How Do Brain Areas Communicate During the Processing of Noxious Stimuli? An Analysis of Laser-Evoked Event-Related Potentials Using the Granger Causality Index

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INTRODUCTION

The processing of noxious stimuli activates a complex neural network of the brain composed of different cortical and subcortical areas (for reviews see Apkarian et al. 2005; Craig 2003a; Peyron et al. 2000; Price 2000; Price et al. 2006). The activation of these areas can be visualized by means of functional imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). These methods have consistently shown that noxious stimuli evoke activation in the secondary somatosensory cortex (S2), the insula (INS), and the anterior cingulate cortex (ACC) (for reviews see, e.g., Apkarian et al. 2005; Peyron et al. 2000). INS and ACC might be further subdivided with respect to pain processing. Thus robust activation to noxious stimuli was found in both the posterior and the anterior parts of INS (e.g., Craig 2003b,c; T. Weiss, T. Straube, J. Boettcher, H. Hecht, D. Spohn, and W.H.R. Miltner, unpublished observations), with the possibility of distinguishing subparts (e.g., Schweinhardt et al. 2006). Similarly, the ACC contains several nociceptive regions that seem to code different aspects of the input (Büchel et al. 2002; Vogt 2005; Vogt et al. 1996). Less consistent results have been obtained for activations of the lateral thalamus and primary somatosensory cortex (S1) contralaterally to the stimulation side, as well as for some other cortical regions, i.e., the posterior parietal cortex, the posterior cingulate cortex, and the prefrontal cortex (Peyron et al. 2000; Treede et al. 1999).

Although fMRI and PET have good spatial resolution, their temporal resolution is rather weak. For the temporal characterization of brain activation in response to noxious stimulation either electrocortical, intracortical, electroencephalographic (EEG), or magnetoencephalographic (MEG) recordings have been used (e.g., Weiss and Miltner 2005, 2006) since all of these methods have a neural temporal resolution within the range of milliseconds. Data based on these methods are suitable for the analysis of interrelations of neural activity between different brain areas and can be submitted to coherence and correlation methods to test how two or several of the regions mentioned earlier interact with each another. However, measures of coherence and correlation do not provide information about the direction of the interaction between two sources of neural activity; i.e., they do not show whether activity of area A positively or negatively affects the activity of area B or area C. One approach shown to solve this problem is the statistical concept of Granger causality (Granger 1969). One element of this approach is based on the concept of predictability. A signal Y is hypothesized as influencing a signal X when the prediction of the future course of X is more accurately based on the history of signals X and Y than based on the history of the signal X alone. Following this principle of predictability, Granger causality is often based on vector autoregressive (VAR) models. For the frequency domain, the directed coherence and partial directed coherence (Baccala and Sameshima 2001; Sameshima and Baccala 1999) as well as the so-called directed transfer func-
tion (DTF) (Kaminski et al. 1997) are appropriate measures. Another approach for the detection of frequency-independent directional interactions among two or more signal components is the Granger Causality Index (GCI), which is used in this study. In recent studies the Granger causality has been successfully used for the analysis of EEG signals (Bernasconi and König 1999; Hesse et al. 2003). For example, Bernasconi, König, and other colleagues investigated the time-invariant directional interrelations between different cortical areas of the cat’s visual system. They investigated signals with stationary behavior. However, in many biological data sets the assumption of stationarity is often not fulfilled. Hesse et al. (2003) extended the time-invariant Granger causality approach for the analysis of time-variant EEG signals by applying an adaptive recursive concept of a VAR model with time-dependent parameters based on a recursive least square (RLS) algorithm with forgetting factor (Möller et al. 2001). For this approach, it was shown that the identification of short-lasting directed interrelations for activated cortical networks is possible (Hesse et al. 2003). A piecewise linear extension was introduced on the basis of self-exciting threshold autoregressive (SETAR) models (Leistritz et al. 2006).

It has been proposed that the different brain areas involved in the processing of noxious input are activated partially in sequential and parallel order (Apkarian et al. 2005; Peyron et al. 2000; Price 2000; Price et al. 2006; Treede 2003) with both types of activation varying from moment to moment. Price et al. suggested that noxious somatosensory input into the brain is first processed simultaneously by S1 and S2 (and possibly also by the posterior cingulate and the insular cortex) and then by the insula and the posterior parietal cortex (PPC) (Price 2000; Price et al. 2006). Additionally, parallel activation will take place in the reticular formation (RF), the hypothalamus (HT), and the amygdala (AMY). These latter structures primarily organize the arousal and autonomic activations that accompany the experience of pain. Although the input to S1, S2, and possibly to INS and PPC most likely establishes and supports the nociceptive sensation, the succeeding and/or parallel activation of the insula, the anterior cingulate cortex (ACC), supplementary motor area (SMA), and prefrontal cortex (PFC) together with the simultaneous activation of the RF, HT, and AMY are hypothesized to organize the perceived intrusion of threat of the noxious event and their behavioral consequences. Finally, it is further presumed that the ACC and PFC are also involved in second-order appraisal processes and the secondary pain affect. A slightly different sequence of activation was suggested by Apkarian and colleagues (2005). Based on fMRI, PET, MEG, and EEG data and on intracortical recordings, they suggested that the S2 contralateral to the side of stimulation is activated first followed by S2 in the ipsilateral and S1 in the contralateral hemisphere. Simultaneously to S2, the INS is also activated followed by the ACC. In addition, Craig (2003a,b) proposed a parallel activation of the posterior INS and the mid-ACC, a proposal that fits well with actual literature from laser-evoked potentials in monkeys (Baumgärtner et al. 2006). These structures might be activated by way of spinal lamina I input via the thalamic subnuclei ventromedial part of the posterior nuclear complex (VMpo) and ventrocaudal part of the medial dorsal nucleus (MDvc) (Craig 2003a, 2004).

Additional confusion about the temporal activation pattern of different areas of the human brain is provided by studies based on the time course of different components of somatosensory-evoked potentials or its dipole sources in response to laser-heat stimulation. For example, S1 and S2 belong to the so-called lateral nociceptive system since their primary nociceptive input comes mainly from the lateral thalamus (Treede et al. 1999). Thus there should be a causal relationship of activation between lateral thalamus, on the one hand, and S1 and S2, on the other hand. Moreover, the INS and the ACC are proposed as belonging to the so-called medial nociceptive system since they receive input from medial thalamic nuclei and from the brain stem (Treede et al. 1999). Thereby, the posterior part of INS takes up an intermediate position since it receives its major thalamic input from medial nuclei but it also receives strong input from the lateral system, especially from S2. There is an ongoing discussion whether the input from S2 to posterior INS or the input from the nuclei of the medial thalamus (more specifically from the VMpo; see, e.g., Craig 2003a, 2004; Frot and Mauguie`re 2003) dominates the processing of the posterior INS. Recent human intracranial recordings suggest that S2 and the posterior part of INS are involved in different aspects of stimulus processing with S2 being more dedicated to finer-grain discrimination task, especially in non-painful up to painful levels, whereas the posterior part of INS is specifically involved in the analysis of painful stimuli and seems to be involved in the triggering of affective cognition and motor reactions (Frot et al. 2007). The INS interacts intensively with limbic structures, e.g., with the ACC (Rainville et al. 1997; Treede 2003; Treede et al. 1999).

An important source of variance for the investigation of brain regions involved in the analysis of a present noxious stimulus is the kind of stimulation performed. Whereas noxious electrical and mechanical stimulations seem to be associated with a strong activation of S1, this activation is questionable for noxious heat stimuli. In this case, an activation of S1 was shown to depend on the stimulus intensity and spatial summation (Apkarian et al. 2005; Peyron et al. 2000). Moreover, intracortical registrations of evoked responses to laser-heat stimuli have shown that the activation of S1 does not precede the activation of S2; rather activation of S2 precedes the activation of S1 and the dorsal INS (Apkarian et al. 2005; Frot and Mauguie`re 2003; Ohara et al. 2004a,b). Therefore it was hypothesized recently (Apkarian et al. 2005) that S2 might activate S1 rather than conversely. To prove this hypothesis, the analysis of a dynamic causal relationship seems necessary. Furthermore, the INS might be subdivided into morphological (Craig 2003b,c, 2004; Mesulam and Mufson 1982) and functional subregions with the posterior part being activated earlier than the anterior part of the INS. The anterior part is thought to be activated by the posterior part and its role has been proposed as being especially important for emotional processing of the nociceptive input (Apkarian et al. 2005; Craig 2003c, 2005).

The aim of the study was to investigate the course of directed and mediated interactions using bivariate and partial time-variant GCIs (tvGCIs) for pairs of electrodes of multichannel EEG recordings obtained while subjects were processing noxious laser-heat stimuli. Partial tvGCI was shown to be a reliable method for the analysis of directed interactions between two signals, where signal X affects the course of signal Y or vice versa. In contrast, bivariate tvGCI also tests for mediated interactions, i.e., interactions that are mediated by a third source Z that couples the activities of sources X and Y.
METHODS

Nine healthy student volunteers aged 19 to 30 yr participated in the present study. All participants were right-handed according to the Edinburgh test of handedness (Oldfield 1971). In accordance with the Declaration of Helsinki, subjects were informed verbally and by written instructions about all parts of the experiment and instructed that participation could be terminated at any time without any negative consequence. Subjects further signed an informed consent prior to the experiment and the study was approved by the ethics committee of the Friedrich Schiller University. All subjects were paid for participation.

Pain stimulation was achieved by a thulium YAG laser stimulator with a laser-beam wavelength of 1.8 μm with a beam diameter of 30 mm². Stimuli consisted of brief heat pulses of 1.4 ms duration applied to the dorsum of the left hand. Stimulus location was visualized by a red helium laser beam pointing onto the dorsum of the hand. To avoid possible tissue damage due to heat accumulation in the skin layers and to comply with regulations of our ethics committee, the following safety maneuvers were applied.

1) Subjects and experimenters wore protective goggles throughout the whole experiment.
2) To avoid tissue damage, the location of stimulus application was slightly shifted after each single stimulus by a stepwise displacing of the optic lens of the laser probe using a computer-controlled step motor. This procedure ensured that no spot of the skin was stimulated twice within succeeding trials of stimulation.
3) To minimize the risk of skin burns or skin irritation, the energy of the laser stimulation was kept below a maximal value of 600 mJ. Subjects who did not experience a clear faint to moderate pain sensation at this maximal stimulus intensity during a test session prior to the experiment proper were excluded from the study (Weiss et al. 2003).

Prior to the experiment proper, each subject’s perception and pain thresholds were determined. In the experiment, subjects received 75 laser-heat stimuli of different intensities on the dorsum of the left hand. At 2.5 s after each single stimulus application, subjects were asked to rate its intensity by a standardized rating scale (Weiss et al. 1997), ranging from 0 (no perception) to 6 (unbearably painful). Pain sensation was defined as the intensity yielding a sharp and painful pinprick sensation whose intensity corresponded to a rating of 3. During the experiment the intensity of laser stimuli was increased slightly above this level and fixed at an energy rate of 4 [mean energy used was 461.33 ± 63.68 (SD)]. This intensity was described as being moderately painful by all subjects. Succeeding stimuli were delivered with an interstimulus interval (ISI) of 25 ± 5 s.

During the experimental session, the EEG was recorded from 62 scalp sites referred to Cz using Ag/AgCl electrodes. The ground electrode was placed between electrodes Pz and Oz. All electrode impedances were kept at <5 kΩ. On-line EEG filters were set to 70 and 0.1 Hz (low-pass and high-pass, respectively) and data were digitized at a rate of 500 Hz. Recordings were subsequently rereferenced to a common averaged reference.

Herein, only data from 24 electrodes will be processed (Cz, C2, C4, C6, T8, TP8, CP6, CP4, CP2, P8, P6, P4, CPz, P3, P5, P7, CP1, CP3, CP5, TP7, T7, C5, C3, C1, according to the extended International 10–20 System of Electrode Placement). All single EEG trials were inspected visually and trials with muscle artifacts and electrode drifts were removed from all further analysis. Ocular correction was applied based on the method of Gratton et al. (1983). Both artifact procedures left ≥50 single-trial recordings per electrode and subject. During off-line analysis, event-related potential (ERP) averages were determined for each electrode and each subject, including 2,048 ms before and 1,024 ms after laser stimulus onset.

With reference to earlier results of intracortical recordings of LEPs (e.g., Lenz et al. 1998a,b, 2000), and to keep the number of statistical comparisons reasonable, we defined five intervals for GCI analysis of LEP activities that reflect activations of different brain areas of cortical stimulus processing: The first time window started at stimulus onset and covered the first 80 ms poststimulus. This time window is considered as one where the cortex does not yet receive laser-heat induced afferent input, thus serving as a precortical activity interval. This interval was followed by a window consisting of the activity of the N2 or N190 component (170–200 ms), which was demonstrated as being generated in the S2; the P2 or P290 component (260–320 ms), proposed as being generated by the ACC; and the S2 and the P3-like or P360 component (322–410 ms), proposed as generated by a widely distributed neural network (Garcia-Larrea et al. 1997; Siedenberg and Treede 1996). For each of the intervals, bivariate and partial GCIs were calculated as described in the following section.

PROCEDURES

Time-varying VAR models. The underlying mathematical framework for the linear Granger causality based on the principle of predictability is given by an autoregressive (AR) model (Granger 1969). This concept assumes that all underlying processes fulfill the assumption of stationarity. However, just this assumption is often not satisfied for EEG signals. Thus there is a need for a natural extension to nonstationary cases, which may be realized using a time-variant vector autoregressive (tvVAR) model, given by

![Figure 1. Recording schema for the laser-evoked event-related potential (LEP) measurements and the 24 electrodes used for the analysis of the Granger Causality Index (GCI) (Cz, C2, C4, C6, T8, TP8, CP6, CP4, CP2, P8, P6, P4, CPz, P3, P5, P7, CP1, CP3, CP5, TP7, T7, C5, C3, C1, according to the extended International 10–20 System of Electrode Placement).](http://jn.physiology.org.org)
\[ X(n) = \sum_{r=1}^{p} A_r(n) \cdot X(n-r) + U(n) \]  

(1)

Thereby, \( A_r(n) \in \mathbb{R}^{M \times M} \) denotes the (time-varying) autoregressive coefficients, \( X(n) \in \mathbb{R}^M \) is the \( M \)-dimensional state space vector of the time-variant autoregressive process \( X \), and \( U \) represents an \( M \)-dimensional zero mean, pairwise uncorrelated, Gaussian noise process. One possible way for fitting the VAR model is to use a short time window algorithm (Ding et al. 2000). A second approach is based on adaptive filtering, e.g., Kalman filtering (Arnold et al. 1998), least mean squares (LMS) approach (Möller et al. 2003; Schack 1999), or the recursive least squares (RLS) approach (Möller et al. 2001). In the present study we prefer the generalized RLS approach because it allows the simultaneous fitting of one mean VAR model for a set of single trials when each of the trials represents a realization of the same process (independent repetition of an experiment). This leads to an improvement of the VAR-parameter estimation when increasing the number of trials. This advantage is useful for ERP studies where a set of single trials is available for each data set (subject).

Time-varying Granger Causality Index. Basically, there are two principal approaches to define a time-varying Granger causality: a bivariate and a multivariate (partial) one.

- Let \( X_i = \{ x_i(n) \} \) and \( X_j = \{ x_j(n) \} \) be two components of an \( M \)-dimensional process \( X \). If knowledge of the past of both process components, \( X_i \) and \( X_j \), improves the prediction of the presence of \( X_i \) in comparison to only having knowledge of the past of \( X_j \), then the component \( X_i \) influences (Granger causes) the component \( X_j \). Consequently, this principle requires models that provide two prediction errors under different past assumptions. Thus (time-varying) VAR models are suitable for the realization of this concept. Working with an underlying time-variant VAR model, a time-variant Granger Causality Index can be effectively utilized. In this case, two models have to be fitted. The first one considers \( (X_i, X_j) \) as a two-dimensional tVAR process and the estimation for a given realization regarding the \( i \)-th component is given by

\[ x_i(n) = \sum_{r=1}^{p} a_{ir}(n) \cdot x_i(n-r) + \sum_{r=1}^{p} a_{jr}(n) \cdot x_j(n-r) + \hat{u}(n) \]  

(2)

The second one considers \( X_j \) as a one-dimensional tVAR process with the estimation

\[ x_j(n) = \sum_{r=1}^{p} b_{jr}(n) \cdot x_j(n-r) + \hat{v}(n) \]  

(3)

Thus the prediction error \( u(n) \) depends not only on the past of signal \( X_i \), but also on the past of signal \( X_j \). In both cases, the accuracy of prediction may be expressed by the corresponding variance of the prediction errors. In fact, we obtain for the univariate case (Eq. 3) a time-variant variance, denoted by

\[ \sigma_{ii}(n) = \text{var}[\hat{u}(n)] \]  

(4)

and for the bivariate case (Eq. 2) a time-variant variance

\[ \sigma_{ij}(n) = \text{var}[\hat{u}(n)] \]  

(5)

Now, the time-variant bivariate Granger Causality Index (tvGCI) is defined by

\[ F_{ij\rightarrow j\leftarrow j}(n) = \ln \frac{\sigma_{ij}(n)}{\sigma_{ii}(n)} \]  

(6)

Obviously, all pairs \( (i, j), i \neq j \), of an \( M \)-dimensional process may be considered, which results in \( M^2 - M \) possible combinations. Since the variance of the bivariate model should be less than or equal to the variance of the univariate model, the tvGCI should not be negative. However, in practice, sometimes it is possible that the tvGCI becomes negative. This effect is based on the parameter estimation procedure. A detailed explanation of this effect and the appropriate use of time-variant tvGCI is described in Hesse et al. (2003).

The multivariate concept considers the \( M \)-dimensional process \( X \) as a whole. To investigate the influence of a component \( X_i \) on the component \( X_j \), again two models are considered: an \( M \)-dimensional (full) model and an \( (M-1) \)-dimensional (reduced) model, where the \( j \)-th component is excluded. Thus for one realization we have

\[ x_i(n) = \sum_{m=1}^{M} \sum_{r=1}^{p} a_{im}(n) \cdot x_m(n-r) + \hat{a}(n) \]  

(7)

for the full model and

\[ x_i(n) = \sum_{m=1}^{M} \sum_{r=1}^{p} b_{im}(n) \cdot x_m(n-r) + \hat{v}(n) \]  

(8)

for the reduced model with the excluded component \( x_j \). Analogously to the bivariate case, we define a partial tvGCI by

\[ F^p_{ij\rightarrow j\leftarrow j}(n) = \ln \frac{\sigma^2_{ij}(n)}{\sigma^2_{ii}(n)} \]  

(9)

with

\[ \sigma^2_{ij}(n) = \text{var}[\hat{v}(n)] \quad \text{and} \quad \sigma^2_{ii}(n) = \text{var}[\hat{u}(n)] \]  

(10)

In practice, Eq. 9 can be estimated by

\[ F^p_{ij\rightarrow j\leftarrow j}(n) = \ln \frac{s^2_{ij}(n)}{s^2_{ii}(n)} \]  

(11)

where \( s^2_{ij}(n) \) and \( s^2_{ii}(n) \) are the estimated variances based on the residuals \( \hat{v}(n) \) and \( \hat{u}(n) \), respectively.

The method can be applied to laser-heat evoked brain potentials (LEPs), each realization of the process \( X \) being defined by the single-subject average (or each single trial). For LEPs, the different electrodes represent source components, whereas time (sample points) is denoted by \( n \).

Determination of model and estimator parameters. For the processing of the event-related potentials, the VAR order \( p \) was chosen according to the AIC criteria, and it was tuned to approach coincidence between the estimated parameter spectrum and the Fourier spectrum of the signal. Finally, we used a fixed order of \( p = 10 \) for all underlying VAR models. To obtain a satisfactory balance between the adaptation speed and variance of the estimation (Möller et al. 2001), a fixed adaptation factor of \( c = 0.03 \) was used. For all nine data sets and all pairwise relationships of the 24 electrodes, the time-variant bivariate GCI according to Eq. 6 and the partial GCI according to Eq. 9 were calculated for all time points.

For statistical analysis and comparison of interactions between electrodes following the stimulus compared with a nonstimulus-affected prestimulus condition, a control interval was investigated within 1,000 ms before stimulus onset. Within each interval, tvGCIs were averaged across time. Wilcoxon tests for paired observations were used with the significance level of \( P < 0.05 \) for noncorrected and to \( P < 0.0125 \) for Bonferroni-corrected (multiple) comparisons to decide whether a directed connection exists in the four intervals. The sum of sources (outgoing interactions) and sinks (receiving interactions) of directed interactions per electrode in each time interval demonstrates the mean direction of the relationship between the different electrodes; i.e., sources are driving regions during this time interval, whereas sinks are receiving brain regions. The numbers of interactions between time intervals were compared using Pearson’s chi-square test.

Finally, a dipolar source modeling was performed on the grand average of the LEPs using brain electrical source analysis (BESA;
Scherg 1990), also known as the spatiotemporal source method (Valeriani et al. 2001). It should be mentioned that we use the term "dipole generator" for the BESA results to distinguish it from the terms "sources" and "sinks" of the GCI analysis (that represent distinct electrodes). The BESA method deals with the so-called inverse problem; i.e., it predicts location, orientation, and time course of the activation strength of the generators from a definite number of recorded channels. This problem is ill-posed since an infinite number of solutions are possible. The use of physiological knowledge (i.e., from intracranial recordings or from functional brain imaging) might help to reduce the number of possible solutions. It has been shown that the results of this approach depend strongly on the user’s strategy (Miltner et al. 1994). A nonlinear least-square estimator is used to calculate the differences between the field distribution, obtained by the dipolar model, and the measured fields. This difference is referred to as residual variance (RV), which is minimized by an iterative procedure. BESA can also be used to verify whether a hypothesized dipolar model accounts for the recorded magnetic fields or potentials (i.e., LEPs). Here, we used a sequential analysis as described in detail by Valeriani et al. (2001). For this analysis, the entire time interval was divided into segments, similarly to those for the tvGCI analysis. In a first step, we used the grand average to test whether different models are sufficient (RV < 5%) to explain the LEP grand average. The first model includes three dipole generators most consistently found to explain LEP generators (García-Larrea et al. 2003): i.e., one dipole in the anterior cingulate cortex (ACC) and two dipoles in the opercular cortex including the secondary somatosensory cortex S2. We then used the two other models, that of Tarkka and Treede (1993) and that of Valeriani et al. (1996). Coordinates given for dipole solutions by Valeriani et al. (1996) were used as starting points; however, two other locations with ±2-cm difference to these starting points were also tested to minimize the risk of receiving solutions in local minima. For RV < 5%, mean dipole strength for each fitted dipole and the SD of its strength were calculated from the estimated dipole strengths of each time point in the baseline interval, from −450 to 0 ms. Then, estimated dipole strength of each time point of the poststimulus interval was compared with the mean value of dipole strength of baseline activity +3SDs during baseline. Dipoles were considered to be active with respect to the processing of the laser stimulus starting with that sample point for which the estimated dipole strength exceeds the mean strength of baseline activity +3SDs. In a second step, the analysis was performed on the averaged LEP of each individual to provide information on the variance of spatial localization. Although the advantage of this approach should not be overestimated due to intrinsic limitations of the approach (for review, see Valeriani et al. 2001), it provides useful information on the possible cerebral sources and voltage fields produced by these sources at each electrode site (e.g., Cruccu et al. 2003; Oppsommer et al. 2001).

**Simulation: mediated interactions—bivariate versus partial tvGCI**

This simulation investigates the performance of both tvGCI methods in the most extreme situation, i.e., in a nonstationary situation when the connections between the signals are suddenly changed in a noncontinuous way. We used a process proposed by Baccala and Sameshima (2001). These authors estimated connections in the frequency domain by applying the partial directed coherence (PDC) method. We extended this simulation insofar as we introduced three additional time intervals without interactions. Thereby the directed interactions according to Baccala and Sameshima (2001) exist only in the middle interval. The structure of interactions is given in Fig. 2. In particular, as proposed by Baccala and Sameshima, the simulated five-dimensional AR(2)-model (autoregressive model of order p = 2) contains directed interactions from signal component 1 to 2, from 2 to 3, from 3 to 4, and from 4 to 5 within the interval (Fig. 2B) from the sample point $n = 513, \ldots, 1,024$. In this time interval, there is also a feedback interaction from signal component 5 to 4. There are no interactions in the first ($n = 1, \ldots, 512$) and third intervals ($n = 1,025, \ldots, 1,536$) (Fig. 2, A and C).

The simulation was performed with 20 trials using the multtrial approach of Möller et al. (2001) for the bivariate and partial GCI. The partial GCI yields the correct interactions introduced to the model; the result is similar to that obtained when applying the PDC in the frequency domain (Baccala and Sameshima 2001). The bivariate GCI shows a strong mediated interaction from node 1 to node 3, which we consider to be the reason for the reduced GCI from node 2 to node 3. Furthermore, there are some mediated interactions in the bivariate GCI from node 3 to node 2, from 1 to 4, from 2 to 4, and from 3 to 5. The partial approach does not yield mediated interactions (Fig. 2D). However, these sudden noncontinuous changes at the transitions between intervals result in an adaptation period because the parameter estimation restarts at each discontinuity.

We have also investigated the undirected connections between the five signals, by using the partial correlations (Withhaker 1990) that also detected the mediated, but undirected, connections (not present here due to space limitations).

**RESULTS**

Application of laser heat was accompanied by clear pain sensations. The mean energy applied was 461 mJ and the mean stimulus intensity was rated as 4 on the verbal description scale.

Figure 3 shows the grand averages of the laser-heat evoked brain potentials (LEPs) for several electrodes. Several components with similar waveforms as in previous studies on LEPs (e.g., Bromm and Chen 1995; Dillmann et al. 2000; Friederich et al. 2001; Legrain et al. 2003; Mouraux et al. 2004) can be identified. These are the N190 and the P290 with maximal magnitudes at the electrode Cz as well as a P300-like component (García-Larrea et al. 1997) with maximal magnitude at CPz. Based on the grand average and previous results of intracortical recordings of LEPs (Lenz et al. 1998a,b; Ohara et al. 2004a,b; Peyron et al. 2002; Vogel et al. 2004), four intervals were selected for all further LEP analyses: 0–80 ms as the uninfuenced interval, 170–200 ms as the N2 (N190), 260–320 ms as the P2 (P290), and 320–400 ms as the P3 (P360).

For both bivariate and partial GCIs Fig. 4 shows significantly higher tvGCIs for these intervals compared with the baseline condition. The arrows indicate the direction of the interaction. Since there are massive interactions for the N2, P2, and P3 intervals, sources and sinks using the bivariate GCI approach are represented in Fig. 5A, whereas sources and sinks for the partial GCI approach are represented in Fig. 5B.

As can be seen in Fig. 4, there are only a few directed interrelations between electrodes within the interval directly following stimulus onset. The bivariate tvGCI approach identified some electrodes over the contralateral centroparietal region (especially CP4, but also P4 and P6) as main sources and indicates that electrodes at the posterior parietal and contralateral sides with regard to hand stimulation are driven by these electrodes. In contrast, the partial tvGCI approach did not identify any significant relationship for this interval.

During the N2 interval, both bivariate and partial tvGCI identified significantly more interactions between electrodes compared with the previous interval directly following stimulus onset (bivariate: $P < 0.001$; partial: $P < 0.01$, respectively). For the bivariate tvGCI approach, several sources can
be identified, mainly at the central electrodes (e.g., C2, C5, C6), whereas sinks emerge at the centroparietal region ipsilaterally to the stimulation side as well as at the temporal region (T8) contralaterally to the stimulation side (Fig. 5). In contrast, the partial tvGCI approach suggests a single source at electrode CP1 with sinks at the centroparietal electrodes on the right hemisphere (Figs. 4 and 5).

For the P2 interval, the number of interactions between electrodes increased further for both bivariate and partial tvGCI approaches; there are significantly more interactions in the P2 interval compared with the interval directly following stimulus onset (both \( P < 0.001 \)). However, a significant increase of interactions from the N2 to the P2 interval was observed only for the bivariate approach (\( P < 0.001 \)). Sources were found mainly at central electrodes (e.g., C2, Cz, CPz), whereas sinks were located at lateral electrodes of both hemispheres (Fig. 5). The main sinks are expressed at C5, C4, C3, and T8. For the partial tvGCI approach, the main source emerges at electrode CPz; additional sources are also present at electrodes C1, C2, and CP1. These source electrodes interact with most other electrodes; however, there is an accentuation of sinks at parietotemporal electrodes of both hemispheres (Fig. 5).

The bivariate tvGCI identified a huge amount of interactions for the P3 interval (Fig. 4). Although the number of interactions increases significantly compared with the P2 interval (\( P < 0.001 \)), the main source electrodes (Cz, C2) and most of the sinks (C5, C4, T8) remain stable compared with the P2 interval. Analogously to the bivariate approach, the partial tvGCI approach also identified a significantly higher number of interactions (\( P < 0.001 \)) between electrodes (Fig. 4); however, the topographical distribution of sources and sinks differs from that of the bivariate approach. For the partial tvGCI, the main source emerges at electrode CPz; additional sources are also present at electrodes C1, C2, and CP1. These source electrodes interact with most other electrodes; however, there is an accentuation of sinks at parietotemporal electrodes of both hemispheres (Fig. 5).

BESA dipole modeling with the three dipoles that were most consistently found to explain LEP generators (two in the opercular cortex and one in the ACC; Garcia-Larrea et al. 2003) showed a residual variance RV of 11.4%. Including an additional dipole in the primary somatosensory cortex S1 (model of Tarkka and Treede 1993) leads to a minimal improvement (RV \( = 10.6% \)). The model of Valeriani et al. (1996) using a five-dipole model yielded an RV of 3.9%. Two dipoles were localized bilaterally in the parietal operculum, a region that includes the secondary somatosensory cortex S2 (Fig. 6). With regard to the stimulated hand, the activity of the first S2 dipole started at the contralateral hemisphere at around 114-ms poststimulus onset (criterion: activity 3SDs above baseline activity in the time interval from 450 to 0 ms). The activity of the second dipole occurred at the ipsilateral hemisphere at around 136 ms poststimulus onset. A third dipole was localized...
in the posterior part of ACC, beginning its activity at around 140 ms poststimulus onset with main activities at 180 and 278 ms. Two additional bilateral dipoles were identified in the INS. Their activity peaked contralaterally at around 160 and 238 ms and ipsilaterally around 184 ms. The analysis also revealed that the contralateral INS contributed considerably to the P2 component of the LEP. RV remained <5% for the time interval from 0 to 440 ms. Furthermore, the addition of one further dipole located testwise in S1 did not improve the RV of the five-dipole model at any time point of the LEP waveform for >0.3% RV.

In a second step, BESA analysis was performed on averaged LEP for each individual to provide information on the variance of spatial localization. We found that the five-dipole model was able to best explain the averaged LEP. Similarly to the analysis on the grand average, two dipoles were localized bilaterally in the parietal operculum, the third dipole in the ACC, and the two remaining dipoles were identified bilaterally in the INS (Table 1).

**DISCUSSION**

Bivariate and partial tvGCIs were calculated for LEPs in five different intervals, i.e., within a baseline period, an interval immediately following stimulus onset, the N2, P2, and the P3 interval. Similarly to simulations, the present analysis demonstrated striking differences between the results of bivariate and partial tvGCIs.

Starting with the interval immediately following stimulus onset, the bivariate tvGCI indicated some interactions between electrodes located over the right (contralateral) posterior parietal cortex, whereas no interactions at these sites were found for partial tvGCI. The posterior parietal cortex was shown as being involved in spatial attention (Forss et al. 2005). This result indicates that spatial attention occurs in preparation for the noxious stimulation, similar to preparatory processes found during aversive conditioning in humans (Arnold et al. 1998; Miltnner et al. 1999). The interactions within the posterior parietal cortex might build up network circuits of information processing, including intracortical interactions as well as communication with subcortical structures such as the thalamus or the lentiform nucleus. Such networks might be influenced by hidden sources, i.e., sources that were not recorded. Such hidden sources might result in mediated interactions detected by both bivariate and partial tvGCIs. However, if all nodes are included in the model, the partial tvGCI does not produce any mediated connection. In contrast, the bivariate analysis includes only interactions between electrode pairs not including the remaining M-2 nodes. The excluded nodes might act as hidden sources for the bivariate model, resulting in mediated interactions between the two considered nodes.

For the N2 interval, bivariate tvGCI revealed several sources mainly at the central electrodes (e.g., C2, C5, C6) with sinks located in the ipsilateral centroparietal region and the contralateral temporal region. This pattern of interactions might represent activity of both S2 and/or the posterior insula (INS). Subdural recordings from the parasylvian cortex (Lenz et al. 1998b) revealed LEPs with a negative N2 component that corresponds ideally with the N2 of scalp electrodes. Moreover, these LEP components, as modeled by dipole sources analysis, appeared in the S2/insula region both ipsilaterally and contralaterally. Such sources were also obtained by several other groups (Bromm and Chen 1995; Kanda et al. 2000; Miyazaki et al. 1994; Tarkka and Treede 1993; Valeriani et al. 2000; Watanabe et al. 1998; for review see Garcia-Larrea et al. 2003). Taking into account the anatomical location of the parietal

![FIG. 3. Time course of the grand average of the laser-heat evoked brain potential (LEP) for the 24 electrodes selected. Thick lines correspond to electrode Cz (black), C2 (dark gray), C4 (black dashed), and CPz (light gray).](http://jn.physiology.org/)

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operculum (S2; see Eickhoff et al. 2006a,b) and the INS, the sources revealed by the bivariate tvGCI approach might depict the driving activity of S2/INS networks (Garcia-Larrea et al. 2003; Peyron et al. 2000). Additionally, source localizations of the N2 component in S1 have been reported for scalp LEPs (Tarkka and Treede 1993), laser-evoked magnetic fields (LEFs; e.g., Kanda et al. 2000), and subdural recordings (e.g., Ohara et al. 2004b). There is evidence that the S1 sources are probably located in Brodmann’s area 3a (for review, see Apkarian et al. 2005; Craig 2003a). Therefore possibly the distribution of tvGCI sources on the contralateral hemisphere to the side of laser stimulation differs from that of the ipsilateral side by an additional source around the S1 representation of the hand even if we did not find an S1 source in our model.

In contrast, the partial tvGCI approach identified only a single source at electrode CP1 with sinks located mainly at centroparietal electrodes on the right hemisphere (Figs. 4 and 5). This is an unexpected constellation. One might speculate whether the source at CP1 represents an early activation of the cingulate cortex. Our dipole modeling revealed an early activity of the ACC source with a maximum at around 180 ms. Furthermore, the dipole orientation would favor a left-sided source (see Fig. 6). However, subdural recordings in patients showed that the negative component in the ACC occurred later in time (211–242 ms; Lenz et al. 1998a), i.e., at a time where our model showed a small local extreme (240 ms). Another hypothesis is that the source at CP1 represents an activation of the thalamus as the driving place for all other cortical regions. It is well known that cortical regions receive nociceptive information from lateral and medial thalamic nuclei (e.g., Treede et al. 1999) whose maximal activity emerges around the time when N2 becomes maximal (Craig 2003a; Lenz and Dougherty 1997). However, it remains an unsolved question whether the Granger causality approach might indeed uncover such deep sources as generators of cortical regions.

For the P2 interval, bivariate GCI revealed several sources mainly at central electrodes (e.g., C2, Cz, CPz), whereas sinks are located at lateral electrodes of both hemispheres with the main sources of sinks at electrodes C5, C4, C3, and T8 (Fig. 5). This pattern might represent the activity of the ACC as well as of both S2 and the insular region. Unexpectedly, the mean interaction does not clearly reflect directed interactions from insular regions toward the ACC, but rather directed interactions from the ACC toward the insular of each hemisphere. In other words, the information processing within the ACC might drive the information processing within the posterior INS in this interval. Taking into account current views that the ACC receives its information not only from thalamus and brain stem but also from the insula (e.g., Price et al. 2000, 2006), this result might add additional information—that the ACC also affects the posterior INS reciprocally and more than previously thought. In line with this interpretation, in subdural recordings from the parasylvian cortex and the ACC Lenz et al. (1998a,b) showed that the positive component within the insular region occurs slightly later than that of the ACC. We propose that this directed interaction from ACC to the posterior INS might represent the inclusion of attentional activation and/or affective dimension into the processing. ACC is well known to play a role for these processes (e.g., Büchel et al. 2002; Vogt 2005). Such an interaction might also be of use for processing within the INS, taking into account its involvement in the processing of interoception and emotional state (e.g., Craig 2004, 2005).

The partial tvGCI approach for the P2 time window identified a main source at electrode CP1 similar to the N2 time window and some additional sources at adjacent electrodes (e.g., CP3). The sinks are located mainly at the lateral electrodes, with a preponderance at the side contralateral to the primary hemisphere.
stimulation (Figs. 4 and 5). Although the source at CP1 has been discussed in detail for the N2 interval and suggested as activity of the ACC, a possible alternative would place this source in S1 that might drive the processing in S2, posterior INS (pronounced on the right side), and posterior parietal cortex. All these sinks seems to be important: S2 and the posterior insula for the analysis of complex somatosensory and affective aspects of the noxious stimulation (Apkarian et al. 2005; Treede et al. 1999) and/or homeostatic emotions (Craig 2003b,c 2005), the posterior parietal cortex for organizing attentional processes toward the noxious stimulation (Forss et al. 2005).

With respect to the difference in the previous time interval, both types of GCI show more similar distributions of sources. Both of them demonstrate directed interactions between medial to lateral electrodes that possibly might be interpreted as directional flow from ACC to the posterior INS and S2 for the inclusion of attentional resources and/or the affective dimension into processing of the noxious stimulus.

For the P3 interval, the bivariate tvGCI revealed a significantly higher number of interactions than that of the previous intervals. Thus the main source electrodes (Cz, C2) and most important sinks (C5, C4, T8) remain nearly stable compared with the P2 component. Therefore results might be interpreted similarly to the P2 interval, i.e., as signs of an intensive information transfer between the ACC, S2, the posterior INS, and the posterior parietal cortex. This processing seems to be intensified, involving broader cortical regions that might contribute to the generation of the intensity rating of stimuli.

The partial tvGCI approach for the P3 interval identified an increasing number of significant interactions with a main source at the electrode CPz and additional sources mainly at C1, C2, and CP1. This might indicate the involvement of the anterior and posterior cingulate cortices (e.g., Bromm and
and projected onto the Talairach space localized by means of a brain electrical source analysis (BESA).

### Table 1

<table>
<thead>
<tr>
<th>Dipole Localization</th>
<th>x, mm</th>
<th>y, mm</th>
<th>z, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral parietal operculum</td>
<td>48.5 ± 2.4</td>
<td>−15.1 ± 4.6</td>
<td>16.8 ± 1.2</td>
</tr>
<tr>
<td>Ipsilateral parietal operculum</td>
<td>−46.6 ± 2.4</td>
<td>−13.1 ± 4.3</td>
<td>19.1 ± 1.2</td>
</tr>
<tr>
<td>Anterior cingulate cortex (ACC)</td>
<td>1.3 ± 3.1</td>
<td>−2.5 ± 6.6</td>
<td>39.9 ± 3.3</td>
</tr>
<tr>
<td>Contralateral insula</td>
<td>29.0 ± 2.5</td>
<td>−32.9 ± 4.3</td>
<td>18.2 ± 3.5</td>
</tr>
<tr>
<td>Ipsilateral insula</td>
<td>−27.3 ± 2.6</td>
<td>−31.0 ± 4.0</td>
<td>20.2 ± 3.8</td>
</tr>
</tbody>
</table>


A second problem arises from the high number \((M)\) of electrodes. On the one hand, one wishes to increase the number of electrodes to cover the whole brain with sufficient spatial resolution for the analysis. However, this results in a higher number of interactions between electrodes to be investigated \([M \times (M - 1)]\). Since computation is considerable and increases quadratically to \(M\), we chose electrodes that cover the centroparietal regions of the brain. These electrodes are known to represent the major components of the LEPs (e.g., Bromm and Chen 1995; Garcia-Larrea et al. 2003). However, it should be emphasized that some regions known to be involved in pain processing (for summary see, e.g., Apkarian et al. 2005; Peyron et al. 2000) are not covered by our choice, i.e., the anterior INS, the anterior parts of ACC, or prefrontal regions. On the other hand, a smaller number of electrodes has some advantages for the VAR modeling. The accuracy of the VAR parameter estimation decreases with an increasing number of investigated electrodes. Furthermore, and strikingly, the dimensionality increases by the number of parameters that should be estimated by the AR model. Although theoretically a higher number of electrodes allows the partial tvGCI approach to better distinguish direct from indirect interactions, it also poses a secondary problem. It is impossible to integrate all relevant components of direct interactions into a single model. The reasons relate to the fact that we are unable to measure all possible sources, such as deep sources (as seen for the N2 component) or tangential sources by the EEG methodology. Additionally, the higher the dimensionality, the higher the number of trials necessary to receive a reliable estimation of a model parameter. However, there are conceptual limitations to increase the number of trials exponentially: first, extremely high numbers of trials do not represent the same processes due, for example, to influences of habituation, and fluctuation of attention and, second, high numbers of trials lead to unacceptably long computation times. In this sense, the bivariate tvGCI approach needs fewer trials (and/or data points) to obtain a sufficiently reliable estimation of model parameters. Therefore one has to decide to use the partial tvGCI approach with a smaller dimensionality to have a more reliable estimation of the model.
parameters and the bivariate tvGCI approach with a higher number of electrodes. In the case of laser-evoked brain potentials, the bivariate tvGCI approach seems to be more useful than the partial GCI approach. The “graphical models”—a methodology to visualize and reveal relationships in multivariate systems (Dahlhaus and Eichler 2002)—might be helpful in interpreting multiple interactions.

The analysis of brain generators of LEPs using BESA revealed that our LEPs could be explained by five dipoles: two bilaterally in S2, one in the ACC, and two bilaterally in the posterior INS. Dipoles of LEPs were previously most often localized in the S2 bilaterally as well as in the ACC (for review, see Garcia-Larrea et al. 2003). The coordinates of the dipoles in these structures agree with various other results (e.g., Bromm and Chen 1995; Garcia-Larrea et al. 2003; Opsommer et al. 2001). Similar to previous results, the contralateral S2 appeared to be activated 10–20 ms before the ipsilateral S2. The interindividual variance is highest in the anterior–posterior direction (y coordinate; see Table 1), which is in line with results from intersubject comparisons (Garcia-Larrea et al. 2003). Interestingly, our model includes two additional dipoles in the posterior INS. These dipoles lay posterior to the S2 dipoles for the grand average as well as for each single individual. These localizations are in line with intracortical recordings of Frot and Mauguie (2003). These authors described intracortical LEP generators to CO2-laser stimulation both in the operculum and in the posterior INS. Moreover, dipole activation patterns reported here closely match the intracortical LEPs described by Frot and Mauguie (2003).

The anatomical accurateness of the dipole localizations of the BESA approach should not be underestimated due to the intrinsic limitations of the approach (Valeriani et al. 2001). A possible way to overcome these limitations might be the use of fMRI to provide anatomical constraints for the dipole analysis. The temporal activation patterns of the diverse dipoles resulting from the analysis of EEG/MEG recordings of the same paradigm can then be used for a succeeding tvGCI analysis. Such an approach not only will solve the question concerning the direction of interactions between the ACC, S2, and INS, but also will reduce the problem of dimensionality in the tvGCI approach.

In summary, results show some similarities, but also some striking discrepancies between the results of bivariate and partial tvGCIs that might be explained by the fundamentally different methodological nature of these two approaches. It is interesting to note that both tvGCI approaches revealed directed interactions from medial to lateral electrodes in the centroparietal region in the intervals of P2 and P3, which may be interpreted as a directed interaction between the ACC, S2, and posterior INS. We propose that these directed interactions serve for the inclusion of attentional resources and/or the affective dimension into processing of the noxious laser stimuli. Using fMRI and EEG/MEG recordings in the same paradigm might result in a more realistic estimation of dipole generators and their activation patterns, which then might be used for a more reliable investigation of directed interactions by the tvGCI approach.

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GRANTS

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DISCLOSURE

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