Modulations of single-trial interactions between the auditory and the visual cortex during prolonged exposure to audiovisual stimuli with fixed stimulus onset asynchrony

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The perception of simultaneity between auditory and visual stimuli is of crucial importance for audiovisual integration. However, the speeds of signal transmission differ between the auditory and the visual modalities and these differences have been shown to depend on multiple factors. To maintain the information about the temporal congruity of auditory and visual stimuli, flexible compensation mechanisms are required. Several behavioral studies demonstrated that the perceptual system is able to adaptively recalibrate itself to audio-visual temporal asynchronies (Fujisaki et al., 2004; Vroomen at al., 2004). Here we explored the adaptation to audio-visual temporal asynchronies at the cortical level. Tone and light stimuli at the same constant stimulus-onset-asynchrony were presented repeatedly to awake, passively listening, Mongolian gerbils. During stimulation the local field potential was recorded from electrodes implanted into the auditory and the visual cortices. The dynamics of the interactions between auditory and visual cortex were examined using the Directed Transfer Function (DTF; Kaminski & Blinowska, 1991). With increasing number of stimulus repetitions the averaged evoked response of the Directed Transfer Function exhibited gradual changes in amplitude. A single-trial analysis indicated that the adaptations observed in the all-trial average were due to modulations of the amplitude of the single-trial DTFs but not to alterations in the trial-to-trial dynamics of DTF peaks.

1. Introduction

Several studies have demonstrated the crucial role of temporal stimulus congruity in the binding of multisensory information (e.g. Morein-Zamir et al., 2003; Bertelson & De Gelder, 2004). Given the differences in physical and neuronal transmission times of auditory and visual signals, the question arises how synchronization of multisensory information is achieved in the brain. An increasing number of studies indicate that temporal perception remains plastic throughout life-time: when stimuli from different sensory modalities are presented repeatedly at a small constant temporal onset asynchrony after a while their temporal disparity is perceived as being diminished in the conscious experience. This chapter explores whether the synchronization dynamics between the primary auditory and visual cortex adapt flexibly to constant timing of auditory and visual stimuli. We applied a rodent preparation designed to mimic relevant aspects of classical experiments in humans on the recalibration of temporal-order judgment.

1.1 The speed of transmission of signals is modality specific

Apparently, precise information about the temporal congruity of multisensory information is not readily available in the nervous system. From the point in time a single event causes an auditory and a visual signal to the point in time a certain brain area is activated by these signals, the information about their relative timing is blurred by different speeds of transmission of the two signals in various ways. The first temporal disparities in signal propagation arise already outside the brain from the different velocities of sound and light. At the receptor level sound transduction in the ear is faster than phototransduction in the retina (see Fain, 2003, for a detailed review). The minimum response latency for a bright flash, ca. 7 ms, is nearly the same in rods and cones (Cobbs & Pugh, 1987; Hestrin & Korenbrot, 1990; Robson et al., 2003). But with low light intensities the rod-driven response might take as long as 300 ms (Baylor et al., 1984; Baylor et al., 1987). In contrast, the transduction by the hair cells of the inner ear is effectively instantaneously via direct mechanic linkage (about 10 microseconds, Corey & Hudspeth, 1979; 1983; Crawford et al., 1985; 1991).

Next, the duration of the transmission of auditory and visual signals depends on the length of the nerves used for their transmission (Von Békésy, 1963; Harrar & Harris, 2005). The relationship of transmission delays between sensory modalities is further complicated by the fact that in each modality processing speed seems to be modulated by both the detailed physical stimulus characteristics, like stimulus intensity (Wilson & Anstis, 1969), visual eccentricity (Nickalls, 1996; Kopinska & Harris, 2004), etc., and additionally by subjective factors, like attention (e.g., Posner et al., 1978, Posner et al., 1980).

1.2 Simultaneity constancy

The ability to perceive stimuli as simultaneous despite their different transmission delays has been termed simultaneity constancy (Kopinska & Harris, 2004). Several studies demonstrated that human beings were able to compensate for temporal lags caused by variances in spatial distance (Engel & Dougherty, 1971; Sugita & Suzuki, 2003; Kopinska & Harris, 2004; Alais & Carlile, 2005). Interestingly, the compensation also worked when distance cues were presented only to a single modality. In the study of Sugita and Suzuki (2003) only visual distance cues were used; Alais and Carlile (2005) varied only cues for auditory distance perception. The question which cues are essential to induce a lag compensation are still a matter of an ongoing debate as there are also several studies failing to find evidence for a similar perceptual compensation (Stone, 2001; Lewald & Guski, 2004; Arnold et al., 2005; Heron et al., 2007).

1.3 Temporal recalibration

The transmission delays of auditory and visual signals depend on multiple factors and cannot be described by simple rules. One way to deal with this complexity could be that the compensation mechanisms remain plastic throughout lifetime so that they can flexibly adapt to new sets of stimuli and their typical transmission delays.

Temporal recalibration to stimuli-onset-asynchronies of multimodal stimuli has been demonstrated in several studies (Fujisaki et al., 2004; Vroomen et al., 2004; Navarra et al., 2005; Heron et al., 2007; Keetels & Vroomen, 2007). In these studies, experimental paradigms typically start with an adaptation phase with auditory and visual stimuli being presented repeatedly over several minutes, consistently at a slight onset asynchrony of about zero to 250 milliseconds. In a subsequent behavioural testing phase auditory and visual stimuli are presented at various temporal delays and usually their perceived temporal distance is assessed by a simultaneity judgement task (subjects have to indicate whether the stimuli are simultaneous or not) or a temporal order judgement task (subjects have to indicate which of the stimuli they perceived first).

Using these procedures temporal recalibration could be demonstrated repeatedly: the average time one stimulus had to lead the other in order for the two to be judged as occurring simultaneously, the point of subjective simultaneity (PSS), was shifted in the direction of lag used in the adaptation phase (Fujisaki et al., 2004; Vroomen et al., 2004). For example, if sound was presented before the light in the adaptation phase, in the testing phase the sound stimulus has to be presented earlier in time than it had to before the adaptation, in order to be regarded as having occurred simultaneously with the light stimulus.

In addition, in several studies an increase in the Just Notable Difference was observed (JND, smallest temporal interval between the two stimuli needed for the participants in a temporal order task to be able to judge correctly which of the stimuli was presented first on 75% of the trials) (Fujisaki et al., 2004; Navarra et al., 2005).

1.4 Outlook on experiments

We applied an experimental paradigm resembling closely the previously described human studies on temporal recalibration: here we presented auditory and visual stimuli repeatedly to awake, passively perceiving, Mongolian gerbils at a constant temporal onset asynchrony of 200 ms.

The neural mechanisms underlying temporal recalibration have not yet been investigated in detail. The idea that temporal recalibration works on an early level of processing is quite attractive: More accurate temporal information is available at early stages as the different processing delays of later stages have not yet been added. But there are also reasons to believe that recalibration works at later levels: recalibration effects are usually observed in conscious perception.

In the last decades the primary sensory cortices have repeatedly been demonstrated to be involved in multisensory interactions (e.g. Cahill et al., 1996; Brosch et al., 2005; Bizley et al., 2006; Kayser et al., 2008; Musacchia & Schroeder, 2009). In the current explorative study we start to search for neural mechanisms of recalibration at the level of primary sensory cortex. We implanted one electrode into the primary auditory cortex and one electrode into the visual cortex of Mongolian gerbils and during stimulation local field potentials were recorded in the awake animals.

There is accumulating evidence that the synchronization between brain areas might play an important role in crossmodal integration (Bressler, 1995; 1996). Directional influences of the auditory and visual cortex were analysed by using the Directed Transfer Function (DTF) (Kaminski & Blinowska, 1991) on the local field potential data. Our main question of interest was whether the interaction patterns between auditory and visual cortex changed with increasing presentation number of asynchronous of auditory and visual stimuli.

A further important question in the investigation of multisensory integration is how unimodal areas can be integrated while maintaining the specialization within the areas. Nonlinear models on complex brain dynamics suggest that this aim might be achieved by constantly changing states of partial coordination between different brain areas (DeGuzman & Kelso, 1991; Tononi et al., 1994; Kelso, 1995; Friston, 1997; 2000). To address this issue we additionally investigated the single-trial dynamics of cross-cortical interactions by characterizing the trial-to-trial variability of the DTF of short data windows.

2. Methods

2.1 Animals

Data were obtained from 8 adult male Mongolian gerbils (*Meriones unguiculatus*). All animal experiments were surveyed and approved by the animal care committee of the Land Sachsen-Anhalt.

2.2 Electrodes

Electrodes were made of stainless steal wire (diameter 185 µm) and were deinsulated only at the tip. The tip of the reference electrodes was bent into a small loop (diameter 0.6 mm). The impedance of the recording electrodes was 1.5 MΩ (at 1 kHz).

2.3 Animal preparation and recording

Electrodes were chronically implanted under deep ketamine anesthesia (xylazine, 2 mg/100 g body wt ip; ketamine, 20 mg/100 g body wt ip). One recording electrode was inserted into the right primary auditory cortex and one into the right visual cortex, at depths of 300 µm, using a microstepper. Two reference electrodes were positioned onto the dura mater over the region of the parietal and the frontal cortex, electrically connected and served as a common frontoparietal reference. After the operation, animals were allowed to recover for one week before the recording sessions began. During the measurements the animal was allowed to move freely in the recording box (20 × 30 cm). The measured local field potentials from auditory and visual cortex were digitized at a rate of 1000 Hz.

2.4 Stimuli

Auditory and visual stimuli were presented at a constant intermodal stimulus onset asynchrony of 200 ms. The duration of both the auditory and the visual stimuli was 50 ms and the intertrial interval varied randomly between one and two seconds with a rectangular distribution of intervals in that range. Acoustic stimuli were tones presented from a loudspeaker located 30 cm above the animal. The tone frequency was chosen for each individual animal to match the frequency that evoked in preparatory experiments the strongest amplitude of local field potential at the recording site within the tonotopic map of primary auditory cortex (Ohl et al., 2000, 2001). The range of the frequencies used reached from 250 Hz to 4 kHz with the peak level of the tone stimuli varying between 60 dB (low frequencies) and 48 dB (high frequencies), measured by a Bruel und Kjaer Sound Level Meter Type). Visual stimuli were flashes presented from an LED-lamp (9.6 cd/m^2) located at the height of the eyes of the animal.

2.5 Experimental protocol

To be able to examine both short-term and long-term adaptation effects animals were presented with asynchronous stimuli for ten sessions with 750 stimulus pres-

entations at each session. For four animals the auditory stimuli were presented first, for the remaining four animals the visual stimuli were presented first.

2.6 Data preprocessing

The local field potential (LFP) time series of each trial were analysed from 1 s before to 1 s after the first stimulus. The LFP data of this time period were detrended, separately for each trial and each channel. In addition, the temporal mean and the temporal standard deviation of the time period were determined for each trial and for each channel and used for z-standardization. Amplifier clippings as they resulted from movement of the animals were identified by visual inspection. Only artefact-free trials were included into the analysis (about 70-90 % of the trials).

2.7. The Directed Transfer Function: mathematical definition

Directional influences between the auditory and the visual cortex were analysed in single trials by estimating the Directed Transfer Function (DTF) (Kaminski & Blinowska, 1991, Kaminski et al., 2001, for comparison of the performance of the DTF with other spectral estimators see also Kus et al., 2004; Astolfi et al., 2007). The Directed Transfer Function is based on the concept of Granger causality. According to this concept, one time series can be called causal to a second one if its values can be used for improving the prediction of the values of the second time series measured at later time points. This basic principle is typically mathematically represented in the formalism of autoregressive models (AR-models).

Let $X_1(t)$ be the time series data from a selectable channel one and $X_2(t)$ the data from a selectable channel 2:

$$X_1(t) = \sum_{j=1}^{\tilde{}} {}_{1\to1}(j)X_1(t-\cdot) + \sum_{j=1}^{\tilde{}} {}_{2\to1}(j)X_2(t-\cdot) + \mathcal{E} \qquad (1)$$

$$X_2(t) = \sum_{j=1}^{\tilde{}} {}_{1\to2}(j)X_1(t-\cdot) + \sum_{j=1}^{\tilde{}} {}_{2\to2}(j)X_2(t-\cdot) + \mathcal{E} \qquad (2).$$

Here, the A(j) are the autoregressive coefficients at time lag j, p is the order of the autoregressive model and E the prediction error. According to the concept of Granger causality, in (1) the channel X_2 is said to have a causal influence on channel X_1 if the prediction error E can be reduced by including past measurement of channel X_2 (for the influence of the channel X_1 on the channel X_2 see (2)).

To investigate the spectral characteristics of the interchannel interaction the autoregressive coefficients in (1) were Fourier-transformed; the transfer matrix was then obtained by matrix inversion:

$$\begin{pmatrix} A_{1\to1}(f) & A_{2\to1}(f) \\ A_{1\to2}(f) & A_{2\to2}(f) \end{pmatrix}^{-1} = \begin{pmatrix} H_{1\to1}(f) & H_{2\to1}(f) \\ H_{1\to2}(f) & H_{2\to2}(f) \end{pmatrix} \qquad (3)$$

where the component of the A(f) matrix are:

$$A_{l\rightarrow} (f) = -\sum_{j=} \rightarrow (j)e^{-2\pi} \qquad (4) \quad \text{when l = m}$$

with l being the number of the transmitting channel and m the number of the receiving channel

$$A_{l\rightarrow} (f) = 1-\sum_{j=} \rightarrow (j)e^{-2\pi} \qquad (5) \quad \text{otherwise.}$$

The Directed Transfer Function (DTF) for the influence from a selectable channel 1 to a selectable channel 2, $DTF_{1\rightarrow2}$, is defined as:

$$nDTF_{1\rightarrow} (f) = \left| H_{1\rightarrow} (f)^2 \right| \qquad (6).$$

In the case of only two channels, the DTF measures the predictability of the frequency response of a first channel from a second channel measured earlier in time. When, for example, X_1 describes the LFP from the auditory cortex, X_2 the LFP form the visual cortex and the amplitude of the $nDTF_{1\rightarrow2}$ has high values in the beta-band, it means that we are able to predict the beta-response of the visual cortex from the beta-response of the auditory cortex measured earlier in time. There are several possible situations of crosscortical interaction that might underlie modulation of DTF amplitudes (see for example Kaminski et al., 2001; Cassidy & Brown, 2003; Eichler, 2006). See the Discussion section for more details.

2.8 Estimation of the autoregressive models

We fitted bivariate autoregressive models to LFP time series from auditory and visual cortex using the Burg method as this algorithm has been shown to provide accurate results (Marple, 1987; Kay, 1988; Schlögl, 2003). We partitioned the time series data of single trials into 100-ms time windows which were stepped at intervals of 5 ms through each trial from one second before the first stimulus to one second after the first stimulus. Models were estimated separately for each time window of the single trials. Occasionally, the covariance matrix used for estimation of the AR-coefficients turned out to be singular or close to singular, in these rare cases the whole trial was not analysed any further.

In the present study we used a modal order of 8, the sampling rate of 1000 Hz was used for model estimation. The model order was determined by the Akaike Information Criterion (Akaike, 1974). After model estimation, the adequacy of the model was tested by analyzing the residuals (Lütkepohl, 1993). Using this model order, the auto- and crosscovariance of the residuals was found to have values be-

tween 0.001 to 0.005 % of the auto and crosscovariance of the original data (data averaged from two animals here). In other words, the model was able to capture most of the covariance structure contained in the data. When DTFs were computed from the residuals, the single-trial spectra were almost flat, indicating that the noise contained in the residuals was close to white noise.

The estimation of AR-models requires the normality of the process. To analyse to which extent the normality assumption was fulfilled in our data, the residuals were inspected by plotting them as histograms and, in addition, a Lillie-test was computed separately for the residuals of the single data windows. In about 80% of the data windows the Lillie test confirmed the normality assumption.

A second requirement for the estimation of the autoregressive models is the stationarity of the time series data. Generally, this assumption is better fulfilled with small data windows (Ding et al., 2000), though it impossible to tell in advance at which data window a complex system like the brain will move to another state (Freeman, 2000).

A further reason why the usage of small data windows is recommendable is that changes in the local field potential are captured at a higher temporal resolution. The spectral resolution of low frequencies does not seem to be a problem for small data windows when the spectral estimates are based on AR-models (for a mathematical treatment of this issue, see for example Marple, 1978, p. 199f).

Using a high sampling rate ensures that the number of data points contained in the small time windows is sufficient for model estimation. For example when we used a sampling rate of 500 Hz instead of 1000 Hz to estimate models from our time windows of 100 ms, the covariance of the residuals increased signaling that the estimation has become worse (the autocovariance of the residuals of the auditory and visual channels at 1000 Hz were about 10 % of the auto- and crosscovariance of the auditory and visual channels at 500 Hz). Importantly, when inspecting the spectra visually they seemed to be quite alike, indicating that AR-models are robust to an extent to a change in sampling rate. When using a data window of 200 ms with the same sampling rate of 500 Hz the model estimation improved (the covariance of the residuals was 20 % to 40 % of the covariance of a model with a window of 100 ms), but at the expense of the temporal resolution.

2.9 Normalization of the DTF

Kaminski et al. (1991) suggested normalization of the Directed Transfer Function relative to the structure which sends the signal, i.e. for the case of the directed transfer from the auditory channel to the visual channel:

$$nDTF_{A\to V}(f) = \frac{\left|H_{A\to V}(f)^2\right|}{\sum_{M=1}^{k}\left|H_{M\to V}(f)\right|^2} \qquad (7).$$

In the two-channel case the $DTF_{A\to V}$ is divided by the sum of itself and the spectral autocovariance of the visual channel. Thus, when using this normalization the amplitude of the $nDTF_{A\to V}$ depends on the influence of the auditory channel

on itself and, reciprocally, the amplitude of the $nDTF_{V\rightarrow A}$ is dependent on the influence of the visual channel on itself. This is problematic in two ways: First, we cannot tell whether differences between the amplitude of the $nDTF_{A\rightarrow V}$ and the amplitude of the $nDTF_{V\rightarrow A}$ are due to differences in normalization or to differences in the strengths of crosscortical influences. Second, analysis of our data has shown that the auditory and the visual stimulus influenced both the amplitude of the local field potential and the spectral autocovariance of both auditory and the visual channels. Thus it is not clear whether changes in the amplitude of the nDTF after stimulation signal changes in the cross cortical interaction or changes in spectral autocovariance of the single channels.

As the non-normalized DTF is difficult to handle due to large differences in the amplitudes at different frequencies we normalized the DTF in the following way:

$$nDTF_{A\rightarrow}(f) = \frac{DTF_{A\rightarrow}(f)}{\left(\sum_{1}\sum_{1}\sum_{1} DTF_{A\rightarrow}(f)\right) / (n_windows * n_trials * n_session)} \quad (8)$$

with n_windows being the number of time windows of the prestimulus interval per trial,n_trials the number of trials per session, and n_session the number of sessions.

Hence, the amplitude of the DTF estimated for each single time window of the single trials was divided by the average of the DTF of all time windows taken from the 1-second- prestimulus interval of the single trials of all sessions.

2.10 Statistical testing

We assessed the statistical significance of differences in the amplitude of the nDTF using the bootstrap technique (e.g. Efron & Tibshirani, 1993) in order not be bound to assumptions about the empirical statistical error distribution of the nDTF (but see Eichler, 2006, for an investigation of the statistical properties of the DTF). The general procedure was as follows: First, bootstrap samples were drawn from the real data under the assumption that the null hypothesis was true. Then for each bootstrap sample a chosen test statistic was computed. The values of the test statistic from all bootstrap samples formed a distribution of values of the test statistic under the assumption of the null hypothesis. Next, we determined from the bootstrap distribution of the test statistic the probability of finding values equal or larger than the empirically observed one by chance. If this value was below the preselected significance level the null hypothesis was rejected.

More specifically, in our first bootstrap test we wanted to test the hypothesis whether the nDTF has higher amplitude values in the poststimulus interval than in the prestimulus interval. Under the assumption of the null hypothesis the nDTF-amplitude values of the pre- and the poststimulus interval should not be different from each other. Thus, pairs of bootstrap samples were generated by taking single trial nDTF-amplitude values at random but with replacement from the pre- and from the poststimulus interval. For each of the sample pairs the amplitudes were

averaged across trials and the difference between the averages was computed separately for each pair. This procedure of drawing samples was repeated 1000 times getting a distribution of differences between the average amplitudes. The resulting bootstrap distribution was then used to determine the probability of the real amplitude difference of the averages between the pre- and the poststimulus interval under the assumption of the null hypothesis.

In a second bootstrap test we assessed the significance of the slope of a line fitted to the data by linear regression analysis. We used the null hypothesis that the predictor variable (here the number of stimulus presentations) and the response variable (here the nDTF amplitude) are independent from each other. We generated bootstrap samples by randomly pairing the values of the predictor and observer variables. For each of these samples a line was fitted by linear regression analysis and the slope was computed obtaining a distribution of slope values under the null hypothesis.

3. Results

3.1 Stimulus-induced changes in the single–trial nDTF, averaged across all trials of all sessions

For a first inspection of the effect the audiovisual stimulation had on the nDTF from auditory to visual cortex ($nDTF_{A\rightarrow V}$) and from visual to auditory cortex ($nDTF_{V\rightarrow A}$) we averaged nDTF amplitudes across all single trials of all sessions, separately for each time window from one second before to one second after the first stimulus. Figure 1 shows time-frequency plots of the $nDTF_{A\rightarrow V}$ (figure 1A) which describes the predictability of the frequency response of the visual cortex based on the frequency response of the auditory cortex and the $nDTF_{V\rightarrow A}$ (figure 1B) which describes the predictability of the frequency response of the auditory cortex based on the frequency response of the visual cortex. Data is shown both for animals receiving the tone stimulus first (figure 1,left) for animals receiving the light stimulus first (figure 1, right) from 200 ms before the first stimulus to 1 s after the first stimulus here. Note that the abscissa indicates the start of a time window (window duration: 100 ms), so the data from time windows at 100 ms before the first stimulus are already influenced by effects occurring after the presentation of the first stimulus.

The significance of the observed changes in the nDTF-amplitude was assessed separately for each animal using the student's t-test based on the bootstrap technique (see Methods). More precisely, we tested whether the amplitudes of the nDTF averaged across trials at different time points after the presentation of the first stimulus were significantly different from the nDTF amplitude of the prestimulus interval, averaged across trials and time from -1000 ms to 100 ms before the first stimulus. To compare the relative amplitudes of the $nDTF_{A\rightarrow V}$ and the

$nDTF_{V\rightarrow A}$, we tested whether the difference of the amplitudes of AV- and $nDTF_{V\rightarrow A}$ averaged across trials at different time points after the presentation of the first stimulus were significantly different from the difference of the amplitudes of $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ of the prestimulus interval. In the following we will describe only peaks of the amplitudes of nDTF which deviated significantly ($p < 0.01$) from the average amplitude of prestimulus interval.

3.1.1 Animals receiving first the light, then the tone stimulus (VA-animals)

At first sight the response of the $nDTF_{A\rightarrow V}$ resembled closely the response of the $nDTF_{V\rightarrow A}$. In animals receiving first the light stimulus and then the tone stimulus we observed two prominent positive peaks in both the $nDTF_{A\rightarrow V}$ (figure 1A, right) and the $nDTF_{V\rightarrow A}$ (figure 1B, right), the first one after the light stimulus started at about - 20 ms and the second one after the tone stimulus began at about 151 ms. After the second peak the amplitude of the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ dropped slightly below the prestimulus baseline and returned very slowly to the prestimulus values within the next second.

Even though the temporal development and the frequency spectra were roughly similar in the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ there were small but important differences. First, there were stimulus-evoked differences in the amplitudes of the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$. After the visual stimulus the nDTF amplitude was significantly higher in the $nDTF_{V\rightarrow A}$ than in the $nDTF_{A\rightarrow V}$, whereas after the auditory stimulus the $nDTF_{A\rightarrow V}$ reached higher values, but only at frequencies above 30 Hz. Second, even though the peaks could be found at all frequency bands in the $nDTF_{V\rightarrow A}$ the first peak was strongest at a frequency of 1 Hz and about 32 Hz and the second peak at frequencies of 1 Hz and about 40 Hz. In the $nDTF_{A\rightarrow V}$ the highest amplitude values after the first peak could be observed at 1 Hz and at about 35 Hz and after the second peak at 1 Hz and about 45 Hz.

3.1.2 Animals receiving first the tone, then the light stimulus (AV-animals)

In animals receiving first the light stimulus and then the tone stimulus, three positive peaks developed after stimulation. As in the VA-animals the $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ were similar to each other (figure 1, left). The first peak could be found between the tone and the light stimulus, at about -40 ms. The second and the third peak occurred after the light stimulus at about 170 ms and 330 ms. And as in the VA-animals in the AV- animals after the auditory stimulus (here the first stimulus) the amplitude of the $nDTF_{A\rightarrow V}$ significantly exceeded the amplitude of the $nDTF_{V\rightarrow A}$ for frequencies above 20 Hz, whereas after the visual stimulus amplitudes were significantly higher in the $nDTF_{V\rightarrow A}$ (figure 1C, left). Thus, the sign of the difference between the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ depended on the type of the stimulus (auditory or visual) and not on the order of stimulus presentation.

The peaks ran through all frequencies from 0 to 100 Hz. The first peak of the $nDTF_{A\rightarrow V}$ was most pronounced at 1 Hz and at about 42 Hz, the second peak at 1 Hz, at about 32 Hz and at 100 Hz. The first peak of the $nDTF_{V\rightarrow A}$ reached highest

values at 1 Hz and at 35 Hz, the second peak had its highest amplitude at 1 Hz and at 28 Hz. For the third peak the amplitude was most prominent at 1 Hz.

3.2 Development of the amplitude of $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ within the sessions

To investigate the development of the effects within the sessions we divided the 750 trials of each session into windows of 125 trials from the start to the end of each session. Averaging was done across the trials of each trial window, but separately for the time windows within the course of each trial. Trials from all sessions were included in the average. As for the majority of the animals the nDTF-amplitude increased or decreased fairly smoothly within the sessions we decided to characterize the effects by linear regression analysis. The slope of the regression line fitted to the observed data points was subjected to statistical testing using the bootstrap technique (for details see Methods).

3.2.1 VA-animals

In figure 3A and 3B the development of the nDTF amplitude of the first and the second peak within the sessions is depicted, averaged across all four animals which received the light stimulus first. Roughly, most of the effects could be observed over the whole range of frequencies tested, in figure 3 we selected nDTF peaks at a frequency of 40 Hz for illustration. Nevertheless, effects did not always reach significance at all frequencies tested, see table 1 and table 2 for more detailed information on the development of peaks at other frequencies).

After the first (visual) stimulus the amplitude of the first peak increased in the $nDTF_{A\rightarrow V}$ and decreased in the $nDTF_{V\rightarrow A}$ (figure 2A, left). At the beginning of the session the amplitude was higher in the $nDTF_{V\rightarrow A}$ than in the $nDTF_{A\rightarrow V}$, thus the amplitude difference between the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ decreased significantly over the session (figure 2A, right).

After the second (auditory) stimulus the amplitude of the second peak increased both in the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ (figure 2B, left). Importantly, the increase of the $nDTF_{A\rightarrow V}$ exceeded the increase of the $nDTF_{V\rightarrow A}$, gradually increasing the difference between the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ (figure 2B, right).

3.2.2 AV-animals

Similar to the nDTF development in VA-animals after the second (auditory) stimulus, in the AV-animals after the first (auditory) stimulus the amplitude increased both in the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ (figure 2C, left). The increase was more pronounced in $nDTF_{A\rightarrow V}$, further increasing the difference between the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ (figure 2C, right).

Interestingly, after the second (visual) stimulus, the behaviour of the nDTF in the AV-animals did not resemble the behaviour of the nDTF after the first (visual) stimulus in the VA-animals. In the AV-animals the amplitude of the $nDTF_{V\rightarrow A}$ increased after the visual stimulus, the amplitude of the $nDTF_{A\rightarrow V}$ decreased slightly

in some animals, in other animals an increase could be observed (see figure 2D left and table 1). As after the visual stimulus the amplitude of the $nDTF_{V\rightarrow A}$ was higher than the amplitude of the $nDTF_{A\rightarrow V}$ already at the beginning of the sessions, the difference between the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ further increased during the course of the sessions (figure 2D, right).

3.3 Trial-to-trial variability of the stimulus-induced changes in the nDTF

The short-time nDTF of single-trials appeared to be highly unpredictable in amplitude. In general, both the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ displayed one or two peaks with the frequency of the peak varying strongly across the whole range of frequencies measured. To characterize the trial-to-trial variability the rates of peak occurrences at different frequencies were determined separately for each time point of the trial. In Figure 3 we show the data of a VA-animal. To illustrate the effect of the stimulation, we subtracted the rates of peak occurrences averaged across the prestimulus interval from the rates of peak occurrences of the poststimulus interval. It can be readily observed that the shapes of the poststimulus distributions of the $nDTF_{A\rightarrow V}$ (figure 3A) and the $nDTF_{V\rightarrow A}$ (figure 3B) were almost identical. Moreover, both distributions were highly similar in shape to the all-trial average of the nDTF of the VA-animals (figure 1): the amplitudes of the all-trial average were high at frequencies we also observed high rates of peak occurrences.

In the all-trial average we observed clear differences between the amplitudes of the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ in that the AV-DTF attained higher values after the auditory stimulus and the $nDTF_{V\rightarrow A}$ attained higher values after the visual stimulus. Interestingly, similar differences could not be observed in the rate distributions (figure 3C). The difference between the two distributions were almost zero at most frequencies and time points.

In figure 3(D and E) we plotted the averaged *amplitudes* of the peaks at different frequencies, again separately for the different time points of the trial. Also the amplitudes of the peaks were highly similar in shape to the amplitudes of the all-trial average. Moreover, in contrast to the rate distributions, for the distributions of peak amplitudes we now observed the differences between the peak amplitudes of the $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ (figure 3F) corresponding closely to those found in the all-trial average: after the auditory stimulus the amplitudes of the peaks of the $nDTF_{A\rightarrow V}$ exceeded the amplitudes of the peaks of the $nDTF_{V\rightarrow A}$, after the visual stimulus the peak amplitudes of the $nDTF_{V\rightarrow A}$ attained higher values than those of the $nDTF_{A\rightarrow V}$.

To sum up, the single-trial dynamics characterized by the rates of peak occurrences appeared to correspond closely in the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$, suggesting that a common waveform occurred in both nDTFs in the single trials. However, within the single trials on average the nDTF differed in amplitude, suggesting directive influences between the cortices (see Discussion for the interpretation of nDTF amplitudes).

When looking only at peak amplitudes, it seems that some of the information contained in the nDTF was lost. However, as the peak was largest it also had the strongest influence on the all-trial average of the nDTF. In figure 4 we averaged the single-trial nDTFs selectively for one of its peaks, both for the pre- and the poststimulus interval. In general the selective averages were largest at its peaks. Interestingly, for nDTF-averages with peaks below 50 Hz the stimulus induced effects was most pronounced around the peak frequency used for averaging. This finding illustrates the clear differences in the evoked responses observed in single-trials.

Next we addressed the question whether the single-trial dynamics would change within a session. In figure 5 we presented rates of peak occurrences from the first 250 trial (figure 5, left) and the last 250 trials (figure 5, middle) of the sessions, averaged for animals receiving first the light and then the tone stimulus. It can be seen that from the beginning to the end of the sessions there were no strong changes in the rate of peak occurrences. In contrast, the amplitudes of the peaks determined from the first 250 trial and the last 250 trials of the session, exhibited changes in amplitude similar to those observed in the all-trial average.

3.4 Development of the amplitude $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ across the sessions

To examine effects of long-term adaptation the nDTF amplitude of the first 100 trials was averaged separately for each session. The development of the amplitude averages across sessions was examined by linear regression analysis and the significance of the slope was tested using the bootstrap technique. In the following, effects are reported for a chosen significance level of 0.05.

Even though some significant trends could be observed, results were not consistent among animals. In the VA-animals in one animal a decrease could be observed in the amplitude of the $nDTF_{A\rightarrow V}$ at the beginning of the first stimulus, but an increase could be found only 20 ms after the beginning of the first stimulus. In a second animal there was an increase in the amplitude of the $nDTF_{A\rightarrow V}$ after the second stimulus. In the amplitude of the $nDTF_{V\rightarrow A}$ of two VA-animals decreases could be observed after the first and after the second stimulus, in a third animal an increase was found after the second stimulus. All these results could be observed for the majority of examined frequencies.

In the $nDTF_{A\rightarrow V}$ of the AV-animals at many frequencies no clear developmental trend could be observed, but at frequencies below 10 Hz in two animals there was an increase in amplitude both after the first and the second stimulus, whereas in one animal a decrease could be found after both stimuli. In the amplitude of the $nDTF_{V\rightarrow A}$ increases could be observed at various frequencies and time points after stimulation.

4. Discussion

When pairs of auditory and visual stimuli were presented repeatedly at a constant stimulus-onset-asynchrony, within the adaptation sessions we observed modulations of the evoked amplitude of the Directed Transfer Function with increasing number of stimulus repetitions. This finding suggests that the modes of interaction between the primary auditory and primary visual cortex adapted to the prolonged exposure with asynchronous pairs of auditory and visual stimuli. Across the adaptation sessions, no coherent development could be observed indicating that there were no long-term effects on the cross-cortical interactions. In the following we discuss possible processes evoked by the repeated asynchronous presentation of audiovisual stimuli and their possible effects on the amplitude of the nDTF. To prepare the discussion some general considerations with respect to the interpretation of nDTF amplitudes seem appropriate.

4.1 Interpretation of DTF amplitudes

Long-range interaction processes have been frequently associated with coherent oscillatory activity between cortical areas (Bressler, 1993; 1995, Roelfsema et al., 1997; Rodriguez et al, 1999; Varela et al., 2001). Moreover, it has been shown that the oscillatory activity in one cortical area can be predicted by earlier measurement of another cortical area using the DTF (Kaminski et al., 1997; 2001; Korzeniewska et al., 1997; 2003; Franaszczuk & Bergey, 1998; Medvedev & Willoughby, 1999; Liang et al., 2000), indicating that the oscillatory activity might signal directional influences between the cortices.

In our data we observed that independent of the stimulus order, when an auditory stimulus was presented, the amplitude of the $nDTF_{A\rightarrow V}$ exceeded the amplitude of the $nDTF_{V\rightarrow A}$, whereas after the visual stimulus the amplitude of the $nDTF_{V\rightarrow A}$ reached higher values. Given these observation, one might feel tempted to conclude that after the auditory stimulus, the auditory cortex sent information about the auditory stimulus to the visual cortex and after the visual stimulus, the visual cortex informed the auditory cortex about the visual stimulus. However, as Cassidy and Brown (2003) demonstrated in a series of simulation studies, there is no straightforward way to infer directed crosscortical interactions from the information provided by the DTF. Specifically, from DTF amplitudes alone it is not possible to tell whether the information flow is unidirectional, bidirectional or even multidirectional including additional brain areas.

For example, let us consider the situation after the presentation of the auditory stimulus when the amplitude of the $nDTF_{A\rightarrow V}$ attained higher values than the amplitude of the $nDTF_{V\rightarrow A}$. Firstly, this result might indicate that there was an unidirectional influence from the auditory to the visual cortex with the size of the amplitude difference positively correlating with the delay in the information transfer. Secondly, this finding could also reflect a reciprocal influence between the auditory and visual cortex, but with the influence from auditory cortex either larger in

amplitude or lagged relative to the influence from the visual cortex. Thirdly, additional unobserved structures might be involved sending input slightly earlier to the auditory cortex than to the visual cortex.

4.2 The development of the nDTF amplitude within sessions

The development of the nDTF after the *auditory* stimulus did not seem to depend strongly on the order of stimulus presentation. Independent of whether an auditory or a visual stimulus was presented first, after the auditory stimulus the peak amplitude of both the $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ increased. Noteworthy, the increase was more pronounced in the $nDTF_{A\rightarrow V}$ than in the $nDTF_{V\rightarrow A}$ further increasing the difference between the amplitudes of the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$. Using the interpretation scheme introduced above, under the assumption of unidirectional interaction, the influence from the auditory to the visual cortex not only increased in strength but also the lag with which the input is sent became larger with increasing number of stimulus repetitions? In case of bidirectional interaction, influences from both sides increased, but the influence form the auditory cortex became stronger relatively to the influence from the visual cortex. Last, in case of multidirectional interaction the influence of a third structure to both the auditory and the visual cortex might become more pronounced, but at the same time the temporal delay input is sent to the visual cortex relatively to the delay input is send to the auditory cortex is increased even further. All these three interpretations have in common that the interaction did not only gather in strength, but also the mode of the interaction changed.

In contrast to the development of the nDTF after the auditory stimulus the development of the nDTF after the visual stimulus clearly depended on the order of stimulus presentation. When the visual stimulus was presented first, contrary to expectations, the amplitude of the $nDTF_{V\rightarrow A}$ decreased with increasing number of stimulus repetitions, whereas the amplitude of the $nDTF_{A\rightarrow V}$ increased in the majority of the animals. Thus, assuming that unidirectional influence underlies our data, this finding might reflect that the visual cortex sends influences to the auditory cortex at increasingly shorter delays. In case of bidirectional interaction the input from the visual cortex decreases whereas the input from the auditory cortex increases. Last, under assumption of multidirectional interaction a hypothetical third structure might still send its input earlier to the visual cortex, but the delay became diminished with increasing number of stimulus repetitions.

When the visual stimulus was presented as the second stimulus the behavior of the nDTF showed some resemblance to the behaviour of the nDTF after the auditory stimulus. More precisely, both the peak amplitude of the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$ increased within the sessions. But importantly, now the increase was stronger in the $nDTF_{V\rightarrow A.}$

4.3 Single-trial variability of the nDTF

The amplitude of the nDTF exhibited a strong trial-to-trial variability: it usually displayed one or two peaks with the frequency of the peaks differing strongly

from trial to trial. The observation that the modes of crosscortical interactions might be highly variable has also been reported in other studies on the dynamics of crosscortical interactions (Bressler & Kelso, 2001, Freeman & Burke, 2003).

The modulations in the rates of peak occurrence observed after stimulation strongly agreed with the changes in the amplitudes observed in the all-trial average: high rates of peak occurrences could be observed at frequencies we also found high amplitudes. When the single-trial nDTFs were averaged selectively in dependence of the frequency of one of their peaks, the evoked response observed in the selective averages deviated from the evoked response found in the all-trial average. Thus we did not find strong evidence for a fixed stimulus-evoked response, constantly occurring in all trials independent of the state of the system. Instead, the trial-to-trial variability did not average out by averaging across trials but appeared to have a strong influence on the shape of the all-trial average.

The evoked changes in the distributions of peaks occurrences were almost identical in the $nDTF_{A\rightarrow V}$ and the $nDTF_{V\rightarrow A}$, indicating that the $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ shared highly similar waveforms in the single-trials and did not differ much in their single-trial dynamics. However, the $nDTF_{A\rightarrow V}$ - and $nDTF_{V\rightarrow A}$ clearly differed in the relative amplitudes of their waveforms indicating the presence of directive interactions between the cortices.

With increasing number of repetitions of the asynchronous stimuli no substantial changes in the rates of peak occurrences were discernable. This observation suggests that the prolonged exposure to the asynchronous stimulation did not affect the constant waxing and waning of spectral activities observed in the ongoing activity. With other words, the amplitude adaptations observed in the all-trial average appeared to be due to changes in the amplitudes of the induced responses within the trials rather than to their trial-to-trial variability.

4.4 Hypothetical processes underlying the observed changes in the nDTF amplitudes

As paired-stimulus adaptation protocols, similar to the one used in the present study, have been shown to induce recalibration of temporal-order judgment in humans (e.g. Fujisaki et al., 2004; Vroomen et al., 2004), some of the described effects on the directed information transfer could possibly underlie such recalibration functions. For example, the characteristic developmental trend after the second stimulus was an increase in both $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$ with the increase stronger in the nDTF sending information from the structure the stimulus had been presented to, namely in the $nDTF_{V\rightarrow A}$ after the visual stimulus, and in the $nDTF_{A\rightarrow V}$ after the auditory stimulus. One might hypothesize that the increase in the interactions between the auditory and the visual cortex after the second stimulus mirrored the integration of the auditory and the visual information resulting in a reduction of their perceived temporal distance.

However, we have to take into account that behavioral studies on recalibration of temporal-order judgment typically demonstrated a shift of the entire psychometric function (i.e. at many stimulus onset synchronies and irrespective of the stimu-

lus order). This finding is remarkable given that the recalibration was induced by presentation of stimuli at a constant order and stimulus-onset-asynchrony. In our data the behavior of the nDTF after the visual stimulus clearly depended on the stimulus order, thus this findings disagrees with the results of the behavioral recalibration experiments.

The independence of the recalibration effect on the stimulus order implies that during the recalibration process the temporal perception of multimodal stimuli is not recalibrated *relative* to each other but perception is simply speeded up or slowed down in one modality.

In our data we did not find any indications for an increase in the speed of stimulus processing in form of a change in the latencies of the nDTF peaks. However, it also might appear surprising if the temporal compensation mechanisms were so simple that they might be readily observed in a decrease in the latency of cross-cortical interactions.

To decide whether the changes in the nDTF we observed were neural correlates of the recalibration of temporal perception the repetition of our experiment in combination with a behavioral test is necessary.

Many other cognitive processes might have been evoked by the paired presentation of stimuli. For example, in accordance to the unity assumption (e.g. Bedford, 2001; Welch, 1999; Welch & Warren, 1980) two stimuli from different sensory modalities will be more likely regarded as deriving from the same event when they are presented in close temporal congruence. The increase in the amplitude of the nDTF after the second stimulus might indicate the binding of the stimuli into a coherent perception. Changes in the nDTF-amplitude before the second stimulus might indicate the expectation of the second stimulus. Several other studies have demonstrated increases in coherent activity associated with anticipatory processing (e.g. Roelfsema et al., 1998; Von Stein et al., 2000; Fries et al., 2001; Liang et al., 2002). To clarify whether the observed changes might have something to do with stimulus association or expectation processes the repetition of this experiment with anesthetized animals might be helpful.

As we presented our stimuli with constant audiovisual lag, also mechanisms of lag detection could have been evoked. There are first studies on the neural correlates of synchronous and asynchronous stimulus presentation (Meredith et al., 1987, Bushara et al., 2001; Senkowski et al., 2007). Senkowski et al. (2007) could observe changes in the oscillatory activity with synchronous or asynchronous presentation of audiovisual stimuli, suggesting that the changes in the oscillatory interaction between cortices we observed in the nDTF might indicate the alerting of lag detectors.

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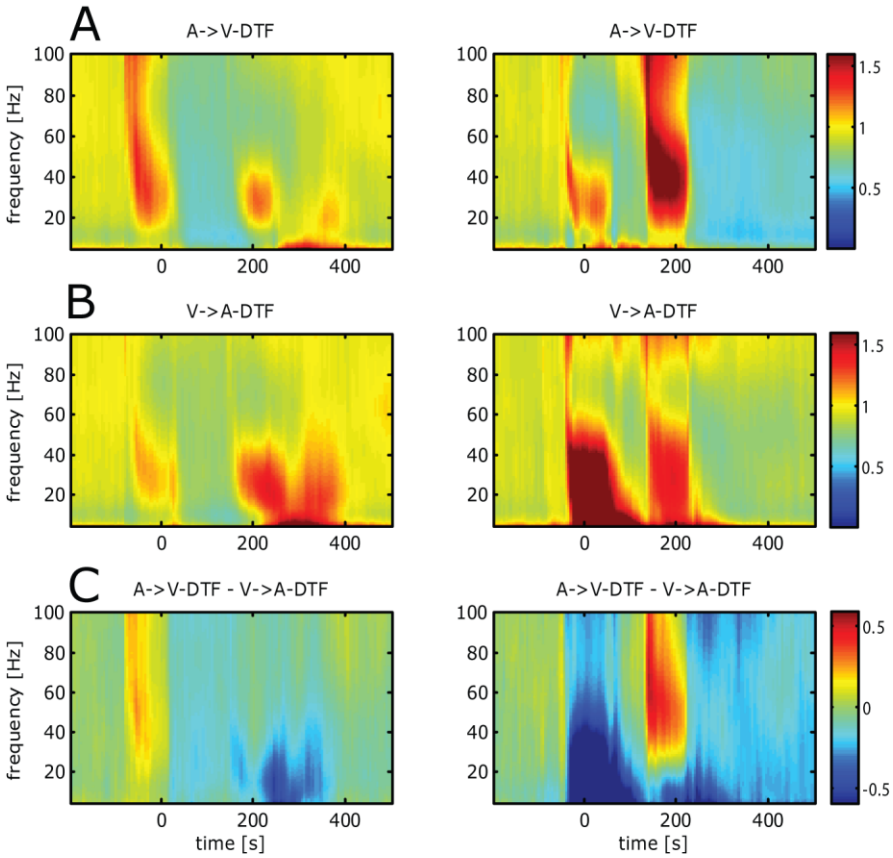


Figure 1: A & B: Normalized $nDTF_{A\rightarrow V}$ (A) and $nDTF_{V\rightarrow A}$ (B) averaged across all trials from all sessions, separately for time windows from -0.2 to 0.9 s after the start of the first stimulus. Left: animal receiving first the tone. Right: animal receiving first the light. C: Difference between averages ($nDTF_{A\rightarrow V}$ - $nDTF_{V\rightarrow A}$).

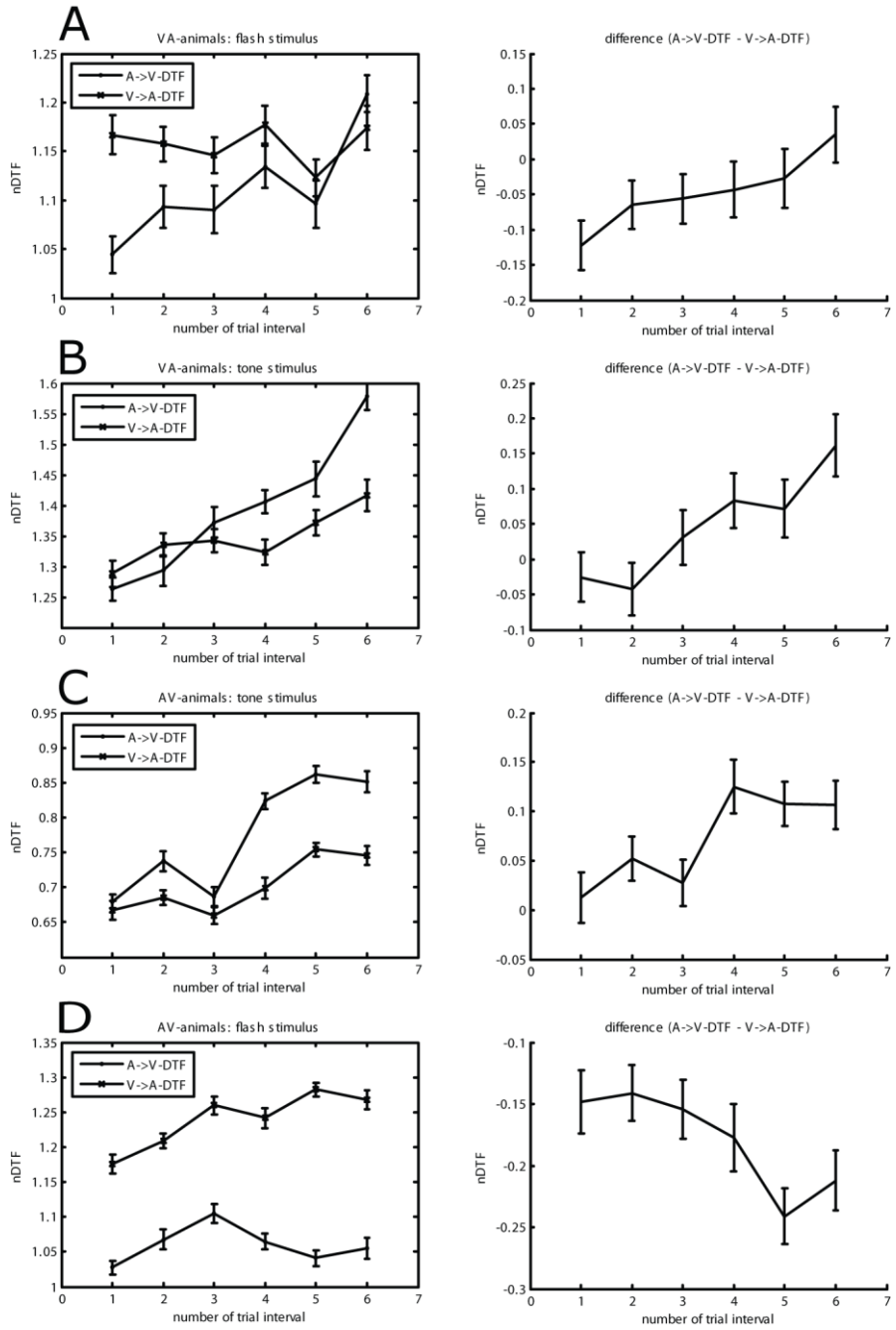


Figure 2: Development of nDTF-peaks at 40 Hz within the sessions averaged across trial windows of 125 trials stepped at intervals of 125 trials through all sessions. AB: animals receiving first the light. CD: animals receiving first the tone. Left: AC: Development of the average amplitude peak after the first stimulus in the $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$. BD: Development of the average amplitude peak after the second stimulus in the $nDTF_{A\rightarrow V}$ and $nDTF_{V\rightarrow A}$. Right: Amplitude of the $nDTF_{V\rightarrow A}$ peak subtracted from the amplitude of the $nDTF_{A\rightarrow V}$ peak shown in the left figures. Error bars denote the standard error of the mean, averaged across animals.

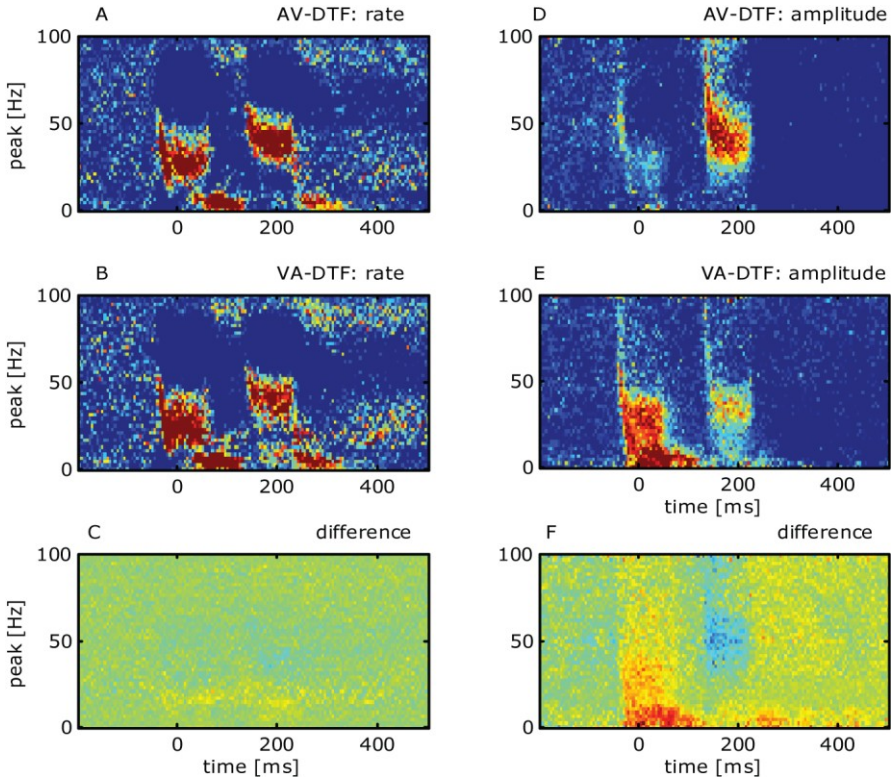


Figure 3: A & B: Rates of occurrence of peaks in the single-trial $nDTF_{A\rightarrow V}$ (A) and $nDTF_{V\rightarrow A}$ (B) at different time points after stimulation. C: rates of peak occurrences of the $nDTF_{A\rightarrow V}$ subtracted from rates of peak occurrences of the $nDTF_{V\rightarrow A}$. D & E: Amplitudes of peaks in the single-trial $nDTF_{A\rightarrow V}$ (D) and $nDTF_{V\rightarrow A}$ (E) at different time points after stimulation. F: Amplitudes of peaks of the $nDTF_{A\rightarrow V}$ subtracted from amplitudes of peaks of the $nDTF_{V\rightarrow A}$. Data from an animal receiving first the light.

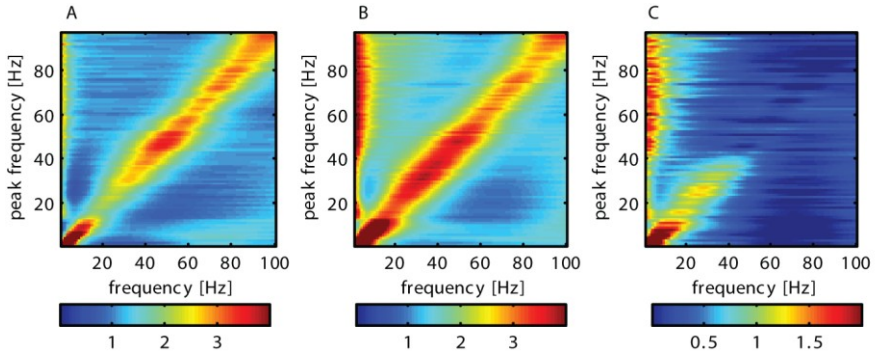


Figure 4: Single-trial $nDTF_{A\rightarrow V}$ selectively averaged for the frequency of one of its peaks. A: data from the prestimulus interval. B: data 0 ms to 200 ms after the auditory stimulus. C. Differences of the averages of the pre- and poststimulus interval.

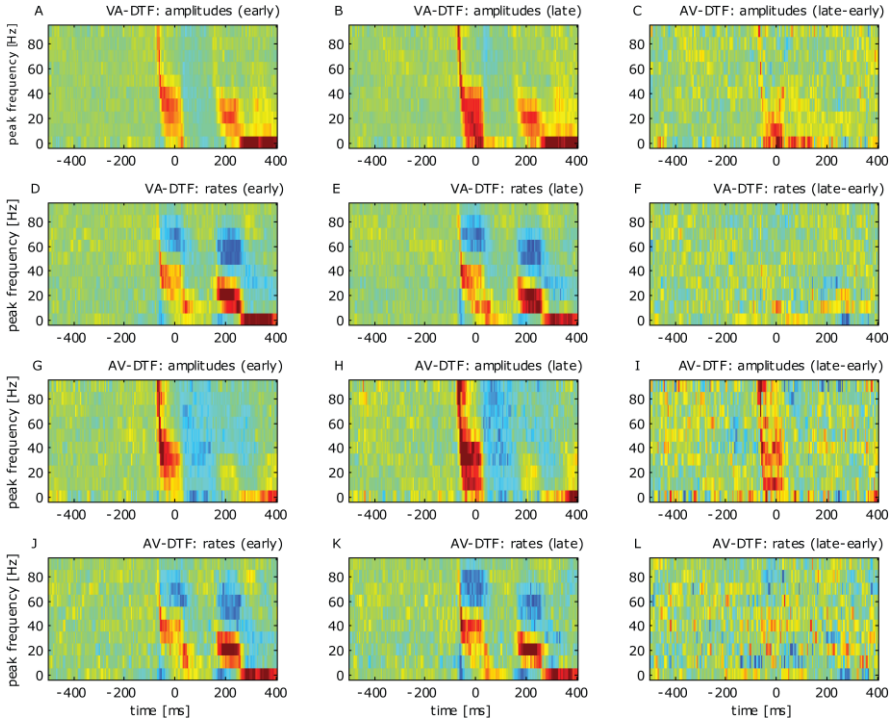


Figure 5: Development of the rates of occurrence (D,E,F,J,K,L) and the amplitudes (A,B,C,G,H,I) of peaks of single-trial nDTFs from the first 250 trials of sessions (A,B,D,G: early) to the last 250 trials of the sessions (B,E,H,I: late). C,F,I,L: difference between early and late trials of the sessions. A,B,C,D,E,F: data from $nDTF_{V\rightarrow A}$. GHIJKL: data from $nDTF_{A\rightarrow V}$. Data from all animals receiving first the tone.