interest (ROIs) for the computation of functional connectivity were identified in the ventral and dorsal portions of visual cortex in each hemisphere. At the group level, two ROIs were identified in each quadrant as showing the strongest visuotopic localizer responses (e.g., in right dorsal cortex for left lower field stimulation) as compared to the average response to stimuli in the other quadrants (group-level voxel-wise random-effect ANOVAs, multiple comparison corrected over the entire brain ($P<0.05$)). These regions are shown in Fig. 1D on a flattened representation of visual cortex in the PALS atlas (Population Average Landmark and Surface) (Van Essen, 2005) and labeled according to their location with respect to the probabilistic borders of visual areas in the same atlas (Table S1). In general, for each quarter field representation in visual cortex, one ROI is ‘early’ in the visual
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hierarchy (near/at V1-V2), while the other is ‘intermediate’ (near at V4-V8 or V3A)(Fig. 1D)(Table S1 for coordinates).

To examine the relationship between pre-training functional connectivity (FC) and the ability to perform the discrimination task, we computed group-level voxel-wise maps of the Pearson correlation coefficient between task fitness and the strength of FC for each visuotopic ROI (defined as FC-PC1 correlation maps) (Methods, SI and Fig. S4). Figure 2A shows that the strength of FC between a representative ROI in right ventral visual cortex (near/at V1-V2) and large swaths of ventral and dorsal peripheral visual cortex in both hemispheres is strongly correlated with task fitness (all voxels Z > 2, P<0.05, Monte Carlo corrected). Observers with stronger pre-training FC between visual regions displayed greater task fitness (Fig. 2B). This relationship was consistent across different ROIs in left and right visual cortex (Fig. S5). To quantify this consistency, a conjunction map was computed that shows the portions of visual cortex with behaviorally predictive FC across multiple ROIs (Fig. 2C). The most consistent regions encompassed both early and intermediate retinotopic areas, including a band outside the foveal region in the near periphery (based on the PALS borders).

To examine whether the regions exhibiting behaviorally significant pre-training FC coded for the stimuli, we quantified the percentage of voxels in the FC-PC1 conjunction map that overlapped with the regions in visual cortex selectively activated by the stimulus array (the sum of the quadrant maps). At a threshold of 4 of 8 ROIs, 72% of the behaviorally predictive voxels from the FC-PC1 conjunction map fell within the borders of the region activated by the stimulus (Fig. 2D). This proportion increased to 86% when the threshold was increased to 5 out of 8 ROIs.
Figure 2 Task fitness and pre-training Functional Connectivity to/from Visual Cortex.

(A) Voxel-wise FC-PC1 correlation map starting from a seed region in the right ventral visual seed (V1-V2) (black border), corresponding to the left upper visual quadrant. The map is projected onto a flattened representation of the posterior occipital cortex using the PALS (population-average, landmark, and surface-based) atlas (Van Essen, 2005). Color scale: yellow/orange indicates positive correlation (Z-stat of Pearson r) thresholded at Z > 2, P<0.05, Monte Carlo corrected. Blue color indicates negative correlation. Blue lines are the same as Fig. 1D. R.H. and L.H.: right and left hemisphere. (B) X-axis: task fitness i.e. principal component scores of PC1; y-axis: FC between a right ventral visual seed V1-V2 (green region in the inset, same as panel A) and a left dorsal visual region (red) extracted from the FC-PC1 correlation map in panel A (Talairach coordinates: -06 -96 +08, 185 voxels). Each diamond represents an observer. r: Pearson Correlation coefficient. (C) Conjunction of eight voxel-wise FC-PC1 correlation maps, one for each visual seed shown in Fig. 1D (Table S1 for coordinates). Color scale: yellow/orange indicates overlap of positive correlations (range: 1-8); cyan/blue indicates overlap of negative correlations. (D) Conjunction map between visuotopic localizer activations (Z-stat >3, P<0.05, Monte Carlo corrected, see Methods) (red) and FC-PC1 conjunction map thresholded 4 out of 8 (green). Overlapped voxels are in yellow.
Computing pair-wise correlations for all ROIs and calculating the correlation with task fitness confirmed these findings. The range of FC-PC1 correlations varied between $r=0.1$ and $r=0.8$; 13 out of 28 ($=8\times7/2$) possible ROI pairs showed a
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significant correlation with task fitness (false discovery rate (FDR), q<0.05 after random permutation test). Thus, both voxel-wise and regional analyses confirmed a significant relationship between task fitness and pre-training FC in portions of visual cortex activated by the visuotopic localizer stimuli. Figure 3A shows the group average strength of FC between ROI pairs arranged by visual quadrant (dorsal, ventral). Figure 3B shows behaviorally significant FC. Behaviorally predictive correlations (FC-PC1) were observed predominantly in heterotopic region pairs, i.e., region pairs in different quadrants either within the same (e.g., left dorsal-to-ventral cortex) or different hemispheres (e.g., left dorsal-to-right ventral cortex) (Figs. 3B and 3C), rather than homotopic region pairs (e.g., right dorsal-to-left dorsal cortex) (Figs. 3B and 3D) or local connectivity (e.g., right dorsal V1-V3-to-right dorsal V3A-LO) (Figs. 3B and 3E) (permutation test on full correlation matrix, FDR q<0.05).

Pre-training functional connectivity between visual and fronto-parietal regions, and task fitness
Behaviorally predictive functional connectivity with visuotopic areas extended also to a small number of regions in higher order frontal and posterior parietal cortex (Fig. S6A). Figure 4A shows functional connectivity between a right dorsal visual ROI and left anterior insula, belonging to the “control network” (Dosenbach et al., 2006; 2007), which was negatively correlated with task fitness. Observers who performed better on the orientation discrimination task tended to have stronger negative correlation (antiphase coherence) between spontaneous activity in visual cortex and anterior insula (Fig. 4B). This result is representative of multiple visual ROIs (4 out of 8 visual ROIs) (Fig. 4C). Interestingly, this region overlaps with an insular region activated by the orientation discrimination task (Fig. 4D, see SI). A similar pattern was detected in the right medial prefrontal cortex, a part of the default mode network (Raichle et al., 2001; Shulman et al., 1997) (Fig. 4E-H). Again, more negative functional connectivity corresponded to greater task fitness (Fig. 4F). This region overlaps with a larger medial prefrontal region de-activated
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Figure 4 Task fitness and pre-training connectivity with visual cortex and frontal regions.

(A) Lateral view of the voxel-wise FC-PC1 correlation map starting from a right dorsal visual seed (V3A-LO), corresponding to the left lower visual quadrant. Color scale: same as Fig. 2. L.H.: left hemisphere. (B) X-axis: task fitness; Y-axis: FC between a right dorsal visual seed V3A-LO (green region in the inset, same in panel A) and left anterior insula (LaI) (red) extracted from the FC-PC1 correlation map in panel A (Talairach coordinates: -38 +18 -07, 171 voxels). Each diamond represents an observer. r: Pearson Correlation coefficient. (C) Conjunction map of FC-PC1 correlation maps from eight visual seeds. Color scale same as Fig. 2. (D) Conjunction map between activation map of orientation discrimination task (trained plus untrained shape greater than fixation, Z-stat >3, P<0.05, Monte Carlo corrected, see Methods) and FC-PC1 conjunction map thresholded at negative 3 out of 8 (green). Overlapped voxels are in yellow. (E) Medial view of the voxel-wise FC-PC1 correlation map starting from a right ventral visual seed (V1-V2), corresponding to the left upper visual quadrant. Color scale: same as panel A. R.H.: right hemisphere. (F) X-axis as panel B; Y-axis: FC between a right ventral visual seed V1-V2 (green region in the inset) and a right ventral medial prefrontal cortex (RvmPFC) (red) extracted from the FC-PC1 correlation map in panel A (Talairach coordinates: +04 +38 -18, 120 voxels). Each diamond represents an observer. r: Pearson Correlation coefficient. (G) Medial view of the same conjunction map in (B). Color scale same as panel (B). (H) Conjunction map between deactivation map of orientation discrimination task (trained plus untrained shape less than fixation, Z-stat >3, P<0.05, Monte Carlo corrected, see Methods) and FC-PC1 conjunction map thresholded negative 3 out of 8 (green). Overlapping voxels are in yellow.
by task performance (Fig. 4H; see SI). Similar negative FC-PC1 correlations, i.e., more negative functional connectivity corresponding to better performance, were detected in other default mode regions, in left middle temporal cortex and right/left angular gyrus (Fig. S6A) (Table 2). All regions in higher order cortex that showed predictive FC with visual cortex overlapped regions recruited by the orientation discrimination task (Fig. S6B-C), albeit in a small proportion of the total extent of cortex recruited by the task.

**Control analyses: auditory cortex**

To examine the modality specificity of these effects, and rule out the possibility that functional connectivity-performance correlations merely reflected a high level of functional connectivity in neighboring areas, a set of control analyses was run on primary and secondary auditory regions (SI and Table S1 for coordinates). The auditory regions were selected based on two criteria: anatomical location and task deactivation during the orientation discrimination task. In fact, primary and secondary auditory regions are typically deactivated during visual tasks (32). From the auditory regions, we computed baseline FC and FC-PC1 correlation maps, which were compared with the visual regions. Both neighboring visual (Fig. S7A) and auditory regions (Fig. S7C) showed strong functional connectivity. However, auditory regions (Fig. S7D), in contrast to visual regions (Fig. S7B), did not show a predictive relationship with task fitness (Fig. S7D). We conclude that the behaviorally predictive pre-training functional connectivity is modality specific, and is not driven by local connectivity.

**Discussion**

We show that certain patterns of resting state functional connectivity within visual cortex, and between visual cortex and higher order cortical regions, represent neural predictors of observer predisposition to perform a novel orientation
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discrimination task. Several previous studies have reported correlations between performance measures and fMRI functional connectivity (Hampson et al., 2006; Koyama et al., 2011; Seeley et al., 2007; van den Heuvel et al., 2009; Zhu et al., 2011). However, this study, as far as we are aware, is the first to demonstrate that behaviorally predictive patterns of functional connectivity, prior to any exposure to a novel task, coincide with areas subsequently recruited by performance of the same task.

**Task fitness: initial performance, rate and duration of learning**

The behavioral component identified by the factor analysis (PC1) combined aspects of initial performance (predisposition), the rate of performance improvement and the quantity of practice required to reach criterion. Our observers were highly variable in their initial performance, a finding concordant with previous studies of complex visual tasks (Halpern et al., 1999) as well as perceptual learning (Fahle and Henke-Fahle, 1996; Fahle et al., 1995; Fahle, 2004; Mukai et al., 2007; Schmitt et al., 2002). Interestingly, task fitness was positively correlated with initial performance, and negatively correlated with both the rate of learning and the number of blocks to criterion. Hence, subjects with high initial performance reached criterion earlier but at a slower rate, consistently with early reports on perceptual learning (Fahle, 1997; Fahle and Henke-Fahle, 1996). Our results, therefore, suggest that the state of the system at the beginning of training may influence the way the observers learn when the task requires extensive cortical processing.

**Predictive intrinsic functional connectivity**

Predictive functional connectivity was observed in region pairs including visual cortex as well as prefrontal and insular areas involved in cognitive control. All predictive regions were a subset of the cortical regions driven by, and in which FC was modulated by, the orientation discrimination task. Two patterns of functional
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connectivity were predictive of task fitness. In visual cortex, observers with stronger ‘heterotopic’ functional connections, i.e., linking cortex representing dorsal and ventral quadrants within or between hemispheres, exhibited higher task fitness. In contrast, the strength of ‘homotopic’ connections, i.e., linking either dorsal or ventral quadrants across hemispheres, or of ‘local’ connections, i.e., linking adjacent regions in visual cortex, was not predictive of performance. This result is noteworthy because local and homotopic functional connectivity typically is stronger than heterotopic functional connectivity.

A possible interpretation is that heterotopic connections linking different quadrants in visual cortex are more important for the dynamic re-weighting of functional connections that occur in the course of learning. The orientation discrimination task required subjects to direct spatial attention to the left lower quadrant. Important processes for acquisition of the task include filtering of distracters at multiple unattended locations (Gál et al., 2009), as well as coding of the locus of attention by gradients of activity across spatial maps (Bisley and Goldberg, 2003; Sylvester et al., 2007). Hence, a high degree of coherence between stimulus-specific regions in visual cortex, prior to any experience, may facilitate the subsequent parsing of relevant from irrelevant information, and facilitate the re-weighting of functional connections among different quadrants in visual cortex. At the end of learning, in agreement with this hypothesis, stronger responses to target shapes were recorded only in the trained visual quadrant, and functional connectivity was differentially modulated in trained and untrained quadrants (Lewis et al., 2009).

The second predictive pattern of functional connectivity was an inverse correlation between spontaneous activity in visual cortex and regions of the default mode (Raichle et al., 2001; Shulman et al., 1997) and control networks (Dosenbach et al., 2006). This finding is also consistent with learning-related changes reported in our previous study (Lewis et al., 2009), and in the work of Sigman (Sigman et al., 2005). We previously found that functional connectivity between unattended
quadrants in visual cortex and default mode regions decreased (became less negative) after learning, and that these decrements correlated with measures of perceptual learning. Sigman et al. reported that decreases in task-evoked deactivation in the default mode network correlated with learning on the same task (Sigman et al., 2005). Observers with stronger negative correlation between visual cortex and default mode regions at baseline may find it easier to filter out distracters at unattended locations early in training, which becomes less important as target selection becomes more automatic. This interpretation is consistent with a role of the default mode network in filtering out unattended stimuli, as suggested by other studies (Shulman et al., 2007; 2003; Weissman et al., 2006). Behaviorally significant negative correlations in functional connectivity between visual cortex and default mode regions have also been reported in relation to reading skills in children and adults, a competency closely related to orientation discrimination (Koyama et al., 2011).

**Putative underlying mechanisms**

One possible substrate for the predictive relationship between task fitness and functional connectivity is individual variability in structural connectivity. The strength of structural connections has been correlated with the strength of functional connectivity at the level of both large-scale networks (Honey et al., 2009) and local micro-circuitry (Ko et al., 2012) and has also been shown to exhibit experience dependent plasticity (Johansen-Berg, 2007). However, in our study, regions exhibiting predictive FC (heterotopic connections in visual cortex, and visual-default-control) showed weaker baseline functional connectivity. The logical inference would be that these areas are less well anatomically connected (Honey et al., 2009). Moreover, in primate studies, subdivisions of visual cortex with behaviorally predictive (heterotopic) functional coupling tend to have weaker anatomical connectivity than non-predictive (homotopic) regions (Gattass et al., 1997).
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Another mechanism that could be related to our results is the recent observation that fMRI functional connectivity is related to slow cortical potentials and band-limited fluctuations of power in higher frequencies (de Pasquale et al., 2010; He et al., 2008; Nir et al., 2007). These relatively slow fluctuations in neural excitability may facilitate synchronization of high frequency activity through a variety of mechanisms (Buzsáki and Draguhn, 2004; Fries, 2005), and enable the coordination of task-relevant circuits. This could explain why observers with stronger functional connectivity within visual cortex, or between visual and other task-relevant areas in prefrontal and insular cortex, can recruit those regions more efficiently when performing a novel task.

We conclude that individual variability of functional connectivity within visual cortex, and between visual and higher-order regions, is related to the predisposition to perform a novel visual discrimination task. These findings suggest a potential role of intrinsic brain activity as a neural predictor of perceptual skill acquisition. This result has general implications for the functional significance of spontaneous activity, and the neural bases of individual behavioral variability. In addition, our findings emphasize the importance of spontaneous activity, and the state of functional connectivity, as a possible ‘neural’ prior for biasing task-evoked activity and behavior (Arieli et al., 1996; Kenet et al., 2003; Sadaghiani et al., 2010).

Methods

Participants

Fourteen healthy, right-handed volunteers (7 females, aged 20-30) with normal or corrected-to-normal vision participated in the study, after providing written informed consent approved by the Institutional Review Board of the University “G. D’Annunzio” of Chieti. All subjects were screened for a history of psychiatric or neurological disorders, and were not taking any medications.
Visual stimuli

The visual stimuli were generated using in-house software (LabScript) implemented in MATLAB (The MathWorks Inc., Natick, MA, USA). They were projected on a NEC 75 F Multisync monitor using a YASHI Pentium 4 computer during the behavioral training sessions, and back projected to a translucent screen by an LCD video projector (NEC 830 G+), which was viewed through a mirror attached to the head coil during fMRI. The timing of stimulus presentation was synchronized to the acquisition of the fMRI frames. The stimulus pattern was an annulus (5 degrees radius) formed by 12 letter Ts (size=1.5 deg visual angle) equally spaced and displayed randomly in four orientations (canonical, inverted 180 degrees, 90 degrees rotated to the left or to the right). The target shape was always an inverted T, whereas Ts of different orientation were used as distracters.

Behavioral training

Observers were instructed to attend to the left lower visual quadrant and report with a key press the presence/absence of a target shape (an inverted T) in a briefly presented radial display of randomly oriented T distracters. Central fixation was monitored with an eye tracker. Criterion for learning was 10 blocks of trials with accuracy =/> 80%. During daily training sessions, participants were instructed to attend to the left lower visual quadrant and report the presence/absence of the target shape while maintaining central fixation (Fig.1 main text). The position of the eyes was monitored with an infrared eye tracker (ISCN ETL-400). Each trial began with a central fixation spot followed by the presentation of the target shape for 2000 ms, and then by the stimulus pattern for 150 ms. In the stimulus pattern the target shape randomly appeared in one of three possible locations in the left lower visual quadrant, whereas the distracters, changing orientation randomly on each trial, were displayed in the remaining eleven locations across the four visual quadrants. Subjects indicated the presence/absence of the target shape by pressing one of two response keys on a Cedrus button box while maintaining fixation on the central spot. Participants performed blocks of 45 trials, of which 36 (80%) contained the
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target, and 9 (20%) did not. In each daily session subjects ran on average ~30 blocks of trials (range: 10-45 blocks). Training was discontinued when each observer reached a criterion of greater than 80% accuracy in at least ten consecutive blocks.

fMRI scanning

Functional images (gradient echo sequence: TR = 2.163 s; TE = 50 ms; FA = 90; slice thickness = 8 mm; 3.75 x 3.75 mm in-plane resolution) were acquired during passive stimulation of each visual quadrant with the same display used for perceptual learning (localizer). Localizer scans were used to define ROIs/seeds for the functional connectivity (FC) analysis of resting-state data obtained prior to any exposure to the task. Prior to behavioral training, subjects were scanned on a 1.5 T Siemens Vision scanner to obtain anatomical and functional scans. Anatomical images were acquired with a sagittal magnetization-prepared rapid acquisition gradient echo T1-weighted sequence (MPRAGE) with repetition time (TR) = 9.7 s; echo time (TE) = 4 ms; flip angle (FA) = 12°; time for inversion = 1200 ms; voxel size = 1 x 1 x 1.25 mm. Functional images were acquired with a gradient echo sequence (TR = 2.163 s; TE = 50 ms; FA = 90; slice thickness = 8 mm) in the axial plane (matrix = 64 X 64, field of view = 240 mm, 3.75 x 3.75 mm in-plane resolution). Sixteen slices were acquired for whole-brain coverage.

The fMRI data were acquired at rest, and during visuotopic localizer scans designed to identify regions in visual occipital cortex responding preferentially to the stimulus pattern in each of the four visual quadrants. During the resting-state scans, subjects were instructed to fixate a small cross in a low luminance environment and remain passive. Six scans of resting-state, each including 128 volumes, were acquired. During the localizer scans, subjects were asked to maintain central fixation and quarter-field stimuli were presented in a blocked design alternating with fixation periods (see Fig. S1A). Each scan consisted of twenty blocks: sixteen stimulation blocks (four for each visual quadrant: left lower, right lower, left upper, right upper), in which an array of 3 Ts was flashed at 6.67
Hz for 13 s, and four fixation blocks that were randomly interspersed among the stimulation blocks. Six runs of visuotopic localizer scans, each including 117 volumes, were obtained. A second scanning session was performed after the perceptual training was completed to define brain regions recruited by the task. In a blocked design, subjects performed the orientation task using either the familiar shape used for training (inverted T) or a novel shape (a T rotated 90 degree either to the left or right). In both conditions, the target was always randomly presented at one of the three locations of the stimulus array in the trained quadrant. The two tasks were run in blocks of trials beginning with a central cue (duration= 2.163 s) indicating the upcoming target (duration= 2.163 s), with each block lasting for 12 seconds (6 trials per block). The target was present on 80% of the trials, as in the behavioral session. Fixation blocks of 6, 10, or 12 s, with equal probability, were randomly interspersed with the active task blocks. Six scans, each including 113 volumes were obtained equal to 18 blocks (or 108 trials) per condition.

**Behavioral score**

Percent accuracy was computed for each block of training as follows: (%Hits + %Correct Rejections)-% False Alarms /(1-% False Alarms) (Sigman and Gilbert, 2000; Sigman et al., 2005). Individual raw learning curves were smoothed by using a 5-point moving average (MATLAB, The MathWorks, Inc), and were fit with a model fit using an empirical two-parameter expression: $a = a_0 + s \cdot \log(k)$, where $a$ is accuracy, $k$ indexes block, $a_0$ is initial accuracy on the first block, and $s$ is a scaling parameter numerically equal to the initial slope, by using Curve Fitting Toolbox 2.0 (MATLAB). This model provided the best fit of the psychophysical performance curves, expressed in terms of variance explained ($r^2$): median $r^2$= 0.68, range: 0.29- 0.93 (Figs. 1B and S1). In addition to $a_0$ and $s$, the number blocks needed to achieve criterion (80%) performance, $k_c$, was evaluated (Fig S2A for individual scores). These three measures were correlated: initial accuracy and
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slope: r=-0.85 (P<0.001); initial accuracy and blocks to criterion: r=-0.68 (P<0.01); slope and blocks to criterion: r=0.27 (P<0.3)(Fig. S2). After normalizing the behavioral parameters (mean =0, standard deviation =1) we attempted to reduce the dimensionality by computing a principal component analysis (PCA).

To obtain individual quantitative indices of performance, \( a_0, s, \) and \( k_c \) were entered into a principal component analysis (Fig. S3). The first component (PC1) explained 75% of the variance. The second component accounted for 15% of the variance, but its eigenvalue was less than 1 (scree plot Fig S3) and it was therefore not further considered (Kaiser, 1960). Accordingly, PC1 was used to compute individual measures of performance, which we here define as “task fitness”, using the expression, \( f = [a_0, s, k_c]^*w \), where \( w \) is the vector of factor weights: 0.6619, -0.5655 and -0.4920 for initial accuracy \( a_0 \), slope \( s \) and number of blocks to criterion \( k_c \), respectively. The first component represents a predictor of our observers’ fitness or aptitude toward performing the orientation discrimination task.

Principal component scores for PC1 from each subject were used as regressors for all the functional connectivity and behavior correlation analysis (Fig S3 B).

Task fitness was defined as the first factor (PC1) of a principal component analysis on the parameters of a natural logarithmic function, plus the number of blocks to criterion, used to quantify observer learning curves. This component accounted for 75% of the behavioral variance and was correlated with initial performance, rate of learning, and number of blocks to criterion.

**fMRI data preprocessing**

Functional data were realigned within and across scanning runs to correct for head motion using an eight-parameter (rigid body plus in-plane stretch) cross-modal registration. Differences in the acquisition time of each slice within a frame were compensated for by sync interpolation. A whole-brain normalization factor was applied to each run to correct for changes in signal intensity between runs (mode of 1000). For each subject, an atlas transformation was computed on the basis of an
average of the first frame of each functional run and MPRAGE structural images to 
the atlas representative target using a 12 parameters general affine transformation. 
Functional data were interpolated to 3 mm cubic voxels in atlas space. The atlas 
representative MP-RAGE target brain (711–2C) was produced by mutual co- 
registration (12 parameters affine transformations) of images obtained in 12 normal 
subjects. All preprocessing steps were performed using in-house software.

**Visuotopic localizer and orientation discrimination task data processing**

The BOLD time course at each voxel, for each subject, was subjected to a general 
linear model with an assumed response function (Boynton hemodynamic model) 
*(Boynton et al., 1996)* using in-house software. Constant and linear terms over 
each BOLD run accounted for baseline and linear drift. Separate task regressors 
coded for each of the event types: 5 for the visuotopic localizer: fixation, left lower 
quadrant, right lower quadrant, left upper quadrant and right upper quadrant; 3 for 
the orientation discrimination task: fixation, trained shape, untrained shape). A 
“residuals” dataset was created by summing the modeled responses (but not the 
constant or linear drift) with the residuals unaccounted for by the linear model. 
Therefore, this dataset contains the original time series minus the constant and 
linear drift terms. Group analyses were conducted using voxel-wise random-effect 
ANOVAs. Statistical images were Monte-Carlo corrected for multiple comparisons 
over the entire brain (*P*<0.05) to obtain z-score maps. Contrast maps were 
computed by subtracting ANOVA effects at each voxel to create z-score images 
from a given GLM. For the visuotopic localizer, voxels responding preferentially to 
each visual quadrant were found by contrasting the z-score image for the desired 
visual quadrant with the average of the z-score images from the other quadrants.

**Additional preprocessing for resting state data**

In preparation for the functional connectivity MRI analysis, data were passed 
through several additional preprocessing steps *(Fox et al., 2005)*: (1) spatial 
smoothing (6 mm full width at half maximum Gaussian blur), (2) temporal filtering
Individual variability predicts performance

retaining frequencies in the 0.009–0.08 Hz band; (3) removal of several sources of spurious variance unlikely to reflect spatially-specific functional correlations through linear regression: (i) six parameters obtained by rigid body correction of head motion, (ii) the whole-brain signal averaged over a fixed region in atlas space, (iii) signal from a ventricular region of interest, and (iv) signal from a region centered in the white matter.

Seed regions

A set of seed regions in visual cortex, dorsal attention network, default mode network and auditory cortex was functionally defined from localizer and task contrast maps using an in-house clustering algorithm. Seeds were initially defined as 15-mm spheres centered on peaks (threshold between z-score 3 and –3); peaks within 15 mm of each other were consolidated into a single region of interest (ROI). Stimulus-specific seeds in visual cortex were defined on the basis of localizer contrast maps: desired quadrant vs. average of all other quadrants. For each quadrant, the two strongest responses were selected. Their location was defined based on their overlap with the probabilistic borders of retinotopic areas in the PALS (Population Average Landmark and Surface) atlas (Van Essen, 2005) similarly to our previous study (Lewis et al., 2009). In general for each quadrant, we obtained a response in early visual cortex (i.e. V1/V2) and one in intermediate visual cortex (i.e. V3-VP/V4-V3A). Moreover, a set of control regions in auditory cortex was defined from the task activation data, by using the contrast Trained + Untrained shape vs. Fixation. During a visual task, rong deactivations are typically observed in auditory cortex (Shulman et al., 1997). Accordingly, two primary and two secondary auditory regions were selected in each hemisphere for the functional connectivity analysis. All the seeds are listed in Table S2.

Resting-state functional connectivity over the whole brain

In each participant voxel-wise resting state FC maps were computed for each seed (e.g. right dorsal V1-V2) by extracting time course from a given seed and then
computing the correlation coefficient (Pearson r) between that time course and the time course from all other brain voxels. Correlation coefficients were converted to a normal distribution by Fisher z transform.

**FC-behavior correlation over the whole brain**

For each of the eight visual ROIs, we computed voxel-wise correlation maps between behavior and Functional Connectivity, i.e. FC-PC1 correlation maps, using individual factor scores of the first component (PC1) of the behavioral PCA (Fig S4). These maps were computed by calculating at each voxel the correlation coefficient (Pearson Correlation (r)) between functional connectivity for a seed region (e.g. right dorsal V1-V3) and the rest of the brain, and behavioral scores over the group of subjects. Considering x equal to behavioral score (e.g. factor score of PC1) and y equal to FC between a visual seed region (e.g. right dorsal V1-V3) and a given voxel, we used the following formula:

$$r = \frac{1}{n-1} \sum_{i=1}^{n} \left( \frac{X_i - \bar{X}}{\sigma_X} \right) \left( \frac{Y_i - \bar{Y}}{\sigma_Y} \right)$$

where $n$ is the number of subjects (14), $X_i$, $\bar{X}$, $\sigma_X$, are the score, sample mean and sample standard deviation for the behavior respectively; while $Y_i$, $\bar{Y}$, $\sigma_Y$, are the score, sample mean and sample standard deviation for the functional connectivity, respectively.

This computation generated a voxel-wise correlation map indicating which voxels showed a significant association (positive or negative correlation) between FC with a given visual seed region (right dorsal V1-V3) and the behavioral score, see Fig. S4 for the analysis flow chart. The r-score maps were transformed first into t-score and then to Z-statistic maps. The final Z-stat maps were corrected for Monte Carlo multiple comparisons, $Z>2$, $P<0.05$ (Fig. S5). These maps are defined as FC-PC1 correlation maps.

The consistency of the topography of the behaviorally significant functional connectivity was quantified by a conjunction analysis of thresholded FC-PC1 maps.
Individual variability predicts performance

across seed regions. A positive value in a given voxel indicates how many visual seeds show positive correlation between behavioral score and its functional connectivity with that voxel. At the same time a negative value indicates how many seeds exhibit negative correlations. Given that we used 8 visual seeds (Fig. 1C, main text), the maximum value in the conjunction maps was ± 8. The same procedure was performed for the four control regions in the auditory cortex.

**FC-Behavior vs. Task-evoked topography**

The overlap of regions showing positive FC-PC1 correlation with those responding either during the visuotopic localizer or the task was quantified by computing the percentage of voxels overlapping between FC-PC1 correlation conjunction maps, and sum maps of task evoked activity. For the visuotopic condition, the four quadrant-related z-score (multiple comparisons corrected) maps were summed. The sum map was thresholded at a value of Z=3 and transformed to a binary map (Fig 2D). For the orientation task, Z-maps with positive or negative modulation above a threshold of Z=±3 for the contrast trained plus untrained vs. fixation were summed. Percentage of voxel overlap between FC-PC1 correlation conjunction, and sum maps of task-evoked activity were calculated at different conjunction thresholds (e.g. 4 out of 8 seeds, or 6 out 8 seeds, etc).

**FC-Behavior correlation at the regional level.**

In addition to computing the FC-behavior correlation over the whole brain, we also conducted FC-PC1 correlation analyses at the regional level. BOLD time series were extracted from visuotopic ROIs (Table S2)(see section on definition of seed regions above), and correlation matrix was created by computing the pair-wise temporal correlation (r Pearson correlation coefficient) across all ROIs. This r-score matrix was then converted by Fisher z transform into a normalized z-score matrix (Fig.3A). Finally, the z-score FC matrix was correlated across subjects with the PC1 scores (Fig. 3B). A correction for multiple comparisons was implemented
by a random permutation test, and thresholded at a false discovery rate of $q<0.05$ for 1000 permutations.

**Functional connectivity-PC1 correlation**

Voxel-wise or ROI pair FC-PC1 correlations were computed as the Pearson correlation coefficient ($r$) between functional connectivity measures and task fitness (see Results text). Functional connectivity was conventionally computed as the Pearson correlation between the timeseries extracted from a predefined ROI (e.g., left ventral visual cortex) and either the rest of the brain (to obtain voxel-wise maps) or another ROI (to obtain ROI-ROI FC) (see SI Methods for detailed information). Voxel-wise statistical significance in correlation maps was evaluated by first expressing the result as equi-probable $Z$ score maps, which were then corrected for multiple comparisons. Significance thresholds for ROI pair FC were computed by means of a permutation test. Presently reported ROI pair results are FDR corrected, $q<0.05$.

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Stimulus induced visual cortical networks are reactivated by spontaneous local and inter-areal rhythmic synchronization.
Abstract

Intrinsic covariation of brain activity has been studied across many levels of brain organization. Between visual areas, neuronal activity covaries primarily among portions with similar retinotopic selectivity. We hypothesized that spontaneous inter-areal co-activation is subserved by rhythmic neuronal synchronization. We performed simultaneous high-density electrocorticographic recordings across several visual areas in awake monkeys to investigate spatial patterns of local and inter-areal rhythmic synchronization. We show that stimulation-induced patterns of inter-areal co-activation were replayed in the absence of stimulation. Replay occurred through both, inter-areal co-fluctuation of local rhythmic activity and inter-areal rhythmic phase synchronization. Furthermore, stimulus induced patterns re-occurred on top of stimulus induced activity, in the trial-by-trial variance of the induced responses. Replay-related synchronization showed distinct peaks in the theta, alpha and gamma frequency bands. These results suggest that networks of intrinsic covariation observed at multiple levels and with several recording techniques are related to rhythmic synchronization.
Introduction

A classical approach to brain research considers the brain as responding to external stimuli and converting them into appropriate behavioral output. In this framework, endogenously driven variance in the brain is considered noise (Shadlen and Newsome, 1994). Yet, this so-called noise has been found to be highly structured and influenced by behavioral context (Cohen and Newsome, 2008). Such variation is a flavor of the ongoing, spontaneous activity of the brain. Both, the variation in the brain’s response to identical stimulation and spontaneous activity in the absence of stimulation are endogenously generated and structured in spatially specific intrinsic networks. Intrinsic networks have been described at essentially all spatial scales: from two individual neurons up to the whole brain. Membrane potentials of nearby neurons show a high degree of spontaneous correlation (Lampl et al., 1999). Neuronal spike rates co-fluctuate across physically identical trials, and this so-called noise-correlation between neurons is related to the similarity in their stimulus selectivity (Luczak et al., 2009). Correspondingly, when entire maps of population activity are investigated, patterns of activation induced by stimuli are found to re-occur spontaneously (Kenet et al., 2003). The same holds across neighboring maps in auditory cortex, where population activity spontaneously reproduces the tonotopic organization (Fukushima et al., 2012). Such co-activations can also be observed with fMRI. Across visual areas, regions selective for either foveal or peripheral stimuli show correlated BOLD activity (Vincent et al., 2007). In human subjects, BOLD signals were found to covary in symmetric, bilateral foci in the two hemispheres (Biswal et al., 1995). Subsequently, brain-wide sets of areas that were typically co-activated or co-deactivated by specific cognitive tasks were described (Fox and Raichle, 2007; Greicius et al., 2003). The finding of robust intrinsic networks using fMRI led to a large number of studies (Fox and Raichle, 2007), and has recently demonstrated the functional importance of intrinsic networks. For example, spontaneous, correlated BOLD signals predict behavior (Hesselmann et al., 2008a), and affect learning.
Stimulus induced networks are spontaneously reactivated (Baldassarre et al., 2012; Lewis et al., 2009).

Most fMRI studies of intrinsic networks involve inter-areal correlations, yet we have only a partial understanding of the underlying mechanisms. Recently, it has been shown that inter-areal BOLD signal correlations between regions of Squirrel monkey somatosensory cortex reflect the somatotopic map and are subserved by millisecond scale spike correlations (Wang et al., 2013). The rhythmic synchronization of neuronal activity is an interesting candidate mechanism for inter-areal interactions. Local rhythmic synchronization likely enhances neuronal impact through coincident postsynaptic input (Fries et al., 2001; Salinas and Sejnowski, 2001). While inter-areal rhythmic synchronization aligns temporal windows of excitability and likely renders communication effective (Fries, 2005; Womelsdorf et al., 2007).

We investigate here whether rhythmic synchronization subserves intrinsic networks between visual areas. Using high-resolution multi-area electrocorticography (ECoG) in awake monkeys, and utilizing the retinotopy of early and intermediate visual areas, we show that intrinsic inter-areal networks replay stimulus induced inter-areal networks, and are subserved by local and inter-areal synchronization in the theta, alpha and gamma-frequency bands. Surprisingly, even though gamma-band activity is not apparent in power spectra of spontaneous activity, intrinsic networks found in the absence of visual stimulation showed a distinct gamma peak. Therefore, rhythmic synchronization is a likely candidate mechanism underlying inter-areal fMRI intrinsic networks. The results link the extensive literature on fMRI intrinsic networks to the insights that have been obtained about the biophysical mechanisms underlying rhythmic synchronization.

Results

We investigated whether intrinsic networks between early (areas V1 and V2) and intermediate (areas V4 and TEO) visual regions are subserved by rhythmic activity
Stimulus induced networks are spontaneously reactivated and synchronization. To this end, we obtained measurements of neuronal activity with millisecond temporal resolution and few-millimeter spatial resolution and simultaneous coverage of both cortical regions. We employed electrocorticography (ECoG) in two awake macaque monkeys (Monkey P, Fig 1A; Monkey K, Fig S1A). For all analyses shown here, the signals from immediately neighboring ECoG electrodes were subtracted from each other to obtain local bipolar derivatives, which are free of the common recording reference, and to which we will refer to as “sites”. We explored intrinsic networks arising from covariations in local synchrony, i.e. correlated variation of endogenous power between areas, or in inter-areal synchronization, i.e. coherence between areas. As intrinsic networks can emerge spontaneously, in the absence of stimulation, or on top of stimulus-induced responses, we examined the correspondence of the spatial structure of the observed networks to the known spatial structure of visual cortex, namely retinotopy. If the observed intrinsic power covariation or coherence is spatially correlated to retinotopy, it is unlikely to be noise and may have a functional role.

**Retinotopic selectivity of ECoG signals**

To assess retinotopic selectivity across all sites of the ECoG grid, monkeys kept fixation for several seconds while visual stimuli were presented randomly interleaved at 60 different positions in the lower right visual quadrant (Fig 1B), corresponding to the portion of visual space covered by our grid. Stimulus-position dependent changes in spectral power (in any frequency band) were established through an Analysis of Variance (ANOVA). The resulting $p$-values are shown in figure 1C (for monkey P; for monkey K, see Fig. S1B) and reveal that retinotopy was mainly found in areas V1/V2 and areas V4/TEO. We selected those sites for further analyses (V1/V2: 68 sites in monkey P, 32 in monkey K; V4/TEO: 17 sites in both monkeys). For these sites, figure 1D shows how well stimulus-position was distinguished based on stimulus-induced power as a function of frequency (both monkeys combined, individual spectra are shown in Fig. S2). This demonstrates that retinotopic selectivity was present predominantly in a low frequency band.
Stimulus induced networks are spontaneously reactivated

Figure 1 High density ECoG layout and receptive field mapping paradigm
(A) Rendering of the brain of monkey P with the ECoG grid overlaid. Lines indicate the covered area with the major sulci. Dots indicate the 218 bipolar electrode derivations. Sites considered as lying in areas V1 and V2 are highlighted in teal and those in areas V4 and TEO are highlighted in purple. (B) Receptive fields were mapped with scrolling gratings at 60 locations in the lower right quadrant, corresponding to the coverage of the ECoG array. (C) Selectivity of all ECoG sites for stimulus position based on stimulus induced power in all frequency bands. (D) Selectivity of position tuned sites as a function of frequency. (E-G) Example average response to stimulation at the position marked in (B). (E) Time-Frequency plot at the site marked by a star in (F). Topographic plot of induced gamma power (80-95 Hz) for each ECoG site. (G) Induced gamma band response across all positions for the site marked in (F).

(1-20 Hz) and a gamma-frequency band (60-100 Hz). These two bands also contained the largest stimulus induced power, as is illustrated in the time-frequency analysis of one example site from area V1 activated by its optimal stimulus (Fig. 1E monkey P, for monkey K see Fig. S1C). While low frequency activity was present both with and without stimulation, gamma-band activity was primarily
Stimulus induced networks are spontaneously reactivated. Correspondingly, we illustrate the gamma-band power for all ECoG sites for the same stimulus position in Fig. 1F (monkey P, for monkey K see Fig. S1D). The well-localized topographical activation suggests that a given site responds only to a select region of visual space (Bosman et al., 2012). Indeed, when the site with maximal response in figure 1F (marked with a star) was selected and the response to all stimulus locations shown in figure 1G (monkey P, for monkey K, see Fig. S1E), there was a clearly defined receptive field (RF).

Figure 2 Retinotopic maps based on gamma band (80-95 hz) activity in monkey P
(A) Map of eccentricity; each recording site is colored to indicate the mean eccentricity of the 5 stimuli giving the largest gamma band response. (B) Map of elevation; each recording site is colored to indicate the mean elevation as estimated above. Inset shows how the 60 stimulus locations are represented across eccentricity and elevation.

To further investigate the retinotopy of the ECoG recordings from V1, V2, V4 and TEO, we grouped the 60 stimulus locations according to eccentricity or elevation. Both eccentricity (Fig. 2A) and elevation (Fig. 2B) were represented in orderly retinotopic maps, that corresponded well with previously determined topographies from repeated recordings with penetrating electrodes (Gattass et al., 2005) or from fMRI (Brewer et al., 2002). Two contiguous maps of space were visible, one behind the lunate sulcus for areas V1/V2, and another one between the lunate and
the superior temporal sulcus for areas V4/TEO. For simplicity, we will refer to ECoG sites in the V1/V2 map as V1, and to sites in the V4/TEO map as V4. We investigated whether these maps determined the intrinsic power covariations and/or the coherence between areas. A schematic of our processing stream is displayed in figure 3A. The main steps are: 1) Quantify the spatial pattern of stimulus induced power correlation; 2) Quantify the spatial pattern of intrinsic power correlation or coherence; 3) Correlate the spatial patterns from 1) and 2). As both stimulus induced (1) and intrinsic (2) metrics could be estimated for a full spectrum of frequencies, the correlation (3) could be determined for all pairs of frequencies. While the gamma-frequency band showed the clearest stimulus related patterns, we will show the full frequency-by-frequency analyses, in order to fully document the results.

**Signal Correlations**

In order to assess the similarity of stimulus preferences between V1 sites and V4 sites, we computed for each inter-areal site pair the correlation between the average induced power per stimulus position, across the different stimulus positions. Figure 3B shows the gamma band (80-95 Hz) response of a single V1 site to 7 repetitions of visual stimulation in 4 different locations. The gamma band response varied systematically as a function of stimulus position in both V1 (Fig. 3C) and V4 (Fig. 3D), as expected from the results shown in Figures 1 and 2. It is common (Gawne and Richmond, 1993) to decompose the response to a stimulus into a signal component, considered to be the average response to multiple identical stimulus presentations (black dots in Fig. 3C for a V1 site and D for a V4 site), and a noise component, considered to be the difference between the average response and the actual response to a given stimulus presentation (variation of the grey dots in Fig. 3C and D around the black dots). In this framework, the correlation between two sites’ average stimulus-induced power across different stimuli is considered the signal correlation (sample site pair shown in Fig. 3E), and we will use this term in the following. The signal correlation is calculated per inter-areal site pair and per
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**Figure 3 Analysis pipeline for stimulation and passive fixation**

(A) Signal processing pipeline: Raw field potentials (left) were filtered in 185 linearly spaced bands between 2 Hz and 200 Hz (center). The power envelope was extracted from the analytic Hilbert representation. (right) Receptive field mapping. (B) Example power responses in the gamma band (80-95 Hz) for one V1 site to 7 repetitions of visual stimulation at 4 positions. (C) Average (in black) and individual trial (in grey) responses of the same V1 site to all 60 stimulus positions. Positions in (B) shown in the respective color. (D) Same as (C) but for an example V4 site. (E) The signal correlation for the two sites shown above. (F) The noise correlation for the same two sites. Signal (G) and Noise (H) correlations were computed between all pairs of V1-V4 sites for all frequencies of interest. Passive fixation. (I) One second of activity in the gamma band for the same V1-V4 sites shown above. (J) Spontaneous correlation was computed for all pairs of V1-V4 sites in each frequency of interest.

frequency band, across the 60 stimulus positions, between the respective average (across repetitions of a given stimulus position) absolute power values of the two sites (Fig. 3G). Therefore, the signal correlation captures the similarity of the two sites’ stimulus selectivity. Figure 4A shows the matrix of signal correlations for all possible V1-V4 site pairs for the gamma-frequency band (between 80 and 95 Hz) for monkey P. The repetitive structure in the matrix along both axes reflects the arrangement of electrodes on both areas in lanes, which have been unwrapped along the x- and y-axes. This matrix captures the spatial pattern of gamma-band co-activation in V1 and V4 that is due to common driving through stimuli at varying positions.

Figure 4B shows the distributions of signal correlation values across all V1-V4 site pairs as a function of the frequency for which the power was taken. While specific frequency bands contain most stimulus-related information, signal correlations occur across a broad spectrum of frequencies.
Figure 4 Signal and noise correlations

(A) Signal correlation matrix in the gamma band (80-95 Hz) for all V1-V4 site pairs. (B) Distribution of signal correlation values for all inter-areal site pairs as a function of frequency. (C) Noise correlation matrix in the same band as in (A). (D) Distribution of noise correlation values as a function of frequency. (E) Correlation of signal and noise correlation across frequencies. Frequency-frequency plane threshold P<0.05 corrected for multiple comparisons.
Noise Correlations

As mentioned above, the difference between the average response and the actual response to a given stimulus presentation has been considered as noise. While we consider that this so-called noise component is actually not noise, but might reflect e.g. top-down influences, we will use the signal versus noise terminology for consistency with previous literature. This noise, i.e. the deviations from the average response, has often been found to be correlated across recording sites, i.e. there is noise correlation. We computed noise correlations in order to assess the degree of power covariation between visual regions that was independent of the stimulus and therefore intrinsically generated (sample site pair, same as above, shown in Fig. 3F). The noise correlation is complementary to the signal correlation because, for each pair of recording sites, it captures the intrinsic covariation in power across repeated trials of identical stimulation as opposed to shared stimulus selectivity. Noise correlation was computed for all V1-V4 site pairs and for each frequency (Fig. 3H). Figure 4C shows the matrix of noise correlation values from all possible V1-V4 site pairs for the gamma-frequency band (between 81 and 96 Hz) for monkey P. This matrix captures the spatial pattern of gamma-band co-activation in V1 and V4 that occurs during stimulation, but reflects intrinsic trial-by-trial variation. Figure 4D shows the distributions of noise correlation values across all V1-V4 site pairs, and reveals similar characteristics as mentioned above for the signal correlations.

Similarity of noise correlations to signal correlations

In order to test whether the spatial pattern of noise correlations resembled the spatial pattern of signal correlations, we calculated the correlation between those two metrics, across inter-areal site pairs. As mentioned above, since both, the signal and the noise correlations were determined as a function of frequency, and we considered all possible frequency pairs, this resulted in a frequency-by-frequency matrix of correlation values. We found that noise correlations correlated
Stimulus induced networks are spontaneously reactivated significantly with signal correlations in specific frequency ranges (Fig. 4E). These frequency ranges correspond to those showing the greatest stimulus selectivity (Fig. 1D). Thus, inter-areal power covariations that are extrinsically driven by different visual stimuli are mirrored in power covariations that occur intrinsically in the small variations around the stimulus response, and this effect is most prominent in the frequency ranges in which retinotopic selectivity is expressed, particularly prominently in the gamma band.

The existence of frequency specific similarity in the spatial pattern of inter-areal covariation cannot be trivially explained by the pattern of signal correlations. It is possible that noise correlations could occur in an unspecific manner, either spectrally or spatially. For example, on a given trial, all visual channels could have high or low power in a narrow or broad range of frequencies, in which case the degree of noise correlation could be the same as observed, but it would not reflect the underlying retinotopic organization. This would be the case if noise correlations reflected unspecific, global, signal variance. However, our results suggest that noise correlations between visual areas obey the functional organization of the underlying cortex.

**Similarity of spontaneous correlations to signal correlations**

The signal and noise correlations investigated so far were derived from the same data, extracting signal-driven variance or endogenous variance and their respective correlation structure. We wondered whether the intrinsically generated covariance resembled the signal correlation also when it was taken from entirely independent data. To this end, we analyzed the fixation periods of separate recording sessions, mostly on different days than the recording sessions analyzed so far. During these fixation periods, the monkey sat quietly in the booth and fixated a central fixation point while awaiting a different task. We calculated correlations between spontaneous power fluctuations across trials, and we refer to them as spontaneous correlations (Fig. 3I and J). Figure 5A shows the spontaneous correlation across the
Figure 5 Spontaneous correlations

(A) Spontaneous correlation matrix in the gamma band (80-95 Hz) for all V1-V4 site pairs. (B) Distribution of spontaneous correlation values for all inter-areal site pairs as a function of frequency. Example topography for spontaneous correlation from the marked V4 site across all visual channels in the (C) theta (6-8 Hz) and (D) gamma (80-95 Hz) bands (same color bar as (A)). (E) Correlation of spontaneous and signal correlation across frequencies. Frequency-frequency plane threshold $P<0.05$ corrected for multiple comparisons. (F) Frequency resolved comparison of spontaneous correlation with signal correlation in the gamma band (80-95 Hz) used to define retinotopic maps. Significance threshold at $P<0.05$ corrected for multiple comparisons. (G) Power spectrum for the activity during fixation used to compute spontaneous correlation. Mean across visual sites shown in dark blue. Shaded region denotes standard deviation across sites.

The complete matrix of V1-V4 site pairs for the gamma-frequency band in monkey P. Figure 5B shows the distributions of spontaneous correlation values across all V1-V4 site pairs as a function of the frequency for which the power was taken. Differences in the two bands were also evident in the topographic distribution of
spontaneous correlation values. Figure 5C illustrates the spatial pattern for the spontaneous correlation from a seed site in V4 in the 6-8 Hz band. The portion of V1 with the highest spontaneous correlation corresponded to the portion with the same retinotopic selectivity as the V4 seed site (cf. Fig. 2). This retinotopic correspondence held also for spontaneous power fluctuations in the gamma band (80-95 Hz, Fig. 5D). Yet, while spontaneous correlation values for the theta band were almost exclusively positive, the values for the gamma band were both positive and negative, as expected from figure 5B.

We found spontaneous correlations to be correlated with signal correlations across V1-V4 site pairs, and this was most pronounced in specific frequency bands as shown in figure 5E for both monkeys combined. As the stimulus selectivity was most pronounced in the gamma-frequency band (Fig. 1D), we further illustrate this with a vertical cross-cut through the frequency-frequency plane, demonstrating how the gamma-band signal correlation is correlated to the spontaneous correlation across all frequencies (Fig. 5F). This spectrum with a clear peak in the gamma-frequency band is in stark contrast to the absolute power spectrum of the LFP during the fixation period (Fig. 5G), which shows a typical 1/f characteristic. Thus, although there was no appreciable gamma-band power, the spontaneous V1-V4 gamma power covariations reflected those that were imposed in the two areas by localized stimuli.

**Spontaneous correlations replay stimulus correlations**

The retinotopic structure of inter-areal spontaneous correlations can be demonstrated directly in topographical form, as shown previously in Fig. 5C and D. In order to assess the degree of this correspondence, we investigated whether the inter-areal pattern of spontaneous correlations allowed us to derive an ordered topography from stimulation-free data, similar to the retinotopic map derived from visual stimulation data.
Figure 6 Retinotopic maps computed from the pattern of spontaneous correlation

(A and C) Map of eccentricity during passive fixation; each recording site is colored based on the mean eccentricity tuning of the two maximally correlated V4 sites during passive fixation in the (A) theta (6-8 Hz) and (C) gamma (80-95 Hz) band. (B and D) Map of elevation during passive fixation as in A and C. The retinotopic maps computed from gamma power as in Figure 2 are shown inset for comparison. (E) Spatial correlation between the maps computed based on the pattern of spontaneous correlation and the stimulus derived retinotopic maps for eccentricity (blue) and elevation (green), as a function of frequency. Spatial correlation was constrained to the recording sites posterior to the lunate sulcus to avoid bias based on V4 seed location. Significance threshold based on $P < 0.05$ corrected for multiple comparisons.

To this end, we grouped sites in V4 into regions of interest (ROI), when they shared similar selectivity either for eccentricity or for elevation. We calculated spontaneous correlations between each V4 ROI and all V1 sites, separately for all frequencies. We hypothesized that a given ROI, with selectivity for either a particular eccentricity or elevation would show strongest spontaneous correlation with V1 sites sharing the same stimulus selectivity. We indeed found this to be the case. We colored each V1 site according to the eccentricity (Fig. 6A and C show...
the maps for power in the 6-8 Hz and 80-95 Hz bands, respectively) or elevation (Fig. 6B and D, as in A and C) of the V4 ROI to which it showed the strongest spontaneous correlation. To quantify the similarity between these topographies and the retinotopic maps from figure 2, we computed the spatial correlation between them. We limited this spatial correlation analysis to sites in V1, because our selection of ROIs in V4 was already based on stimulus preference and so similarities inside V4 were trivial, whereas the pattern seen in V1 was solely the result of topographic specificity of the spontaneous correlations. We performed this analysis for all frequencies and correspondingly obtained a spectrum of spatial correlations between spontaneous-correlation derived topographies and stimulation-derived retinotopic maps, separately for eccentricity and elevation (Figure 6E, combined spectrum for both monkeys). These spectra were highly similar to those computed in the previous correlation analyses across inter-areal site pairs, and further confirm that intrinsic power variations have spatial structure that recapitulates the functional topography of the underlying cortex.

**Similarity of spontaneous coherence and directed influences with signal correlation**

Given that both noise correlations and spontaneous correlations showed a similar spatial pattern as signal correlations, we asked whether this spatial pattern could also be found in the spontaneous inter-areal coherence, i.e. in a metric of phase synchronization during spontaneous activity recorded during pre-stimulus fixation periods. It is possible that the oscillatory power between areas could be correlated, but that the inter-areal signals would not exhibit a specific phase relationship, particularly given that the signals showed no noticeable departure from a 1/f spectrum during the fixation period. To our surprise, we found that inter-areal phase coherence between visual regions obeyed retinotopic organization in frequencies bands similar to those showing similarity in noise correlations and spontaneous correlations (Fig. 7A). Thus, even though no clear gamma peak was noticeable in the power spectrum, the correlation analysis revealed that there was
spontaneous gamma-band coherence between V1 and V4 that occurred selectively between regions of retinotopic correspondence.

Given that this was the case, we attempted to assess the directionality of the interaction by measuring the granger causal (GC) influences in both directions. We found that signal correlation determined the spatial structure of inter-areal GC influences both in the direction from V1 to V4 (Fig. 7B) and in the direction from V4 to V1 (Fig. 7C).

**Figure 7 Spontaneous coherence and directed interaction**

Correlation of (A) spontaneous coherence, (B) spontaneous feed-forward GC, and (C) spontaneous feed-back GC with signal correlation for all frequencies. Frequency-frequency plane threshold set at P<0.05 corrected for multiple comparisons.
Discussion

In summary, we found intrinsic networks subserved by local and inter-areal rhythmic synchronization, whose spatial structure correlates significantly with stimulus driven patterns of activation. Concretely, we found that the spatial structure of signal correlations was recapitulated in 1.) the trial-to-trial variability of stimulus induced power (noise correlations), 2.) the correlation of spontaneous power fluctuations during fixation (spontaneous correlations) and 3.) the spontaneous coherence and GC influences during fixation. We take the specificity of these intrinsically generated patterns of synchronization as a robust indicator of highly organized endogenous rhythms. Notably, the same spatial pattern of intrinsic covariation was present both during stimulation, i.e. in the noise correlation, and in the absence of stimulation, i.e. in the spontaneous correlation. This suggests that in addition to the role intrinsic networks may play during passive states, the respective intrinsic networks may influence the pattern of activity during perception and action, and thereby, impact behavior. The temporal structure of the observed intrinsic networks showed characteristic frequency bands, corresponding to the rhythms most specifically modulated by visual stimulation, namely the theta, alpha/beta and gamma rhythms.

Despite similarity in the topography of inter-areal co-variation, the state of stimulation and spontaneous activity vary in their spectral properties and in the distribution of inter-areal correlation coefficients. The distribution of signal correlation values is considerably broader than the distribution of either noise correlations or spontaneous correlations, suggesting that stimulation has a different overall effect on inter-areal power covariation. Specifically, stimulation drives brain dynamics into more strongly correlated patterns of activity. This is not surprising, because stimulation and task engagement often induce pronounced oscillatory peaks that deviate substantially from a 1/f spectrum, suggesting the emergence of resonant modes of activation. Additionally, the distribution of
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spontaneous correlation coefficients is distinct for the two frequency ranges most correlated with retinotopy. Inter-areal correlations below 20 Hz are skewed to positive values with very few inter-areal site pairs showing negative correlations. In contrast, correlations in the gamma band are largely symmetric around zero with positive and negative correlations of equal magnitude. The topographic distribution of gamma band correlations demonstrated that positive correlations were limited to points of high retinotopic correspondence flanked by zones of negative correlation, reminiscent of the focal activations seen during stimulation.

One potential concern is that the signal correlation is correlated to intrinsic networks in a spectrally specific way only because the signal correlation is spectrally specific to begin with. Yet, a close examination of the data does not confirm this. For example, figure 4E shows peaks in the congruence between signal and noise correlation for signal correlations in the frequency bands of roughly 10-20 Hz and of 100-120 Hz. Figure 4B shows the distributions of signal correlations and reveals that signal correlations in the 10-20 Hz band are among the weakest, and signal correlations in the 100-120 Hz band are in the middle of a broad band of all frequencies above 40 Hz, for which the distribution of signal correlations gets progressively narrower. Thus, the frequency bands, in which the signal correlation is particularly congruent to intrinsic correlation patterns, are not particularly conspicuous in the spectral distribution of signal correlation values. Very similar arguments can be made for the congruence between signal correlation and spontaneous correlation, coherence or GC influences. The frequency bands of highest congruence do not correspond to frequency bands with particularly strong signal correlation. The same reasoning holds for the distributions of noise correlations and spontaneous correlations, which are shown in figures 4D and 5B, and also lack conspicuous structure in the ranges in which they show spatial congruency to the signal correlation. Furthermore, the statistical tests for establishing congruency between signal correlations and intrinsic networks rested on the inter-areal spatial correlation pattern. By randomizing the spatial relation
between inter-areal sites, we generated surrogate distributions of congruency metrics. Importantly, this randomization left the underlying distribution of the signal correlation unchanged.

The frequency-specific interactions during spontaneous activity occurred in the absence of clear peaks in the LFP power spectrum. The retinotopically specific band-limited inter-areal correlation patterns were embedded within the 1/f frequency spectrum. This suggests that: 1) the involved networks have the tendency to structure their activity in rhythmic modes even when they are not strongly activated, and 2) the conventional power spectrum is an insensitive tool to detect rhythmicity. This latter point is supported by other recent findings. For example, the relative phases between simultaneously recorded LFPs are highly structured in the gamma-frequency band, both during visual stimulation and in the pre-stimulus baseline, when there is no peak in gamma power (Maris et al., 2013). Furthermore, the spike times of putative inhibitory interneurons are selectively locked to the phase of activity in the gamma-band during the pre-stimulus baseline, again in the absence of any appreciable gamma power peak (Vinck et al., 2013).

Interestingly, the two bands of high correspondence between intrinsic covariation and stimulus driven covariation correspond well with previous findings. Activity in the gamma band is more selective for stimulus properties than oscillations in other bands (Frien and Eckhorn, 2000; Frien et al., 2000). Furthermore, when natural movies were shown to anesthetized monkeys, most information about the movies was contained in the power time courses of LFP components between 1-8 Hz and between 60-100 Hz (Belitski et al., 2008). These bands correspond roughly to the ones in which we find consistency between retinotopy and intrinsic covariation, suggesting that those frequency bands may generally be involved in the representation of visual features within areas and their communication between areas. In fact, within areas, stimulus representation is more accurate for the spikes that are optimally aligned to the gamma rhythm as opposed to spikes that occur at
random phases of the gamma cycle (Womelsdorf et al., 2012). Similarly, gamma-band synchronization might facilitate the inter-areal communication of representations: 1) Inter-areal gamma phase locking leads to enhanced inter-areal interactions (Womelsdorf et al., 2007); 2) The selective inter-areal communication of attended stimuli appears to be subserved by a corresponding selective inter-areal gamma-band synchronization (Bosman et al., 2012; Grothe et al., 2012). These converging results indicate that the intrinsic patterns we report here may play a role in the brain’s endogenous sampling and routing of visual information.

Intrinsic networks defined by structured endogenous activity have been observed at many spatial and temporal scales. Luczak et al investigated the similarity of evoked and spontaneous patterns of population activity within auditory or somatosensory cortex of both anesthetized and awake rats (Luczak et al., 2009). They found that spike timing patterns were conserved across states of stimulation and spontaneous activity, and that the spatial patterns observed during stimulation constitute a subset of all spatial patterns visited by the network during spontaneous activity. Further, they demonstrated the similarity of pair-wise correlations between individual cell’s firing rates during evoked and spontaneous activity as well as in noise correlations across repeated stimuli. Similar observations have been made for the entire map of activity across cat primary visual cortex (Arieli et al., 1995; Kenet et al., 2003; Tsodyks et al., 1999). The distribution of activity in the absence of visual stimulation resembled maps evoked by specific visual stimuli more frequently than expected by chance. Between monkey visual areas, retinotopically corresponding regions show spontaneous co-variations in the fMRI BOLD signal (Vincent et al., 2007). Similar inter-areal intrinsic networks of BOLD covariation have been found abundantly in human subjects and have been related to different functional brain networks. The finding of intrinsic, whole brain networks with fMRI has expanded the scope of patterned spontaneous activity from the level of sensory maps to general inter-regional interactions. Further, the ability to investigate intrinsic networks in humans has led to studies that directly demonstrate the functional role
of these networks: Human intrinsic networks have been shown to be shaped by experience and to affect neuronal and behavioral responses. Related effects of experience have also been shown for local circuits in animals (Berkes et al., 2011). These results expand the implications of intrinsic networks and illustrate their relevance for understanding both healthy and diseased brain function.

Electrophysiological correlates of intrinsic networks observed with BOLD have been sought in order to begin to bridge the gap between detailed accounts of endogenous dynamics within cortical areas to the patterns of activity observed with fMRI across the whole brain. In visual cortex, BOLD signal fluctuations show a positive correlation primarily with LFP power in the gamma frequency range (Logothetis et al., 2001; Mukamel et al., 2005; Niessing et al., 2005; Scheeringa et al., 2011; Schölvinck et al., 2010). LFP power in the gamma frequency range can reflect both, rhythmic neuronal synchronization at the gamma rhythm (Eckhorn et al., 1988; Fries et al., 2001; Gray et al., 1989) and the broadband spectral signature of basic biophysical processes like spikes and/or postsynaptic potentials (Einevoll et al., 2013; Miller et al., 2009a), a distinction that is increasingly made explicit (Ray and Maunsell, 2011). The BOLD signal appears related to both, the strength of broadband gamma-range power (Mukamel et al., 2005) and the strength of the band-limited gamma rhythm (Scheeringa et al., 2011; Schölvinck et al., 2010). Correspondingly, LFP power in the gamma-frequency range is correlated between corresponding regions of the two hemispheres (Nir et al., 2008), fluctuates spontaneously according to tonotopic maps in auditory cortex (Fukushima et al., 2012), and reflects regional boundaries in somatomotor cortex, in close correspondence to BOLD signal fluctuations (He et al., 2008). When activity in BOLD-signal defined intrinsic networks is directly correlated to EEG band-limited power, distinct networks relate to various frequency bands (Mantini et al., 2007). Conversely, power fluctuations in different frequency bands of the EEG signal are related to distinct spatial patterns of brain activation shown with simultaneous fMRI (Laufs et al., 2003a; 2003b). Power co-
fluctuations were also demonstrated directly with source-projected MEG, revealing distinct spatial networks for power in different frequency bands (de Pasquale et al., 2010; Hawellek et al., 2013; Hipp et al., 2012). Related findings have been reported in animal experiments recording from cat brain regions homologous with two well-described human fMRI intrinsic networks, the task-on and task-off networks (Popa et al., 2009). Between those two networks, total LFP power during spontaneous activity showed an overall difference, and periods in which band-limited power time courses were anti-correlated. In the task-off network, reduced power was surprisingly correlated to enhanced neuronal firing rates, illustrating the importance of combined multi-level investigations.

We have shown that the intrinsic networks are subserved by specific rhythms. This is evidenced by clear spectral peaks that exclude broadband power changes as underlying causes and rather correspond to classical frequency bands. In particular, the gamma-frequency band has been linked to inter-areal communication. Communication is likely supported by gamma-band coherence. Surprisingly, gamma-band coherence, normally considered to exist exclusively during stimulus driving, was found in spontaneous activity to reflect the spatial pattern of signal correlations. This finding provides a potential link from the putative mechanism of communication-through-coherence to the organization of endogenous activity in intrinsic networks. The link could be shown due to ECoG recordings that combined the high temporal and high spatial resolution with coverage of two visual areas. The high temporal resolution was necessary to reveal the spectral specificities, the high spatial resolution allowed to reveal the topographic specificity and the coverage was required to allow the inter-areal correlation between spatial patterns of spontaneous and stimulus driven activity. It will be an intriguing possibility for future research to capitalize on these features and investigate whether e.g. the influence of intrinsic networks on stimulus responses or behavior is spectrally specific, or whether experience shapes intrinsic networks particularly or differentially in different frequency bands. Furthermore, it will be crucial to extend
Stimulus induced networks are spontaneously reactivated coverage to both hemispheres to allow investigation of bilateral symmetric patterns, which have been the hallmark of many fMRI demonstrations of intrinsic networks, and finally to add single cell recordings that will allow the integration of population dynamics into large-scale patterns.
Experimental procedures

Neurophysiological Recording Techniques and Signal Preprocessing

All procedures were approved by the ethics committee of the Radboud University, Nijmegen, NL. Neuronal recordings were made from two left hemispheres in two monkeys through a micro-machined 252-channel electrocorticogram-electrode array implanted subdurally (Bosman et al., 2012; Brunet et al., 2013; Rubehn et al., 2009). Briefly, a 6.5 x 3.4 cm craniotomy over the left hemisphere in each monkey was performed under aseptic conditions with isoflurane/fentanyl anesthesia. The dura was opened and the ECoG was placed directly onto the brain under visual control. Several high-resolution photos were taken before and after placement of the ECoG for later co-registration of ECoG signals with brain regions. After ECoG implantation, both the bone and the dural flap were placed back and secured in place. ECoG electrodes covered numerous brain areas, including parts of areas V1, V2, V4 and TEO. As mentioned in the main text, retinotopic mapping revealed two contiguous maps of space, one behind the lunate sulcus for areas V1/V2, and another one between the lunate and the superior temporal sulcus for areas V4/TEO. For simplicity, we will refer to ECoG sites in the V1/V2 map as V1, and to sites in the V4/TEO map as V4. After a recovery period of approximately 3 weeks, we started neuronal recordings. Signals obtained from the electrode grid were amplified 20 times by eight Plexon headstage amplifiers, then low-pass filtered at 8 kHz and digitized at 32 kHz by a Neuralynx Digital Lynx system. LFP signals were obtained by low-pass filtering at 200 Hz and down-sampling to 1 kHz. Power line artifacts were removed by digital notch filtering. The actual spectral data analysis included spectral smoothing that rendered the original notch invisible.

Visual Stimulation

Stimuli and behavior were controlled by the software CORTEX. Stimuli were presented on a cathode ray tube monitor at 120 Hz non-interlaced. When the
monkey touched a bar, a gray fixation point appeared at the center of the screen. When the monkey brought its gaze into a fixation window around the fixation point (0.85 radius in monkey K; 1 radius in monkey P), a pre-stimulus baseline of 0.8 s started. If the monkey’s gaze left the fixation window at any time, the trial was terminated.

Several sessions (either separate or after attention-task sessions) were devoted to the mapping of receptive fields, using 60 patches of drifting grating, as illustrated in Figure 1B. Gratings were circular black and white sine waves, with a spatial frequency of 3 cycles/degree and a speed of 0.4 degrees/s. Stimulus diameter was scaled between 1.2 and 1.86 degrees to account for cortical magnification factor. Receptive field positions were stable across recording sessions (Figure S1D).

**Data Analysis General**

All analyses were performed in MATLAB (MathWorks) using FieldTrip (Oostenveld et al., 2011) (http://fieldtrip.fcdonders.nl). We calculated local bipolar derivatives, i.e., differences (sample-by-sample in the time domain) between LFPs from immediately neighboring electrodes. We refer to the bipolar derivatives as “sites.” Bipolar derivation removes the common recording reference, which is important when analyzing power correlations and/or coherence. Subsequently, per site and individual epoch, the mean was subtracted, and then, per site and session, the signal was normalized by its standard deviation. These normalized signals were pooled across sessions with identical stimulus and task, unless indicated otherwise. In order to select solely visually selective recording sites, an ANOVA was computed across frequencies (Fig. 1X).

Time series of band-limited power were computed for each trial by filtering the data with a Butterworth filter of order 2. Data were filtered into 185 frequency bands with center frequencies between 3 Hz and 193 Hz. We used different pass bands in computing the analysis to confirm the robustness of the results, however, in the results presented here, we chose three different pass bands for three different
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groups of center frequencies (cf) : 3 Hz for low (cf = 3-11 Hz), 6 Hz for medium (cf = 13-53 Hz) and 15 Hz for high (cf = 59-193 Hz). After filtering, we calculated the Hilbert transform and took the absolute value of the analytic signal. All fixation trials were then concatenated into a pseudo-continuous time series for calculation of the inter-areal correlation coefficient.

**Retinotopic maps**

In order to construct retinotopic maps for eccentricity and elevation, the mean power was computed across trials for each stimulus location. First, each trial was cut to the time between 0.3 s after stimulus onset to avoid the early transient activity, and 0.9s. Frequency analysis was performed by computing the fast Fourier transform of the entire stimulation epoch. Each site was assigned an eccentricity and an elevation based on the position of the stimulus giving the maximal response in the gamma band. The overall pattern of retinotopic organization was robust to different manners of computing the preferred eccentricity and elevation and so the maximal stimulus was chosen because of its simplicity.

**Signal and Noise Correlations**

In order to compare the spatial patterns of stimulus-driven correlations and stimulus-independent correlations, frequency-resolved signal and noise correlations were computed. Signal correlation was computed by first calculating the mean spectral responses per stimulus position, across trials, and then determining the Spearman rank correlation across the mean stimulus responses, between site pairs. Noise correlation was computed by first calculating the trial-by-trial deviation from the mean responses, and then determining the Spearman rank correlation across trials, between site pairs. Across all site pairs, we then calculated the Spearman rank correlation between signal correlations and noise correlations. The Spearman rank correlation was used to avoid assumptions about underlying distributions. Yet, results were essentially the same when Pearson correlation coefficients were used.
Spontaneous correlation

For the analysis of spontaneous activity, we used the period of passive fixation during an attention task, which contained stimuli at two fixed positions, one of them contralateral to the recorded left hemisphere. Recordings during the attention task occurred in different sessions and often on different days than retinotopic mapping. We defined fixation as the time period from 0.3 s after fixation point onset and after the monkey acquired fixation until 0.1 s before the first stimulus appeared on the screen. As with signal and noise correlations, spontaneous correlations during fixation were computed for each frequency of interest and for each channel pair.

Spontaneous Retinotopy

Retinotopic maps were computed during passive fixation by first determining the eccentricity and elevation preference of recording sites in V4. Once this was determined, V4 sites were grouped into regions of interest (ROIs) with similar eccentricity (6 ROIs) or elevation preference (10 ROIs). For each ROI, average time series of band-limited power were computed and correlated across V1 sites. This resulted in 6 maps of correlation for eccentricity and 10 maps of correlation for elevation. In order to compute the topography in V1 during the period of spontaneous activity, each recording site in V1 was assigned the value of the retinotopic preference of the V4 ROI it was most strongly correlated with. This resulted in maps of intrinsically generated retinotopy for each frequency of interest. In order to quantify the extent to which spontaneous retinotopy corresponded with stimulus driven retinotopy in area V1, we computed the spatial correlation of those maps computed during spontaneous activity with stimulus derived retinotopic maps for eccentricity and elevation.

Spontaneous Coherence and Directed Influence

For the analysis of spontaneous coherence and directed influence, we used the
same passive fixation periods as during spontaneous correlation. We computed the coherence and Granger causality (GC) using two separate analysis approaches, one for frequencies less than or equal to 30 Hz and the other for frequencies between 31 Hz and 190 Hz. We did this because high frequency components characteristically have less power and broader spectral bands than low frequency components. Therefore, it is advantageous to apply different frequency domain smoothing to these two distinct frequency bands. For both analysis we cut the fixation data into 500 ms segments and computed the cross-spectral density matrix (CSD) using the fast Fourier transform. In the low frequency band we used a Hanning taper in order to insure our time domain signal was symmetric. For the high frequency band we used multi-tapering with 11 tapers from the discrete prolate spheroid sequence (Mitra and Pesaran, 1999). This gave us a spectral smoothing of +/- 12 Hz. After calculation of the CSD, we either calculated coherence or GC. GC was calculated using non-parametric spectral factorization (Dhamala et al., 2008). The pattern of inter-areal coherence and GC were then compared with the pattern of stimulus selectivity for each combination of frequencies.

Statistical Testing

Wherever possible, data from both monkeys were combined in order to minimize the false negative rate. This amounts to a fixed-effect analysis for our sample of two animals. The false positive rate was controlled by our statistical testing procedure as follows. In order to compute significance thresholds for both, frequency planes and line spectra, surrogate data were used which broke the pattern of inter-areal intrinsic interaction but kept all other features (e.g. the distribution and inter-areal pattern of signal correlations and the distribution of intrinsic correlation and coherence) unchanged. This was done by creating 1000 random pairings of inter-areal site pairs and then computing the correlation across site pairs, between the signal correlations and 1) the noise correlation, 2) the spontaneous correlation, 3) the coherence, and 4) the GC influences. Each
realization of the randomized structure resulted in a distribution of correlation coefficients. In all cases we set our threshold for significance across all tests at P<0.05. In the case of noise correlation, since this came from the same dataset as the index for stimulus selectivity, we used an omnibus based multiple comparison correction (Maris et al., 2007; Nichols and Holmes, 2002). In this case, we retained the maximum surrogate correlation coefficient (absolute value) across all combinations of frequencies and corrected our empirical distributions by thresholding at the P<0.05 level from this distribution of surrogate maxima. In the other cases, since the analysis were from independent data, we used the less conservative False Discovery Rate correction (Genovese et al., 2002). To compute FDR corrections, we retained all correlation values across all randomizations in a single distribution and took our significance threshold from P<0.05 level of this complete distribution.
Two frequency bands contain the most stimulus-related information in visual cortex.
Abstract

Sensory cortices represent the external world through the collective activity of diversely tuned cells. How the activity of single cells is coordinated within local populations, across whole cortical areas, and between different levels of a sensory hierarchy in order to reliably represent the external world is largely unknown. Cortical oscillations may play an important role in coordinating the activity of local and distributed neuronal groups. By combining recordings from large-scale electrocorticography (ECoG) grids with acute multi-electrode recordings, we investigated how parameters of visual stimulation could be extracted from the local field potential (LFP) and how this compared with the information available from small groups of cells. We found that information related to visual stimulation was spatially limited to the recording sites in visual areas covered by the ECoG array. Further, by decoding stimulus parameters in a frequency- and time-resolved fashion, we were able to investigate the frequency bands with the greatest stimulus related information as well as the temporal evolution of this activity. We found that visual features were extractable from the LFP in two distinct bands; one centered between 10-20 Hz and the other between 50-160 Hz. These same bands were found for different visual features both from ECoG recordings and acutely placed tungsten electrodes. Most surprisingly, we were able to decode the identity of natural scenes in these same frequency ranges, while the monkey freely viewed a static display.
Introduction

Two perspectives have dominated the theory of sensory processing, especially with respect to vision. The first, serial feature extraction, posits that the features of externally arising stimuli are represented by collections of individual cells in specialized cortical domains. In this view, individual cells in different areas extract diverse families of stimulus features and sensory processing occurs in a serial fashion, whereby the features extracted from early sensory areas are conveyed to higher order areas, where they are elaborated and combined through local processing (DiCarlo et al., 2012). This is most commonly illustrated by the example of multiple low level cells that converge onto individual higher order cells. This convergence of features from a lower order area can convey both enhanced selectivity, through the conjunction of multiple low level features; or, invariance, through the repetition of a given feature across multiple cells which span some portion of a different feature dimension.

While this approach has shaped our understanding of sensory processing to a great extent, its general applicability to sensory processing has been recently questioned by some of it’s originators (Carandini et al., 2005). At issue is the failure of the approach to explain the response properties of sensory neurons in higher order cortical areas when multiple stimuli are simultaneously present, the response of cells to naturalistic visual stimulation, as well as the lack of horizontal and feedback interactions in such a model, despite the fact that such connections are prevalent in the brain. The necessity of lateral and feedback connections in computational models able to perform object recognition or reproduce the selectivity of early sensory areas, has led to a second major account of sensory processing, sparse coding (Olshausen and Field, 2005). In the case of primary visual cortex, the vast majority of models that can account for the diversity of individual cell tuning do so by performing optimization on a constraint of the sensory space, which the brain is presumed to realize (Simoncelli and Olshausen,
Two frequency bands contain the most information

2001). The realization of efficient or sparse encoding models requires an explicit constraint to be placed on the population of sensory cells. Under such a constraint, models can account for the localized, oriented gabor-like receptive fields of v1 cells when applied to a set of natural images (Olshausen and Field, 1996; 2004). However, the physiological realization of a sparsity constraint that regulates activity across a population of cells in order to efficiently encode the space of naturalistic sensory images has so far been left unspecified. Models that exploit such a constraint have been very successful at reproducing empirical features of cortical receptive fields and have provided an intuitive notion of how sensory processing may optimally represent naturalistic images. However, despite the success of these models and the suggestion that such a sparse code may provide an elegant solution to cortical representation within the framework of serial feature extraction, the two approaches afford considerably different views on how sensory representation may occur.

Whereas serial feature extraction typically operates in a regime in which the effects of lateral and feedback connectivity are negligible, the invisible hand of sparse-coding suggests that such recurrent connectivity may be critical in the formation and function of neurons under naturalistic paradigms. Sparse coding of stimuli and the associated receptive fields may be accounted for by local inhibitory circuits that balance the activity of excitatory cells and constrain population activity based on the spatial and temporal input characteristics (Haider et al., 2010). As such, single cells, rather than reflecting disjointed feature extractors, are rather integral members of the local population, which reflects the coordinated orchestration of temporal and spatial context.

The failure of feed-forward models to account for the diversity of empirically described receptive fields, the behavior of cells under naturalistic stimulation protocols, the modulation of responses by temporal, spatial or cognitive context, and the need for an external constraint on joint activity in order to computationally
Two frequency bands contain the most information explain receptive field structure in early sensory cortices, all call for a reconsideration of this approach to sensory processing (Gilbert and Li, 2013; Gilbert and Sigman, 2007). An alternative approach must combine the successes of the existing framework with the lateral and feedback connections critical to models of sensory processing in order to synthesize a coherent perspective on sensory representation in cortex. One essential element is the integration of single unit activity into the local ensemble, with a particular emphasis on how inhibition constrains population activity. The critical role of inhibition and recurrent connectivity in realizing sparse coding models suggest this as a particularly fruitful point of initiation. Further, the association of both inhibition and recurrent activity with oscillatory activity suggest that specific oscillatory patterns may structure and sparsify local activity patterns in a dynamic manner conducive to natural vision. Local circuits have been demonstrated to oscillate in a highly specific manner that correlates with high-level features of sensory stimuli. Such oscillatory activity may therefore serve as a flexible substrate by which to parse transient ensembles into coherent representations. Further, the hypothesized role of oscillatory activity in dynamically regulating effective connectivity suggests that oscillatory packaging of local ensemble activity may provide a parsimonious mechanism for the distributed representation and communication of information from the sensorium.

In order to begin to formulate such a theory, we investigated the extent to which local oscillatory activity contains information related to specific features of sensory stimulation. Specifically, we assess the degree to which stimulus information is contained in local oscillatory activity on a single-trial basis. We further compare this to the information contained in local population activity in order to begin to understand what role rhythmic activity may play in the coordination of sensory processing in local and distributed cortical circuits.
Two frequency bands contain the most information

Results

We investigated the extent to which characteristics of visual stimulation were recoverable in specific frequency bands of the local field potential. In order to evaluate the generality of our findings, we combined recordings from large-scale electrocorticography (ECoG) grids covering large portions of one hemisphere in 3 awake, behaving monkeys with acute multi-electrode recordings from primary visual cortex of 3 additional behaving monkeys. Across a variety of stimulus features, viewing contexts, recording techniques, and 6 monkeys, we found two frequency bands that reliably had the most stimulus-related information. These two bands, centered between 10-20 Hz and 50-160 Hz exhibited distinct temporal dynamics that were reproducible across recording techniques, decoded feature and stimuli. The 10-20 Hz band exhibited fast, transient performance that was largely attenuated during the sustained period of stimulation. In contrast, the 50-160 Hz band showed both a fast, transient performance, as well as sustained performance through the duration of the stimulus.

Retinotopic selectivity of ECoG signals

To assess retinotopic selectivity across all sites of the ECoG grid, monkeys kept fixation for several seconds while visual stimuli were presented randomly interleaved at 60 different positions in the lower right visual quadrant (Fig 1B), corresponding to the portion of visual space covered by our grid. Stimulus-position dependent changes in spectral power (in any frequency band) were established through an Analysis of Variance (ANOVA). The resulting $p$-values are shown in figure 1C/D (for monkey P/K) and reveal that selectivity for stimulus position was mainly found in areas V1/V2 and areas V4/TEO. We selected those sites for further analyses (V1/V2: 68 sites in monkey P, 32 in monkey K; V4/TEO: 17 sites in both monkeys).
Figure 1 High density ECoG layout, receptive field mapping paradigm and spatial specificity of stimulus position information from local field potentials.

(A) Rendering of the brain of monkey P with the ECoG grid overlaid. Lines indicate the covered area with the major sulci. Dots indicate the 218 bipolar electrode derivations. Sites considered as lying in areas V1 and V2 are highlighted in green and those in areas V4 and TEO are highlighted in purple. (B) Receptive fields were mapped with scrolling gratings at 60 locations in the lower right quadrant, corresponding to the coverage of the ECoG array. (C) Selectivity of all ECoG sites for stimulus position based on stimulus induced power in all frequency bands in monkey P. (D) Same as C, but for monkey K.

Frequency specificity of positional information

After confirming that power in the local field potential could differentiate the position of visual stimuli, we investigated whether this effect was broad-brand, occurring equally across many frequencies, or occurred in specific, band-limited rhythms. We therefore limited our analysis to visually selective recordings sites and investigated the spectral structure of positional information. For these sites, figure 2 shows how well stimulus-position was distinguished based on stimulus-
Two frequency bands contain the most information induced power as a function of frequency (A, monkey P; B, monkey K). This demonstrates that retinotopic selectivity was present predominantly in a low frequency band (1-20 Hz) and a gamma-frequency band (60-100 Hz). These two bands also contained the largest stimulus-induced power. While low frequency activity was present both with and without stimulation, was more transient and could increase or decrease in power, gamma-band activity was primarily stimulus induced and sustained throughout stimulus presentation. This confirmed that information that is selective for stimulus position was both spatially and spectrally specific.

![Figure 2 Frequency specificity of stimulus position information from local field potentials.](image)

(A) Selectivity of position-tuned sites as a function of frequency for monkey P. (B) Same as in A, for monkey K.

**Time frequency dynamics of positional information and classification error**

Due to the fact that two frequency bands both contained a high amount of stimulus-related information, we investigated the temporal evolution of band-limited stimulus information. To do this, we first computed single trial estimates of spectral power in a time-resolved manner. We then applied naïve-Bayes classification to our single-trial power estimates in order to produce a read-out of stimulus position
Two frequency bands contain the most information given the power at a specific point in time and for a specific frequency. This resulted in training a single classifier for each point in the time-frequency plane, which could estimate stimulus position based on the spatial pattern of local field potential power at a given frequency. We used an iterated cross-validation approach, which allowed us to control the generalization and robustness of our classifier. In the results presented here, we trained our classifier on 75% of the available data and tested the classification accuracy on the remaining 25% of the data. Our results were highly reproducible across a broad range of training set sizes. Further, for each time-frequency point, we repeated training and classification 100 times on randomly selected trial sets in order to insure our classification was robust to variations in samples from the training and test sets. The resulting time-frequency classification plot is shown in figure 3A.

Our classifier was able to calculate the likelihood associated with each of 60 stimulus locations. Based on the distribution of likelihoods, we chose the maximum value and based our classification accuracy on this. For a given pattern of power at a specific frequency and point in time this resulted in a single selection of the most likely stimulus position. Chance performance for such a calculation is 1/60 (1.67%), however, each of the two bands which showed the highest stimulus related-information based on our ANOVA, was able to estimate stimulus position with an accuracy more than 20 times greater than chance. Further, the two bands showed distinct temporal patterns. While both bands showed fast transient accuracy, which allowed determination of stimulus position within 0.1s, the low frequency band contained stimulus specific information for only a brief period after stimulatus onset. By contrast, the gamma-frequency band continued to show high performance throughout the stimulation period. Additionally, the gamma band showed two distinct spectral signatures during the transient and sustained periods. The transient response was more broadband and temporally narrow, while the sustained response was more band-limited, but maintained stimulus information in a continuous manner.
Two frequency bands contain the most information.

Figure 3 Time frequency resolved stimulus decoding and errors.
(A) Time frequency plot showing position decoding accuracy for a classifier trained separately on each time-frequency combination. Peak decoding performance is reached by 100 ms. (B) Example decoding performance, showing that misclassifications are spatially close and often overlapping with the veridical stimulus. Red dots show the stimulus-position likelihoods estimated based on a stimulus shown at the position marked by the white asterisks. (C) The mean likelihood-weighted distance for all trials for the classifiers trained as in A. This is a measure of the average distance between the actual stimulus position on each trial and the classifier read-out, weighted by the classifier confidence on that trial. (D) The mean distance of all misclassified (error) trials. This is a measure of the average distance between the actual stimulus position on each trial and the classifier read-out restricted to trials with a misclassification.
Two frequency bands contain the most information

Given that the local field potential contained stimulus information in such a specific manner, we next investigated the size of errors made during misclassifications. This was important because a well-behaved classifier should make minimal errors in classification if the information contained in the feature set is well-ordered based on the characteristic of interest. The classification performance for an example stimulus can be seen in 3B. The veridical stimulus was presented in the location marked by the white asterisks. The circular outlines represent the size of the different stimuli and the red circles highlight the relative proportion the classifier identified the location. The fact that misclassifications occur in a clustered fashion around the veridical stimulus location and the fact that they mostly occur from stimuli that overlapped with the veridical stimulus suggest that the position information available in the LFP is highly structured. In order to assess this scatter across all stimulus positions, we calculated the mean distance between the veridical stimulus position and the position estimated by the classifier. We first computed a measure of the mean error distance weighted by the classification confidence on each trial. For each trial, the classifier provided an estimate of the stimulus position, as well as a value between 0 and 1 indicating the confidence that the stimulus was in a specific position on that trial. We therefore computed the average of this likelihood-weighted distance across all trials in a time and frequency dependent manner. The results of this analysis are in figure 3C and show that mean likelihood-weighted distances were less than 1 degree of visual angle in the two frequency bands with highest accuracy. To further assess the performance of our classifier, we performed a similar analysis, limited to trials in which the classifier had generated a misclassification. Like above, we calculated the distance between the position for all misclassified trials and the veridical stimulus position across all error trials in a time- and frequency-resolved fashion. The time-frequency plot of mean distance restricted to error trials in shown in figure 3D. In the frequency bands of interest, error trials generally resulted in a misclassification of less than 1.5 visual degrees. Importantly, as shown in figure
Two frequency bands contain the most information

1B, our stimulus set contained stimuli that spatially intersected each other and this may have limited the resolution of our classification.

**Spatial pattern of positional decoding weights**

To further understand the performance of our classifier and how localized stimulus-specific responses were, we investigated the spatial pattern of the read-out weights across area V1. Because the read-out weights of our classifier correspond to the degree to which LFP power from a particular V1 location is able to provide useful information about stimulus position, we reasoned that our classifier could be tapping into the retinotopic organization of V1 in estimating stimulus position. Therefore, in order to compute the retinotopy of the ECoG recordings from V1, V2, V4 and TEO, we grouped the 60 stimulus locations according to eccentricity or elevation. Both eccentricity (Fig. 4A) and elevation (Fig. 4C) were represented in orderly retinotopic maps, that corresponded well with previously determined topographies from repeated recordings with penetrating electrodes (Gattass et al., 2005) or from fMRI (Brewer et al., 2002). Two contiguous maps of space were visible, one behind the lunate sulcus for areas V1/V2, and another one between the lunate and the superior temporal sulcus for areas V4/TEO. For simplicity, we will refer to ECoG sites in the V1/V2 map as V1, and to sites in the V4/TEO map as V4.

Once we had confirmed the presence of an ordered retinotopic map in V1 based on the induced gamma power, we checked to see if our classifier was explicitly taking advantage of this organization. For each V1 site, we displayed the eccentricity or elevation of the maximally weighted read-out for our decoder. These topographic maps are shown in figure 4B and C. The read-out weights from our stimulus-position classifier in both the gamma and low frequency range were highly ordered and both the mean weight, as well as the decoder uncertainty (not shown; uncertainty, measured by variance, is correlated with the mean weight.) reflected
Two frequency bands contain the most information

Figure 4 Retinotopic maps based on gamma band (80-95 Hz) activity in monkey P.

(A) Map of eccentricity; each recording site is colored to indicate the mean eccentricity of the 5 stimuli giving the largest gamma band response. (B) Distribution of decoder weights in is able to reproduce the retinotopic map. (C) Map of elevation; each recording site is colored to indicate the mean elevation as estimated above. Inset shows how the 60 stimulus locations are represented across eccentricity and elevation. (D) Same as in B, but for elevation.

the spatial organization of position tuning in V1. While such an ordering may seem trivial, the fact that decoding weights reflected areal organization speak to both the local nature of the recorded signals, as well as the interpretability of our decoding mechanism. While machine-learning algorithms can often lead to strange and uninterpretable results, our classifier seems to correspond to the functional organization of the underlying cortical areas.
Subsampling performance and the accuracy of single sites

The fact that our decoding of stimulus position was so accurate, we sought to understand the scaling of performance with the number of recorded sites. To this end, we investigated the increase in classification accuracy by including different numbers of sites in the decoder. The results of our recording site subsampling are displayed in figure 5A for area V1 and in figure 4B for area V4. Performance in V1 was significantly higher than that in V4, even when considering a V1 decoder trained on the smaller number of recording sites available for V4 (28% in V1 versus 17% in V4). This indicates that position is more accurately represented by LFP in V1, which is likely a consequence of the difference in receptive field size between the two areas.

In both areas we were able to perform significantly above chance level using only the information from a single recording site. The performance of individual recording sites in both areas are shown in figure 5C. Again, the increased accuracy of positional information in V1 is evident from the single channels analysis. These results suggest that populations of V1 cells may information about visual stimulation occurring far from their classical receptive field. However, this high single channel performance could be due to the subset of stimuli lying near the classical receptive field. In order to rule out this possibility, we performed a control analysis for single channel performance restricted to stimuli more than 2.5 visual degrees outside of the classical receptive field defined for each site. For this analysis, the number of stimuli was limited to 20 and we used the 45 most or least eccentric v1 recording sites. As shown in figure 5D, many single channels were able to determine the position of the stimulus with performance 2-3x greater than expected by chance (max = > 4x chance). This was also true of the average across all tested recording sites. The fact that individual channels have information that allows relatively accurate performance across the sampled visual quadrant suggests that the local field potential may reflect more global aspects of stimulation.
Two frequency bands contain the most information.

Figure 5 Subsampling performance and single channel decoding.
(A) Effect of classifying stimulus position based on subselected channels in V1. (B) Same as A, but for V4. (C) Performance of classifier trained to distinguish position based on single channels. Single V1 channels have higher performance than V4. Single channels contain a significant amount of information about stimuli lying outside of the classical receptive field. (D) Performance of single channels in v1 restricted to the 20 stimuli > 2.5 degrees from the classical receptive field. Mean performance across all channels is higher than chance and many channels show performance 2-3x that expected by chance.
Figure 6 Orientation and direction decoding from LFP and MUA are comparable.
Comparison of average LFP and MUA decoding performance across sessions and animals. (A) Time-frequency classification performance as shown in Figure 4, but based on LFP recorded from intracortical microelectrodes and decoding stimulus orientation. (B) Accuracy of a decoder trained on the same channels as shown in A, but using the multi-unit response. The shaded regions depicts the 95% confidence interval across sessions. (C) Same as A, but for stimulus direction. (D) Same as B, but for stimulus direction. The shaded regions depicts the 95% confidence interval across sessions. All results are average performance across multiple sessions in 3 monkeys.
Two frequency bands contain the most information

**Time frequency dynamics of orientation and direction information and classification error**

In order to better evaluate the quality and generality of stimulus-related information carried by the LFP, we investigated the representation of orientation and direction from localized moving gratings. Further, we were able to compare the information available in the LFP to the spiking activity of small groups of neurons recorded from the same electrodes. We found that the same frequency bands carrying positional information also carried the most information related to both the orientation and direction of moving gratings. Likewise, the temporal dynamics of the two bands was highly similar to the pattern observed in the case of position (Figure 6A shows orientation related information from one session in monkey J, chance = 1/8 (12.5%); Figure 6C shows direction-related information from the same data, chance = 1/16 (6.25%).) Most interestingly, the amount of information available from the band-limited LFP was on the same order as that obtained from time-resolved application of our decoder to the estimated spike density (Figure 6B and figure 6D show orientation and direction decoding based on MUA recordings for the same session as in 6A and C).

Given that the LFP and MUA activity contained stimulus information in such a specific manner, we next investigated the size of errors made during misclassifications. The classification performance across 1 session for both LFP and MUA decoding of orientation are displayed in figure 7A and B. Both the LFP and MUA are able to classify the orientation of a given stimulus with high accuracy and errors are in general clustered around the veridical orientation. The classification performance across the same session for both LFP and MUA decoding of direction are displayed in figure 7C and D. Like orientation, errors in the estimation of direction from LFP and MUA are both small, with the interesting fact that LFP shows a higher incidence for decoding the movement in the opposite direction. As with positional information, the decoded orientation and direction information were highly clustered and estimation was robust in the frequency
Two frequency bands contain the most information. Finally, in order to quantify the error in orientation decoding across the population of sessions and monkeys, we calculated the average distance in radians from the veridical stimulus orientation and the time-resolved estimation from both gamma-band LFP and MUA. The time resolved average error for LFP is shown in figure 7C and that for MUA is shown in figure 7D. Errors were in general less than 1.75 radians for LFP and 1.5 radians for the MUA.

**Comparison of LFP versus firing-rate on a single trial basis**

In order to assess the degree to which LFP and MUA performance co-varied on a trial-by-trial basis, we directly compared the ability of our decoders to differentiate orientation on fast time scales. Both LFP and MUA were able to accurately classify orientation in approximately 60ms. Surprisingly, the LFP reached peak performance more quickly than the decoder based on MUA, while the overall variation in decoding performance between the two conditions was largely correlated in time. The correlation of LFP and MUA single trial performance as a function of time are shown across recording sessions from a single monkey in figure 8A. Figure 8B displays the time-resolved single trial correlation of MUA and LFP performance across the population of sessions and monkeys. Across the data, periods of low classification accuracy in the LFP commonly co-occur with periods of decreased MUA based decoding. However, these values are not perfectly correlated and suggest that independent information may be carried by the two measures of local activity. This fact can be seen most clearly in figure 8C which displays the proportion of joint error trials as a function of time. This shows that approximately 60-70% of errors in single-trial orientation decoding based on LFP (green line) co-occur with an error in orientation based on the MUA. Likewise, ~75% of errors in decoding the orientation based on the MUA also have errors in decoding based on the LFP. While the two signals show similar errors and information, independent information in contained within the different signals.
Two frequency bands contain the most information.

Figure 7 Misclassification errors are small and comparable between LFP and MUA.

(A) Polar plot showing the proportion of classified orientations (true stimulus orientation aligned to 0) using LFP in the gamma band. (B) Same as in A, but using spike density. (C) Same as A, but for stimulus direction. (D) Same as B, but for stimulus direction. Errors made in classifying the orientation of stimuli from the LFP and MUA were highly similar, as shown in figure 7A and B. In the case of direction, the LFP decoder exhibited a higher tendency to misclassify stimuli as occurring in the opposite direction as compared to MUA. (E) Mean error distance for LFP decoding of orientation as a function of time. The average distance between the veridical stimulus orientation and the estimated orientation is shown in radians. Shaded regions show 95 percent confidence interval across sessions and monkeys. (F) As (E), but for MUA decoding.
Two frequency bands contain the most information

Figure 8 Trial-wise comparison of LFP and MUA decoding performance. (A) Correlation of misclassified trials between LFP and MUA across sessions in one monkey. The time course of orientation classification for both LFP and MUA were compared in order to estimate the degree of correspondence in misclassification as a function of time. Both signals show a relatively high degree of correspondence in misclassified stimuli. (B) Population misclassification correspondence between LFP and MUA. Shaded region shows 95 percent confidence interval across monkeys and sessions. (C) Joint error trials. The percent of trials in which LFP (green) or MUA (blue) misclassification occurred together with an error in the simultaneously recorded complementary signal. Errors in LFP and MUA often occurred in unison. Results show population values across sessions and monkeys.

Classification of natural scene and object identity from LFP

Finally, we investigated the presence of stimulus specific patterns of local field potential power in V1 and V4 during free viewing of natural images. As above, we estimated time-resolved single trial power for V1 and V4 recordings sites acquired while two monkeys freely viewed a static natural image. We limited our analysis to images that were repeated at least 10 times so that we had enough stimulus
Two frequency bands contain the most information repetitions to train a decoder. The monkeys fixated while waiting for a natural image to appear and we were able to determine the identity of the image with peak accuracy above 60% (chance = 1/16 (6.25%)). Critically, the same frequency bands that had the greatest position, orientation and direction information also had the greatest information about natural scene and object identity. Although highest performance is limited to the transient activity during free viewing, this may be a result of the trial design. Monkeys fixated a small dot in the center of the screen prior to stimulus presentation and afterwards they were free to move their eyes, while remaining on the image. This may explain low classification performance during the sustained period of the free viewing protocol. However, the fact that similar frequency bands contain a high degree of stimulus specific information also during free viewing, suggests that the spatial pattern of band-limited activity across whole cortical areas accurately encodes the identity of visual scenes.

![Figure 8 Identification of natural scenes and objects from ECoG.](image)

(A) Time frequency classification performance for color natural images presented for free viewing to two monkeys (Chance is 1/16 = 0.0625). (B) Time frequency classification performance for black and white natural images presented for free viewing to two monkeys.
Discussion

We found two frequency bands that reliably contained the most stimulus-related information across a variety of stimulus features, viewing contexts, recording techniques, and monkeys. By combining multiple datasets using different recordings from large-scale ECoG arrays to acute recordings from multiple single electrodes, we were able to demonstrate the robust existence of band-limited stimulus information in the local field potential. By comparing the performance of a decoder trained on the band-limited local field potentials, we were able to directly compare stimulus-related information available from the LFP to that in the spike rate. Overall, similar amounts of stimulus-related information were in the low frequency and gamma bands as was in the spike rate. Further, in the case of stimulus-position decoding, the pattern of activity learned by the trained decoders was organized according to areal topography, suggesting that these weights might function as a means to construct functional maps in sensory areas. The fact that LFP and spike decoders both showed considerable stimulus selective information during the initial transient indicates that downstream areas, which may use these signals in order to further process incoming information, can differentiate the parameters of stimulation on behaviorally relevant time scales. Finally, the fact that we could distinguish image identity from band-limited LFP during free viewing suggests that the spatial distribution of LFP power across cortical areas may reflect the coordination of cortical activity into a cohesive representation of the sensory environment.

More work is needed in order to determine the extent to which the spatial distribution of LFP reflects bottom-up features of the sensory stimulus, such as local indices of contrast, orientation, or luminance, and to which extent it reflects lateral or feed-back activity which constrains the bottom up signal into a fixed perception. It will also be important to determine the extent to which the information contained in spike rates and LFP are redundant and the degree to
which they carry synergistic information. Using measures of spiking separate from rate could also add substantial information. The specific sequence of spikes across the population, spike-LFP phase, or even the distribution of the inter-spike-interval for single units may provide additional information about stimulus parameters. Likewise, our analysis of the LFP was limited to power, but phase information could contribute important information to the representation of stimuli.

These all lead to the question of what role the local field potential and spiking play in the representation of visual features. Given that band-limited rhythms in the LFP arise from recurrent activity between excitatory and inhibitory neurons in local populations (Olufsen et al., 2003; Salinas and Sejnowski, 2001), it seems plausible that the input volley of visual information from the lateral geniculate nucleus to the primary visual cortex is accompanied by simultaneous feed-forward excitation and inhibition. If the spatial pattern of the input volley contains stimulus information in its distribution, as seems likely given the topographically ordered projection from LGN to V1, then the recurrent dynamics initiated by the feed-forward excitation and inhibition will create a loose assembly of oscillatory dynamics. Whether these dynamics are spatially constrained and uncoordinated across V1, or whether they cohere into a single assembly is unclear, but some evidence suggests that the latter applies. During free viewing of natural images, coherent gamma band oscillations occur across broad swaths of primary visual cortex (Brunet et al., 2013). These oscillations are stimulus specific and may reflect the unified representation of the visual scene. Regardless, the further exploration of sensory representation by means of joint LFP and spiking activity is essential to increase our understanding of this important process.
Experimental Procedures

Electrocorticography Recording Techniques and Signal Preprocessing

All procedures for the 3 monkeys implanted with ECoG arrays were approved by the ethics committee of the Radboud University, Nijmegen, NL and have been previously published. Neuronal recordings were made from three left hemispheres in three monkeys through a micro-machined 252-channel electrocorticogram-electrode array implanted subdurally (Bosman et al., 2012; Brunet et al., 2013; Rubehn et al., 2009). Briefly, a ~6.5 x ~3.5 cm craniotomy over the left hemisphere in each monkey was performed under aseptic conditions with isoflurane/fentanyl anesthesia. The dura was opened and the ECoG was placed directly onto the brain under visual control. Several high-resolution photos were taken before and after placement of the ECoG for later co-registration of ECoG signals with brain regions. After ECoG implantation, both the bone and the dural flap were placed back and secured in place. ECoG electrodes covered numerous brain areas, including parts of areas V1, V2, V4 and TEO. As mentioned in the main text, retinotopic mapping revealed two contiguous maps of space, one behind the lunate sulcus for areas V1/V2, and another one between the lunate and the superior temporal sulcus for areas V4/TEO. For simplicity, we refer to ECoG sites in the V1/V2 map as V1, and to sites in the V4/TEO map as V4. After a recovery period of approximately 3 weeks, neuronal recordings commenced. Signals obtained from the electrode grid were amplified 20 times by eight Plexon headstage amplifiers, then low-pass filtered at 8 kHz and digitized at 32 kHz by a Neuralynx Digital Lynx system. LFP signals were obtained by low-pass filtering at 200 Hz and down-sampling to 1 kHz. Power-line artifacts were removed by digital notch filtering. The actual spectral data analysis included spectral smoothing that rendered the original notch invisible.
Two frequency bands contain the most information

Visual Stimulation for Receptive Field Mapping

Stimuli and behavior were controlled by the software CORTEX. Stimuli were presented on a cathode ray tube (CRT) monitor at 120 Hz non-interlaced. When the monkey touched a bar, a gray fixation point appeared at the center of the screen. When the monkey brought its gaze into a fixation window around the fixation point (0.85 degree radius in monkey K; 1 degree radius in monkey P), a pre-stimulus baseline of 0.8 s started. If the monkey’s gaze left the fixation window at any time, the trial was terminated. Several sessions (either separate or after attention-task sessions) were devoted to the mapping of receptive fields, using 60 patches of drifting grating, as illustrated in Figure 1B. Gratings were circular black and white sine waves, with a spatial frequency of 3 cycles/degree and a speed of 0.4 degrees/s. Stimulus diameter was scaled between 1.2 and 1.86 degrees to partially account for the cortical magnification factor. Receptive field positions were stable across recording sessions.

Visual Stimulation for Natural Images

The monkeys were trained to perform different tasks, while having their head fixed. For the data reported in this study, monkeys were required to fixate for 0.63 s on a fixation point (0.12 by 0.12° black square) centered on a gray background, after which a natural image was presented, which was again centered on the background screen. We used 49 grayscale images and 16 color images, with grayscale and color images presented in separate sessions. Grayscale images subtended 16-by-16°, and color images 18.5-by-18.5°. Grayscale images were shown for 3.5–6 s (flat random distribution), and color images for 1.5 s. Once the image had appeared on the screen, the monkey could view it freely. If the monkey kept its gaze on the image as long as it was presented, it was given a juice reward after stimulus offset. Because this task was very easy for the monkeys, almost every trial was rewarded. Each grayscale image was presented for an average of 15 trials, and each color image for an average of 22 trials. Eye position was recorded
Two frequency bands contain the most information with an infrared camera system (Thomas Recording ET-49B system) at a sampling rate of 230 Hz. Unless stated otherwise, we selected electrodes over V1 and V4 that were strongly driven by stimuli within the central 4° of eccentricity. Due to placement of the ECoG grid onto the dorsal parts of V1 and V4 in the left hemisphere, receptive fields were in the lower right visual quadrant. Correspondingly, we accepted analysis epochs when the gaze of the monkey was at least 4° away from the lower and the right border of the natural image for at least 90% of the epoch duration. This ensured that the responses of the recorded sites were due to the natural image rather than the screen background or the edge of the image. In monkey P (monkey A), we used 43 (42) electrodes on V1 and 16 (14) on V4. The assignment of electrodes to visual areas was based on intraoperative photographs and brain atlases, and used primarily sulcal landmarks. For most of the electrodes, the area assignment was unequivocal. Yet, some of the most anterior electrodes assigned to V1 might as well be over V2, and the most lateral electrodes assigned to V4 might as well be over the temporal-occipital area (TEO). Exclusion of those electrodes left the results qualitatively unchanged.

Acute Recording Techniques and Signal Preprocessing

All procedures for the 3 monkeys with acute tungsten recordings were approved by the German local authorities (Regierungspräsidium, Hessen, Darmstadt) and were in full compliance with the guidelines of the European Community for the care and use of laboratory animals (European Union directive 86/609/EEC) and have been previously published. Experiments were performed as described in detail in Lima et al. and Vinck et al. (27) on three awake adult monkeys. Recordings were performed in area V1 using 2–7 tungsten/platinum electrodes. Monkeys passively viewed drifting gratings of varying orientations (16 directions in steps of 22.58°).

Before the experiment, each monkey was surgically implanted with a head post, a scleral search coil, and a recording chamber. Recordings were made from the
Two frequency bands contain the most information in the opercular region of V1 (receptive field centers: 2–5.8 degrees of visual angle in eccentricity) and from the superior bank of the calcarine sulcus (receptive field centers: 8–12.8 degrees of visual angle in eccentricity). Recordings proceeded with 2–5 Quartz-insulated tungsten/platinum electrodes inserted independently into the cortex through transdural guide tubes with five precision hydraulic microdrives mounted onto an X-Y stage (MO-95; Narishige Scientific Instrument Laboratory). Spiking activity and the local field potential (LFP) were obtained by amplifying (1,000×) and band-pass filtering (multiunit activity: 700–6,000 Hz; LFP: 0.7–170 Hz) the recorded signals using a customized 32-channel headstage and preamplifier (headstage HST16025; headstage and preamplifier from Plexon Inc. Additional 10× signal amplification was performed by onboard amplifiers (E-series acquisition boards; National Instruments). LFPs were acquired with a resolution of 1.0 ms. Spikes were detected online by amplitude thresholding. Spike events and corresponding waveforms were sampled at 32 kHz, and spike waveforms were recorded for 1.2 ms.

Visual Stimulation and Behavioral Task

Stimuli were presented as movies at 100 or 120 frames per second using a standard graphical board (GeForce 6600 series; NVIDIA). The CRT monitor used for presentation (CM813ET; Hitachi) was gamma corrected to produce a linear relationship between output luminance and gray values, and subtended a visual angle of 36 × 288 (1,024 × 768 pixels). At the beginning of each recording session, receptive fields were mapped using an automatic procedure, in which a bar was moved across the screen in 16 different directions (160 trials). Receptive field position was estimated from the global maximum of a response matrix, at a resolution of ~6 min of arc. Subsequently, monkeys passively viewed drifting gratings during fixation of a small central fixation spot. Gratings had spatial frequencies ranging from 0.5 to 2.0 cycles per degree and velocities ranging from 0.5 to 3.0 degrees per second. Grating drift directions were generated randomly.
Two frequency bands contain the most information from a total of 16 directions (steps of 22.58). The stimuli were centered over the receptive fields within a circular aperture of 8.08 degree. After the monkey acquired fixation, there was a prestimulus baseline of 800–1,000 ms, after which the stimulus was presented for 800–1,400 ms. To obtain a reward, monkeys had to release the lever within 500 ms after the fixation point had changed color. Trials were aborted upon fixation breaks, or when the lever was released before the color change. Eye position was monitored continuously by a search coil system (DNI; Crist Instruments) with a temporal resolution of 2 ms.

Data Analysis General

All analyses were performed in MATLAB (MathWorks) using FieldTrip (Oostenveld et al., 2011) (http://fieldtrip.fcdonders.nl). For the ECoG datasets, we calculated local bipolar derivatives, i.e., differences (sample-by-sample in the time domain) between LFPs from immediately neighboring electrodes. We refer to the bipolar derivatives as “sites.” Bipolar derivation removes the common recording reference, which is important when analyzing power correlations and/or coherence. Subsequently, per site and individual epoch, the mean was subtracted, and then, per site and session, the signal was normalized by its standard deviation. These normalized signals were pooled across sessions with identical stimulus and task, unless indicated otherwise. In order to select visually selective recording sites, an ANOVA was computed across frequencies. Time-frequency resolved power was computed for the LFP by multiplying the time-domain data by a hanning taper in over-lapping windows of a length scaled to the frequency of interest (3 cycles/window) and with a variable frequency smoothing (+/- 0.5 x the center frequency) in step sizes of 1 Hz and 0.01 s.

Retinotopic maps

In order to construct retinotopic maps for eccentricity and elevation, the mean power was computed across trials for each stimulus location. First, each trial was
Two frequency bands contain the most information cut to the time between 0.3 s after stimulus onset to avoid the early transient activity, and 0.9 s. Frequency analysis was performed by computing the fast Fourier transform of the entire stimulation epoch. Each site was assigned an eccentricity and an elevation based on the position of the stimulus giving the maximal response in the gamma band. The overall pattern of retinotopic organization was robust to different manners of computing the preferred eccentricity and elevation and so the maximal stimulus was chosen because of its simplicity.

**Spike Rate**

Instantaneous spike density was computed on a single-trial basis by convolving the binary spike time-series by a Gaussian kernel with a standard deviation of 10 ms. Each trial consisted of 3-5 individual time-series from independent electrodes. Each binary time-series was separately convolved with the kernel in order to derive a continuous variable, which divided by the time window, led to a quasi-instantaneous spike density estimate.

**Naïve-Bayes Decoding**

Single-trial estimates of power or instantaneous spike density for each condition were used to train probabilistic decoders. The feature vector was composed of one time slice across channels. Our training set consisted of 80% of the available trials for the condition with the fewest number of trials. This avoided introducing prior biases into our decoder. We trained on 60% and 90% of the available data and this did not qualitatively change our results. Values displayed for the decoding plots shown here display the mean value from 100 iterations of the decoder to avoid any extraneous effects of the training and test sets.
Chapter 7

Summary, conclusions and future directions
Summary

This thesis has presented evidence that intrinsic activity possesses a high degree of functional relevance and specificity, far from random fluctuations that can be categorized as noise. These results link intrinsic patterns of inter-areal connectivity with individual behavior, learning and a potential electrophysiological mechanism. Intrinsic activity in networks selectively engaged in a perceptual task was demonstrated to reflect recent experience. The coupling of specific brain areas between intrinsically defined networks was modified in a manner and to a degree that reflected learning and was reflective of individual improvement in performance. The state of intrinsic connectivity within and between functional networks prior to learning a new task was also predictive of the rate at which an individual acquired competency in the task. Together, these results suggest that the state of intrinsically defined networks may reflect recent experience and provide a diagnostic for differences in aptitude or fitness to particular tasks.
While increasing evidence supports the functional importance of intrinsic networks defined by brain imaging techniques, there is still little known about the neuronal activity that underlies the brain-wide networks observed in this fashion. In order to investigate the electrophysiological bases of these intrinsic networks, I assessed the characteristics of intrinsic inter-areal coupling in electrocorticography measurements. I showed that the retinotopic organization of primary and intermediate visual areas was echoed in the absence of visual stimulation. Both the intrinsically generated trial variance of visual responses, as well as spontaneous variation in power and inter-areal synchronization occurred in a coordinated manner between visual areas. Rather than occurring in a temporally unspecific manner, this correspondence occurred at specific rhythms known to be involved in sensory processing. Importantly, these same rhythms contained the most stimulus related information, suggesting that intrinsic variation in these frequency bands may be functionally relevant and advantageous.

In order to further explore the role of these frequencies in stimulus processing, I presented an analysis of their ability to distinguish various sensory stimuli on a single-trial basis. By using a probabilistic decoder to classify single trials of stimulus presentation, I was able to demonstrate the functional relevance of these frequency bands across several visual features and categories. Single trial examples of power in these frequencies were able to distinguish the spatial position of grating stimuli, the identity of natural images and the orientation of gratings. Interestingly, responses in the gamma band, which showed the highest degree of intrinsic organization and has been related to the fMRI signal, showed orientation and direction selectivity that was equal to that contained in single-trial neuronal spike-rate data from the same sites. These results suggest that intrinsic activity may shape and contribute to sensory processing and reflect individual experience in the maintenance of distributed brain networks.
Conclusions

The brain’s intrinsic activity is likely to provide essential insight into brain function. The fact that intrinsic activity is organized in discrete, functionally relevant patterns and that this organization occurs at specific frequencies, suggests that intrinsically generated variability shares some organizational principles with the patterns of stimulus- and task-related activation more commonly studied. The question remains as to what role intrinsic activity may play in typical brain function and the degree to which it is actively shaped on short or long time scales. A compelling bit of evidence comes from the role that spontaneous activity plays in the formation of sensory maps (Buonomano and Merzenich, 1998; Shatz, 1996).

The formation of retinotopic organization in the visual thalamus and cortex has contributed a good deal to the study of neural development and has implicated spontaneous activity in the formation of sensory maps. During development, prior to eye-opening, activity in the retina undergoes a highly ordered sequence of events during which spontaneous waves of activity generate coherent patterns of cellular depolarization along anatomical axes (Meister et al., 1991). It has been demonstrated that these spontaneous waves are necessary for the formation of ocular dominance columns, as well as retinotopically ordered maps in the thalamus and cortex (Ackman et al., 2013). Additionally, the patterns of the spontaneous waves directly lead to the spatial patterning of cell responsivity in these downstream areas. This suggests that, at least in development, spontaneous activity can operate in a feed-forward manner in order to specify anatomical connectivity (Katz and Crowley, 2002; Shatz, 1996).

Additional insight into the potential role of intrinsic activity in brain function comes from work on homeostatic mechanisms in maintaining a stable working point for neurons and networks. Cells and circuits must operate within a stable regime in order to reliably maintain and transmit information (Marder and Goaillard, 2006; Turrigiano and Nelson, 2004). If circuits are unbalanced,
information is endlessly amplified as a result of runaway excitation; alternatively, it is quenched as a result of too little excitation. In a balanced regime, cells maintain a relatively fixed firing rate by scaling their synapses and regulating their excitability. The same situation holds for recurrent networks of cultured cortical cells or computational models, in which homeostatic mechanisms are important in regulating network activity. It has been suggested that the excitatory/inhibitory balance within local populations is also important in order to maintain a stable working point in which the population can optimally process incoming signals and reliably pass them on. While this perspective is growing in influence, recent results suggest that differences in both the relative timing of synaptic events, as well as the excitatory/inhibitory balance may shift between behavioral states. In awake cortex, inhibitory conductances seem to dominate excitatory currents, especially during stimulation (Haider et al., 2013). This shift in excitatory/inhibitory balance results in brisker, more selective sensory responses during awake stimulation as compared to under anesthesia. Relatedly, the transition from spontaneous activity to stimulation in awake sensory cortex shifts the timing of synaptic events from a synchronous to an asynchronous regime, leading to distinct distributions in membrane voltage for these two conditions (Tan et al., 2014). Although much remains to be understood about the balance of excitation and inhibition across behavioral states, these mechanisms appear essential for controlling cortical activity. The balance of excitatory and inhibitory drive may balance the counteracting effects of synaptic plasticity, such as long-term depression or potentiation. It is feasible that both local and large-scale dynamics of intrinsic activity may serve such a homeostatic function on a circuit or brain-wide level. Such a link may allow for a mechanistic bridge between the diverse spatial scales on which intrinsic activity plays a role. One compelling piece of evidence comes from the similar developmental sequence of neurons, glia, and vasculature, all of which share guidance mechanisms and cofactors (Eichmann et al., 2005). The active generation of circuits through spontaneous activity may drive the formation of coupled hemodynamic units that regulate local activity patterns (Kleinfeld et
al., 2011). While little evidence exists to make an explicit connection, the active role that astrocytes play in maintaining homeostasis in local circuits and their tight regulation of the blood-brain barrier provide enticing hints (Blinder et al., 2013; Moore and Cao, 2008). Spontaneous activity may play an active role in shaping the brain’s circuits, after which time synaptic plasticity and homeostatic mechanisms maintain circuit activity in a balanced state so that local and distant populations of cells are regulated in a coordinated fashion. Indeed, at the level of individual cells, spontaneous activity maintains a tight coordination of excitation and inhibition (Okun and Lampl, 2008). As noted above, more questions than answers currently exist concerning these important questions. However, it is appealing to hypothesize that, by serving such a balancing role on brain activity patterns, intrinsic activity could reflect the path of development and the active processes of synaptic plasticity could continue to shape anatomical and functional connectivity throughout the lifetime. In such a scenario, the patterns of intrinsic activity could reflect the history of brain co-activation of brain-wide networks, and the coordinated activity within and between these networks could reflect learned biases or internal models of perceptual, motor and cognitive expectation or prediction.

Likewise, at the scale of small populations, the intricate intrinsic dynamics could maintain the network at a balanced working point so that learning is maintained and newly arriving input is coordinated into the local activity pattern. Although spontaneous activity is thought to shape topographic maps in a largely feed-forward direction, a perspective supported by the disruption of ordered topographies after interference with spontaneous activity in the retina, the sufficiency of feed-forward mechanisms has not been established in the absence of down-stream spontaneous activity. It is possible that spontaneous activity in higher order areas could occur between previously co-activated circuits, which could enhance the stability or specificity of feed-forward patterns, as well as maintain the structure and function of lateral and feedback connections. Activity within these
networks during perception, action or task engagement could then provide historically weighted context signals that modulate and shape the input signal. Intrinsic dynamics would therefore provide an essential ingredient to the processes of state-based computation that would enable the integration of historical biases and expectations with newly arriving input patterns. Far from the posited reflex arc, the brain must embody a rich model of the external world, the effector and motor systems of the body and the repertoire of likely outcomes and future events. Adaptive behavior requires the intricate interaction of both external, sensory information and endogenous, intrinsically generated signals that reflect the learned state of the system.

**Future directions**

Although the perspective championed in this thesis has been expressed multiple times and existed in some form for centuries, new technologies and experimental facts have led to a recent resurgence in interest. This interest stems in part from the pressing need for new conceptual frameworks in which to understand brain dynamics. As interest has shifted away from feed-forward models of neural circuitry to recurrent networks with rich dynamics, the need to understand processing within these complex circuits has become unavoidable. Likewise, as brain-recording technology has made it possible to record from larger and larger portions of the brain, *in vivo*, the importance of intrinsic dynamics, and the ability to monitor the internal state of the brain are pushing intrinsic activity to the forefront.

Better understanding of intrinsic activity will come from increased investigation of the neural processes involved in perception and goal-directed behavior. As we move away from studies of single neurons to the investigation of large neuronal populations in local circuits and the coordination of activity across distributed brain regions, we will begin to understand how the brain’s intrinsic dynamics affect the activity of individual cells and how this interaction enables adaptive behavior. We
Summary, conclusions and future directions

will start to see the variable response of individual cells as a reflection of the distributed activity of the network they are embedded in, rather than bothersome measurement noise.

In the past few decades, neuroimaging techniques have complemented electrophysiological methods in the study of perception and cognition. These methods, which allow a greater field of view of many brain areas or many cells within a confined area, have highlighted the importance and order of intrinsic dynamics and spurred forward the appreciation of integrated networks over localized brain regions in brain dynamics and behavior. However, the limited spatial and temporal resolution of these techniques has often left mechanistic understanding of the underlying neural processes lagging behind. As the ability to record from larger and more widespread neural populations becomes more common, it will be important to continue to integrate the perspective on networks and intrinsic activity into our understanding of single cells and small populations.

Likewise, if the results presented in this thesis reflect aspects of the actual, functional significance of intrinsic activity, it will be important to investigate the effect of intrinsic activity on the behavior of animals and single cells, as well as to monitor it, ideally in concert with measures of synaptic connectivity during learning and behavior. Some evidence already points to a constructive role of intrinsic activity in the course of development. However, much is left to resolve regarding the role of intrinsic activity in modeling the external world and coordinating population activity. Much of our current knowledge of brain function has derived from the application of reductionist methodology to the study of individual cells in isolation. Additionally, the success of the reductionist approach to deriving localized functions for single cells has led to a dogmatic stance regarding the receptive field properties of sensory cells. These facts have often led to a selection bias in the electrophysiology literature in which investigators advance single electrodes in search of cells with a particular response pattern.
While these studies have provided a wealth of information, the advent of dense electrode arrays, which sample from local circuits in an unbiased way will provide additional insight into the heterogeneity of individual cell responses even in early sensory cortices. As electrophysiological studies push forward to the study of larger and larger populations across multiple brain regions, this trend promises to add more understanding and appreciation for intrinsic brain dynamics and the varied, non-classical response properties of sensory cells.

Finally, the growing appreciating of intrinsic activity needs to be supported by new conceptual models to replace the dominant perspective on feed-forward, stochastic computation. Methods must be developed which take account of single-trial, rather than average responses. The brain must compute on single trials and so our methods should attempt to suss out the operation of such a brain. Work in recurrent networks is starting to achieve this mission, and promising results regarding an active role of intrinsic activity have already been promoted (Buonomano and Maass, 2009; Lazar et al., 2009; Maass et al., 2002; Sussillo and Barak, 2013).

Likewise, the utility of a heterogeneous population of cells with highly non-classical response properties has been described and initial empirical evidence suggests they may exist. However, the dynamics of recurrent networks are currently beyond our comprehension and no practical analytic methods exist which allow the dissection of function from models of recurrent connectivity, let alone the recurrent dynamics of biological neural networks. Methods for data analysis are beginning to incorporate the effects of local connectivity into more traditional feed-forward models, and as these techniques expand, new mathematics will be required in order to begin to understand how computation occurs in highly connected, asymmetric networks. The growing evidence in support of the critical role that intrinsic dynamics play in brain function should serve as a cautionary tale for those quick to categorize variation from models, implicit or explicit, as noise. Rather than dismissing the sources of variance in our measurements, we should try to formulate descriptive models that can incorporate these variables as explanatory
parameters and endeavor to construct an over-arching paradigm in which to approach brain function. While systematic investigation of a well-defined phenomenon is laudable, the complexity of the brain seems to demand a holistic approach that integrates all sources of information in order to begin to make sense of its coherent madness.
Appendices

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Samenvatting (Dutch Summary)

Acknowledgements

Curriculum Vitae

Publications

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Appendices


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Appendix


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Samenvatting in het Nederlands

De dagelijkse ervaringen hebben hoogstwaarschijnlijk de impressie gegeven dat de hersenen niet statisch of stil zijn. Ver van de steriele inerte passiviteit van een digitale computer, bruisen je hersenen met perpetuele energie met een eigen leven op zich. Gedurende verschillende gedragstoestanden, van intense activiteit tot rust en slaap, zijn onze hersenen in gelijke mate aan het werk, continu bezig met gedachtengangen, gevoelens en lichamelijke opdrachten, waarin wij ons als ons zelf herkennen.

Ondanks dit evidente feit, heeft de meerderheid van het onderzoek naar hersenfuncties de intern geproduceerde activiteit van de hersenen zelf ten gunste van het begrijpen van de hersenactiviteit gebonden aan externe stimulatie of bewust gedrag, genegeerd. Dit heeft tot gevolg dat het meeste onderzoek zich concentreert op de analyse van het gedrag van de hersenen zoals het zich gemiddeld voordoet. Dat wil zeggen, de hersenactiviteit wanneer vele dezelfde prikkels worden vertoond of wanneer vele identieke processen van een specifieke opdracht worden uitgevoerd. Het op deze manier berekenen van gemiddelde hersen reacties biedt een tal van wenselijke kenmerken. Ten eerste geven herhalingen, omdat hersenreacties vaak klein en moeilijk te meten zijn, een robuuste schatting van de meest betrouwbare activiteitspatronen die door een gegeven experimentele gebeurtenis opgewekt worden. Bovendien zijn er vele variabelen die de hersenactiviteit beïnvloeden en door zo veel mogelijk variabelen over herhaaldelijke gebeurtenissen parametrisch the controleren, heeft de wetenschapper de mogelijkheid vanuit het complexe mengsel de verschillende unieke componenten van elkaar te onderscheiden. Tenslotte bevatten alle fysieke metingen een mate van technische ruis. Willekeurige variatie in de gemeten waarden, veroorzaakt door bronnen uit de omgeving of door instrumenten, en ongerelateerd aan het systeem, worden ook bestudeerd. Het analyseren van gemiddelde waarden, ten opzichte van individuele metingen, waarborgt dat de ruis componenten die ongecorreleerd zijn tussen de metingen worden verminderd.

Om deze redenen heeft het hersenwetenschapsveld lange tijd vertrouwd op de analyse van gemiddelde hersenreacties in het onderzoek naar, en het maken van, conceptuele modellen van hersenfuncties. Het werken met gemiddelde waarden heeft voor een enorme hoeveelheid empirische feiten gezorgd en relateert een grote variatie aan hersenreacties, aan vele experimentele manipulaties van fysieke of cognitieve variabelen. We hebben inzicht gekregen in hoe hersenreacties gemoduleerd worden door stimulatie parameters en de potentiele rollen van verschillende hersengebieden in sensorische verwerking, cognitie en gedrag. Het succes van deze aanpak en van de afgeleide fenomenologische modellen heeft geleid tot de wijdverspreide acceptatie van conceptuele modellen die veronderstellen dat het middelen over reacties een potentieel mechanisme voor neuronale berekeningen is. Deze modellen stellen voor dat neuronen functioneren
door te middelen over de ruisige invoer afkomstig van individuele pre-synaptische partners. De overgang van variatie in de gemeten signalen tot het postuleren van neuronale ruis, heeft de implicatie dat het brein werkt op een stochastische wijze en suggereert dat de gemiddelde hersenrespons een "signaal" representeren, en afwijkingen van het gemiddelde respons "ruis" vertegenwoordigen. Het op deze manier bekijken van de hersenen heeft een grote invloed op neurowetenschappelijk onderzoek en discussies over hersenfunctie, neurale representatie en berekening gehad.

De transformatie van de signaal middeling als een bruikbare analytische strategie tot een theorie van hoe de hersenen opereren heeft echter een beperking als het gaat om interne dynamiek van de hersenen en de rol van de hersenfunctie. Vanwege het feit dat de intrinsieke activiteit van de hersenen feitelijk ontoegankelijk is voor experimentele controle of monitoring, draagt het noodzakelijkerwijs bij aan de variabiliteit in de hersenen signalen die niet tot uiting komen in de gemiddelde reacties. Intrinsieke activiteit wordt dan ook voorgesteld als de bron van neuronale ruis. Onder dit conceptuele milieu, heeft de verzakelijking van onderzoeksvariabiliteit als systemische ruis in de hersenen geleid tot een grote familie van hersenen theorieën die de baby met het badwater weggooien. Verder heeft het van dit standpunt afgeleide perspectief op hersenfunctie ernstige beperkingen en is een re-evaluatie van intrinsieke activiteit en de operationele modus van de hersenen noodzakelijk.

Dit proefschrift presenteert een oeuvre dat ons begrip van deze kritieke grenzen tussen intrinsieke dynamiek en de door externe bronnen opgelegde hersenenactiviteit voort probeert te duwen.

Ik beargumenteer dat deze intrinsieke activiteit, verre van ruis, een robuuste structuur heeft, ervaringen reflecteert, gefrag beinvloedt en de perceptie vormt. Ik ben ook een voorstander voor een aanpak die hersen functie onderzoekt rekening houdend met variabelen buiten onze experimentele controle en ik probeer manieren voor te stellen waarin we deze in werkende modellen van hersenfuncties kunnen integreren.

In hoofdstuk 1 en 2 beschrijf ik vorig werk dat tot een conceptuele overstap van analyse met gemiddelden, tot een analyse met individuele metingen heeft geleid en dat de rol van intrinsieke activiteit in het moduleren van hersen reacties benadrukt. Vervolgens leg ik de gebruikte experimentele methoden en de analytische benadering toegepast in de rest van het proefschrift uit. Hoofdstuk 3 beschrijft een studie van een leerproces en pogingen om veranderingen in intrinsieke activiteit te koppelen aan veranderingen in gedrags prestaties die optreden als gevolg van intensieve training op een nieuwe perceptuele taak.
In hoofdstuk 4 wordt ingegaan op de stand van de intrinsieke activiteit in de hersenen voorafgaand aan het leren van een nieuwe perceptuele taak en wordt gebruik gemaakt van deze activiteit om individuele metingen van competentie en taakverwerving te voorspellen. Hoofdstuk 5 onderzoekt ritmische synchronisatie verschijnselen tussen visuele gebieden die stimulus eigenschappen tijdens visuele stimulatie reflecteren en opnieuw optreden in de afwezigheid van stimulatie.

Hoofdstuk 6 vergelijkt de aanpak op de stimulus selectiviteit voor stimulus geïnduceerde patronen van hersenactiviteit van individuele metingen (gebaseerd op methoden uit de "machine-learning") met meer traditionele, meting middelende methoden. Tenslotte persenteer ik in hoofdstuk 7 een aantal conclusies die getrokken kunnen worden uit dit werk en kijk ik uit naar toekomstige vragen die moeten worden aangepakt en nieuwe richtingen van onderzoek die inzicht kunnen bijdragen aan deze nieuwe conceptualisering.
Acknowledgements

Science doesn’t happen in a vacuum, but is a social enterprise. I am fortunate to have been a member of a handful of wonderful labs. First, in the Media and Machines Lab at the Engineering School of Washington University, I was given free reign of rather large and mobile robots. These roaming monstrosities were great fun to program and even more fun to send careening autonomously through the department hallways. I learned a great deal about the hands-on, pragmatic work of engineering and empirical research. Next, at the Neuroimaging lab at the Washington University School of Medicine, I was exposed to the messy realities of data and the delights of study planning, spirited debate and great camaraderie. My time spent in Chieti, with my wonderful colleagues at ITAB, gave me a love of Italy and the projects and people I met while there finally pushed me to pursue neuroscience head-on. Likewise, the Donders Centre in Nijmegen provided a terrific environment in which to begin a new line of work and the support and interaction with my colleagues there continues to this day. Finally, at the ESI I’ve been able to learn a tremendous amount and indulge in exhilarating projects with an exceptional group of lab-mates and friends.

At each stop, the people made the place. I would like to thank my mentors, Maurizio Corbetta and Pascal Fries for guiding me into exciting projects, giving me space to try for myself, and pushing me to flourish. Chad Sylvester and Gaurav Patel inducted me into neuroscience research swiftly and with great mirth. Carlo Sestieri, Francesco de Pasquale, and Paolo Belardinelli ushered me into Italian life. Antonello Baldassarre worked through all the kinks.

Finally, I would like to thank my parents for their bottomless support; for taking me outdoors when I might have preferred to stay on the computer and for introducing me to building, learning, laughing and loving. My brother, Michael, has been a constant source of inspiration, humor and insight. Eric Wood has been a close companion on many intellectual and entertaining diversions over past two decades. And lastly, to Andreea, for understanding and often agreeing.
Appendix

Curriculum Vitae

Christopher Lewis was born in Saint Louis, Missouri on February 10, 1980. It was cold. He graduated from Saint Louis University High School in May, 1999 and enrolled at Purdue University, West Lafayette, Indiana. Between September, 1999 and May, 2001, he studied Electrical and Computer Engineering and Physics at Purdue and worked on production line automation at Watlow Electric. He transferred to Washington University in September 2001, in order to pursue interdisciplinary studies between the engineering and neuroscience faculty. While enrolled at Washington University, he studied Computer Science and Engineering in addition to participating in the joint Philosophy, Neuroscience and Psychology program. During this time, he worked on object recognition and navigation in mobile robots, as well as the propagation of errors in binary networks under the guidance of Professor Robert Pless in the Media Lab. In 2003, he began to work in the group of Professor Maurizio Corbetta at the Neuroimaging Lab of the Washington University Medical School. In the Neuroimaging lab, he developed algorithms for the registration of functional and anatomical volumes from Magnetic Resonance Imaging, as well as methods for fiber tractography from diffusion tensor imaging data. Between the summer of 2005 and May, 2006, he was a software engineer at the Boeing Company, where he worked on the estimation and scheduling of aerospace engineering tasks. He left Boeing in order to help start a functional imaging lab at the Institute for Advanced Biomedical Technology, University G. d’Annunzio, in Chieti, Italy. While in Chieti, Chris investigated perceptual learning in relation to spontaneous brain activity. During this time he started to work with Professor Pascal Fries on the analysis of large-scale electrophysiological data from awake primates. After leaving Chieti, Chris joined the group of Professor Pascal Fries for a short stay at the Donders Centre for Cognitive Neuroimaging, Radboud University, Nijmegen, The Netherlands. In January, 2010, Chris joined the newly formed department of Professor Fries at the Ernst Strüngmann Institute (ESI) for Neuroscience in Frankfurt, Germany. At ESI, Chris has worked on the development of new electrophysiological techniques for chronic, large-scale recordings in animal models. This work continues.
Appendix

List of Publications


Appendix

Donders Graduate School for Cognitive Neuroscience Series


25. Treder, M. S. (2010). *Symmetry in (inter)action.* Radboud University Nijmegen, Nijmegen, the Netherlands.


32. Van Dijk, J.P. (2010). On the Number of Motor Units. Radboud University Nijmegen, Nijmegen, the Netherlands.
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