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Special section on music in the brain: Research report

Simultaneous recording of electroencephalographic data in musicians playing in ensemble

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ABSTRACT

Here we describe a methodological approach for the simultaneous electroencephalographic (EEG) recording in musicians playing in ensemble. Four professional saxophonists wore pre-wired EEG caps (30 electrodes placed according to an augmented 10–20 system; cephalic reference and ground). Each cap was connected to a single multi-channel amplifier box [Brain Explorer (BE), EB-Neuro®]. The four boxes converged to a single workstation equipped with a software (GALILEO NT, EB-Neuro®) allowing the simultaneous recording of sounds, digital trigger, and EEG–electrooculographic (EOG)–electromyographic (EMG) data, and providing a separate output file for each individual. Noteworthy, the subjects were electrically decoupled to satisfy international safety guidelines. The quality of the EEG data was confirmed by the rate of artifact-free EEG epochs (about 80%) and by EEG spectral features. During the resting state, dominant EEG power density values were observed at alpha band (8–12 Hz) in posterior cortex. The quality of EMG can be used to identify “on” and “off” states of the musicians’ motor performance, thus potentially allowing the investigation of the relationships between EEG dynamics and different characteristics of the specific performance. During the music performance, alpha power density values decreased in amplitude in several cortical regions, whereas power density values enhanced within narrow high-frequency bands. In conclusion, the present methodological approach appeared to be suitable for simultaneous EEG recordings in musicians playing in ensemble.

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1. Introduction

Previous studies using electroencephalographic (EEG), magnetoencephalographic (MEG), and functional magnetic resonance imaging (fMRI) techniques have investigated cortical responses to the execution, imagination, and perception of music events (Elbert et al., 1995; Petsche et al., 1996; Maess et al., 2001; Nirkko et al., 2001; Koelsch et al., 2001, 2002; Langheim et al., 2002; Kristeva et al., 2003). In the study of these responses, EEG and MEG techniques provide a high temporal resolution (msec) but a low (cm) and medium (bit more than a cm) spatial resolution, respectively. On the contrary, fMRI provides a high spatial resolution (mm) but a low temporal resolution (more than a sec). On the whole, these techniques have shown that primary and premotor sensorimotor cortical areas are active during both preparation (time preceding the execution) and execution of a music performance (Kristeva et al., 2003; Koelsch et al., 2001, 2002; Maess et al., 2001). Furthermore EEG and MEG techniques have shown that the imagination, observation, and learning of music performances are characterized by peculiar patterns of cortical activity (Kristeva et al., 2003; Elbert et al., 1995; Petsche et al., 1996; Langheim et al., 2002; Nirkko et al., 2001).

Given the above framework, EEG (MEG) techniques allow not only a fine temporal evaluation of music-related cortical responses but also can help to pinpoint the role of emerging cortical oscillatory activity in the neural processes related to music information processing. Of extreme interest is the modulation of a dominant cortical oscillatory activity which occurs at about 8–12 Hz (alpha rhythms). This rhythm has been found to be tightly related to cortical information processing of emotional stimuli and music experience (Schmidt and Trainor, 2001; Altenmüller et al., 2002).

A limitation of the aforementioned EEG, MEG, and fMRI studies is that the evaluation of the cerebral processes related to music performance has been investigated in individual musicians, therefore ‘outside’ the typical context of an ensemble music performance. Due to this limitation and to all the technical issues involved in setting up fMRI, MEG or EEG machines for the simultaneous recording of neurophysiological data in musicians playing in ensemble, the neural underpinnings of music performance in musicians playing in ensemble are poorly known. In addition, the use of metal objects is banned in rooms where MEG and fMRI devices are seated and this is a serious limitation for studies addressing musical performance with real music instruments, although this issue has been successfully dealt with and overcome in some cases (Limb and Braun, 2008; Berkowitz and Ansari, 2008, 2010). Furthermore, while slow displacements of subject’s head respect to the MEG and fMRI sensors may compromise the quality of recordings, the tight contact between exploring EEG sensors and subject’s head allows the recording of artifact-free neurophysiological data, in musicians that have been trained to minimize head–body movements during their performance.

To overcome the aforementioned obstacles, the present study aims at describing a methodological approach for the simultaneous EEG recording in expert musicians playing in ensemble, allowing the synchronous storage of environmental

sounds, digital trigger, and neurophysiological data including EEG, electrooculographic (EOG), and electromyographic (EMG) signals. In contrast to fMRI and MEG techniques which need a shielded room of limited dimensions, EEG data can be recorded in an environment allowing music performance of several musicians. This approach potentially allows online data acquisition in order to test several hypotheses about peculiar cortical responses characterizing sensorimotor, emotional (including inter-individual empathy), and cognitive processes related to ensemble musical performance.

We therefore simultaneously recorded EEG data from a quartet of professional saxophonists during a music performance in ensemble in order to demonstrate that this methodological approach allows the recording of high-quality EEG data in four subjects playing in ensemble. The quality of the EEG data was evaluated by the rate of artifact-free EEG epochs and by the features of dominant alpha rhythms (about 8–12 Hz) during both resting state and music performance. Noteworthy, due to the limited amount of observations, we could not test specific hypotheses, namely the relationships between EEG dynamics and musicians’ performance or between EEG dynamics and musicians’ psychological traits. For this reason, we report exclusively the global cortical activity of the musicians during the whole performance. Furthermore, we used the musicians’ score to empathy tests (Lawrence et al., 2004) in a descriptive manner in order to emphasize the potential value of the present methodological approach for the neuroscientific research in the field of human social interactions.

2. Methods

2.1. Subjects

A quartet (four men) of professional saxophonists with highly international credentials was recruited. They had been practicing for more than 22 years at least five times a week, and are constantly playing together in public concerts since 13 years. The mean subjects’ age was 44 years (± 2.2 standard error (SE); range: 38–48 years). All subjects gave their informed consent according to the Declaration of Helsinki. They were free to withdraw from the study at any time. The procedure was approved by the local Institutional Ethics Committee.

2.2. Design of the system for the simultaneous electrophysiological data recording in the saxophone quartet

Fig. 1 plots a sketch illustrating the system for the simultaneous recording of electrophysiological data. The system is formed by several interconnected items. Noteworthy, these items are commercial products whose technical features and quality are well known (they were licensed by European community regulatory agencies at the time they were approved for the commercialization etc.). The novelty of the present methodological approach is their interconnection for the new scientific purposes. Specifically, four pre-wired EEG caps are used. Each cap includes 30 electrodes placed according to an augmented 10–20 system (cephalic reference and

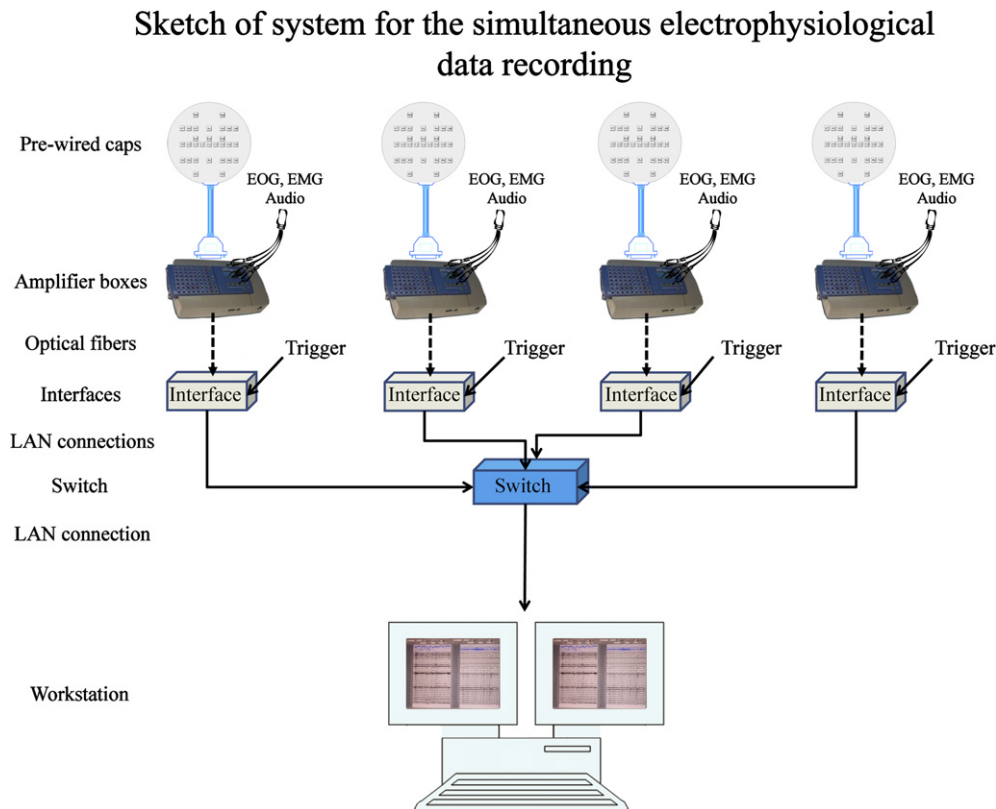


Fig. 1 – Sketch illustrating the system for the simultaneous electrophysiological data recording during music performance in ensemble.

ground), and is connected to a single multi-channels amplifier box [Brain Explorer (BE), EB-Neuro[®]] that also receives the individual bipolar EOG and EMG signals. Furthermore, this box receives audio signals relative to environmental sounds, which are revealed by two dynamic microphones (Shure Beta 57[®]) placed on proper stands, pre-amplified and mixed by a commercial analog mixer. The mixer also gives real-time these audio signals to a professional multi-track hard-disk recording system (two channels, 24 bit, sampling rate 48 KHz). The registration of the audio signals allows a descriptive tracking of the music performance synchronized with EEG data and a clear rendering of the music performance.

In the amplifier boxes, analog/digital conversion of EEG–EOG–EMG and audio signals occurs. The four multi-channels amplifier boxes are connected via optical fibers to four BE net interfaces (BE-Net EB-Neuro[®]), which also receive digital trigger signals coming from the experimenter or computer (trigger represents a common reference signal in the data sets of the four musicians). These four interfaces send (via a local area network, LAN) all converted signals to a commercial switch. The switch collects and redirects (data transfer rate 100 Mb/sec) the converted signals from the four musicians toward a single workstation devoted to the data acquisition. Of note, the only possible cause of jitter of the EEG signals recorded from the four musicians was the LAN interconnection inside the switch device, since the delay of these signals was practically equal in the four identical EEG

amplifiers and EB-net interface used by the system. The maximum jitter, defined as the maximum variation in the arrival/recording time of the four set of EEG signals supposedly synchronous, was in the order of hundreds of microseconds (i.e., less than the actual temporal resolution of the present EEG recordings), as reported by the technical report of the commercial switch device used for this study. The operative system of this workstation allows the opening of four sessions of data acquisition software (GALILEO NT, EB-Neuro[®]), for the simultaneous storage of environmental sounds, digital trigger, and EEG–EOG–EMG data. A single file is obtained for each individual. Noteworthy, the above system is characterized by the fact that the four subjects were electrically decoupled to satisfy international safety guidelines and to record high-quality EEG–EOG–EMG signals.

2.3. Experimental procedure

A training session of about 10 min was performed before the EEG recording session. The musicians had to learn how to minimize head–body movements during their performance, thus allowing the recording of artifact-free EEG data associated to the music performance. During the training period, the musicians also warmed up fingers and instruments.

The musical performance concerned a classic music piece of Domenico Scarlatti (Allegro, from Sonata in A moll L. 223 Kirk. 532, in the elaboration for four saxophones by Salvatore Sciarrino), and lasted 1.5 min

2.4. Data recordings

The EEG data were collected simultaneously from the four musicians (bandpass: .1–100 Hz, sampling rate: 512 Hz; EB-Neuro Be-family[®], Firenze, Italy) by the 30 scalp electrodes of the pre-wired EEG caps (see Fig. 2 for an overview of the four musicians playing in ensemble during the simultaneous EEG recording). Electrode impedance was kept below 5 k Ω . EEG data were recorded at eyes-open resting state condition (4 min). Afterwards, the EEG data were recorded while the quartet was playing a classical music piece taken from its repertoire (duration: about 1.5 min, balanced distribution of phrase attacks between subjects). In parallel, we performed the recording of bipolar EOG data (band pass: .1–100 Hz; sampling rate: 512 Hz) for the monitoring of blinking and eye movements. Furthermore, EMG activity (band pass: .1–100 Hz; sampling rate: 512 Hz) of orbicularis oris, mentalis and right and left extensor digitorum muscles were recorded by bipolar surface electrodes, in order to monitor movements during music performance. For the extensor digitorum communis (EDC), bipolar electrodes were placed 1/4–1/3 of the distance between the midpoint between the radial and ulnar styloid processes, as measured from the wrist dorsum and the olecranon process. For the orbicularis oris muscle, the electrodes were positioned on the left side along a medio-lateral axis so that the circular edges of each electrode were aligned with the vermilion border of the upper and of the lower lips respectively (Cole et al., 1983; Blair and Smith, 1986; McClean, 1987). For the mentalis muscle (MM), one surface electrode was positioned in the midline above the chin, while the other about 1 cm under the chin on a virtual vertical line. Low impedance of the EMG electrodes (<1 k Ω) at the skin–electrode interface was obtained by shaving, abrading, and cleaning the skin with alcohol. Special caution was given to the standardization of the experimental procedure (electrode position), to reduce the susceptibility of EMG to cross-talk (Kellis, 1998).

An additional recording channel was used for triggering the reference instants of the event on the on-going EEG–EOG–EMG data of the four musicians, namely the start



Fig. 2 – Overview of the four musicians playing in ensemble during simultaneous EEG recordings.

and stop instants of the music performance (sampling rate: 512 Hz, no hardware filter). These instants – generally the attacks of musical phrases – could be used for the choice of the same EEG periods in the four musicians.

2.5. Safety

The four multi-channels amplifier boxes (BE, EB-Neuro[®]) were produced and assembled following all the principal guidelines for the manufacture of medical electrical equipment (British Standard BS EN 60601-1-1, 2001), with a special care for safety of the subjects and disturbances rejection. Furthermore, all audio devices were powered using 1:1 transformers as ground decoupler, thus avoiding any risk for the safety of subjects. At the same time, hum, noise, and other disturbances due to ground loops were filtered.

2.6. Testing for empathy

Subjects' empathy as a personality trait was evaluated by empathy quotient test (EQT). This test was developed by Simon Baron-Cohen (Lawrence et al., 2004), and measures the person's level of empathy by means of a questionnaire (60 items). Basically, this questionnaire probes how the person emotionally reacts to various situations. The score of this test ranges from 0 (minimum) to 80 (maximum) to index the ability of understanding how other people feel and respond appropriately in several circumstances.

In addition to the EQT and only for descriptive purposes (i.e., an example of the potentiality of the present methodological approach for the study of social interaction), we prepared and employed a simple questionnaire to evaluate the empathic feelings (especially rhythmic precision and musical interpretation) experienced by every player with respect to the others (1) in general (i.e., empathic feeling as the result of all previous experience of playing in ensemble with that quartet) and (2) during the experimental music performances (i.e., empathic feeling relative the particular music performance of the present experiment). The subjective scale ranged from 0 (no empathy) to 100 (maximum empathy imaginable). The empathy values for each colleague were averaged to form the empathy index related to the musicians involved in the music performance.

2.7. EEG data processing

Recorded EEG data were segmented to single epochs lasting 2 sec (across subjects' mean of 46.5 ± 1.5 SE). The 2 sec-lasting EEG epochs with ocular, muscular, and other types of artifact were preliminarily identified by a computerized automatic procedure (Moretti et al., 2003). The EEG epochs contaminated by ocular artifacts were then corrected by an autoregressive method (Moretti et al., 2003). Finally, two expert electroencephalographers (F.V. and N.M.) manually confirmed this automatic selection and correction, with special attention to residual contaminations of the EEG epochs due to head, trunk, and eye movements. Therefore, only the EEG epochs totally free from artifact residuals were accepted for the subsequent analyses. Across subjects, the artifact-free 2-sec EEG epochs were $36.7 (\pm 2)$ SE, namely 79% of the recorded 2 sec-lasting

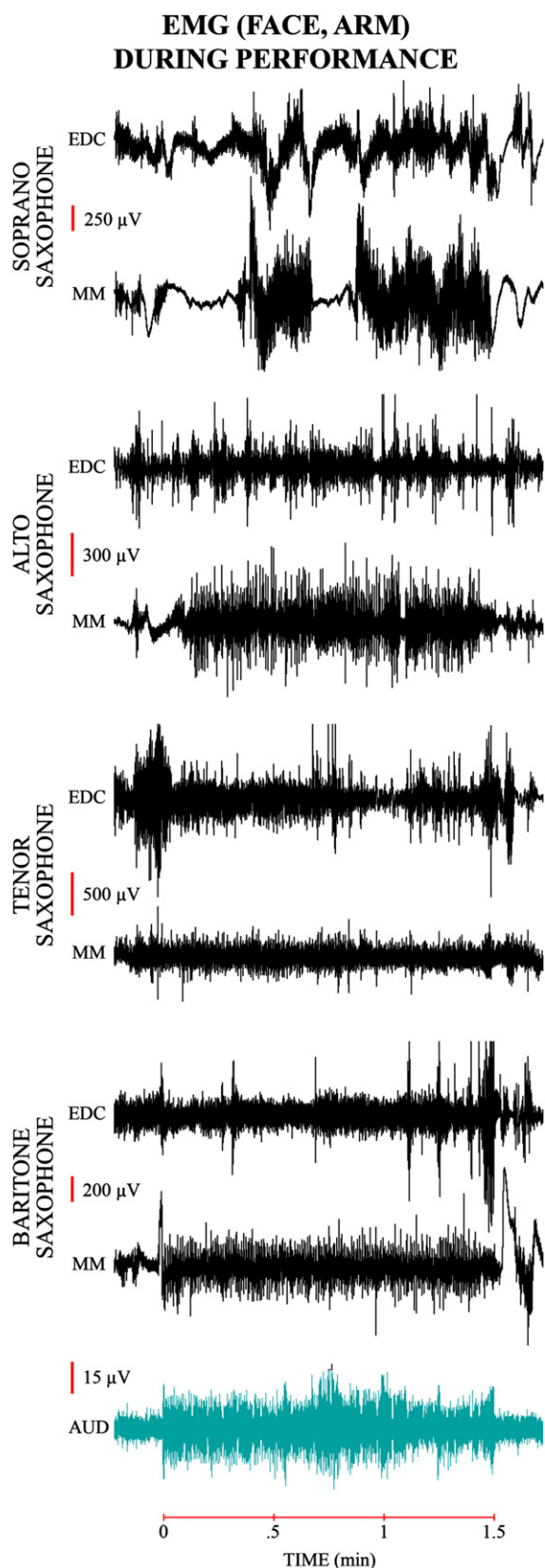


Fig. 3 – EMG signals collected from the EDC and MM for the four musicians (i.e., soprano, alto, tenor and baritone saxophone players) playing in ensemble during simultaneous EEG recordings. The last line presents the audio channel of the performance.

EEG epochs. These EEG epochs were referred to a common average reference for further analyses.

2.8. Frequency analysis of alpha rhythms

For the analysis of EEG power density spectrum, artifact-free EEG epochs were re-referenced to a common average and were analyzed by a standard Fast Fourier Transform (FFT) approach using Welch technique and Hanning windowing function (1 Hz frequency resolution). EEG relative power density was obtained normalizing the EEG absolute power density at each frequency bin and electrode for the mean of the EEG absolute power density across all frequency bins and electrodes. For the determination of the alpha sub-bands, individual alpha frequency (IAF) peak was identified on closed eyes data according to literature guidelines (Klimesch, 1996, 1999; Klimesch et al., 1998). The IAF is defined as the frequency within the 6–13 Hz range of the EEG spectrum showing the maximum power. With reference to the IAF, the alpha sub-bands of interest were as follows: low-frequency alpha band as IAF-2 Hz to IAF, and high-frequency alpha band as IAF to IAF+2 Hz (for example, IAF = 10 Hz gives low-frequency alpha band ranging from 8 to 10 Hz and high-frequency alpha band from 10 to 12 Hz). The mean IAF value across the four musicians was 10 Hz (± 4 SE).

2.9. Cortical source analysis of the EEG rhythms by sLORETA

The artifact-free EEG data were given as an input to the original standardized low resolution brain electromagnetic tomography (sLORETA) software for the EEG source analysis (Pascual-Marqui, 2002; <http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm>). sLORETA is a functional imaging technique belonging to a family of standardized linear inverse solution procedures, modeling three-dimensional (3D) distributions of the cortical source patterns generating scalp EEG data (Pascual-Marqui, 2002). With respect to the dipole modeling of EEG cortical sources, no a priori decision of the dipole position is required by the investigators in sLORETA estimation. In a previous paper, it has been shown that sLORETA is quite efficient when compared to linear inverse algorithms like minimum norm solution, weighted minimum norm solution or weighted resolution optimization (Pascual-Marqui, 2002). Furthermore, sLORETA has been successfully used in recent EEG and MEG studies (Babiloni et al., 2009; Wagner et al., 2004; Sekihara et al., 2005; Greenblatt et al., 2005; Du et al., 2007).

sLORETA computes 3D linear solutions (sLORETA solutions) for the EEG inverse problem standardized with respect to instrumental and biological noise as mathematically defined in the original paper by Pascual-Marqui (2002). sLORETA solutions are computed within a three-shell spherical head model including scalp, skull, and brain compartments digitized as the mean of the MRIs of 152 subjects at the Brain Imaging Center of the Montreal Neurological Institute (MNI) – (Talairach and Tournoux, 1988). The brain compartment is restricted to the cortical gray matter/hippocampus of a head model co-registered to the Talairach probability brain atlas. This compartment includes 6239 voxels (5 mm resolution), each voxel containing an equivalent current dipole. The head

model for the inverse solution uses the electric potential lead field computed with a boundary element method (BEM) applied to the MNI152 template (Fuchs et al., 2002). The electrode coordinates were based on the average location of the 10-5 system placement system (Jurcak et al., 2005).

2.10. Computation of task-related decrease/increase (TRPD/TRPI) of alpha sources

The sLORETA solutions were used to index the magnitude of the brain rhythmicity. Changes of the alpha power density during the EXECUTION condition (task) referenced to the period of eyes-open resting state condition (baseline) was calculated following the same line of reasoning widely used for the computation of event-related desynchronization/synchronization (ERD/ERS; Pfurtscheller and Aranibar, 1979; Pfurtscheller and Neuper, 1994; Pfurtscheller et al., 1997; Pfurtscheller and Lopes da Silva, 1999). Specifically, the formula was

$$\text{TRPD/TRPI\%} = \left(\frac{\text{task} - \text{baseline}}{\text{baseline}} \right) \times 100$$

The procedure was repeated for low- and high-frequency alpha sub-bands. Percent negative values (i.e., weaker alpha power density during the task than baseline condition) represented the alpha TRPD (Manganotti et al., 1998). On the contrary, percent positive values (i.e., stronger alpha power density during the task than baseline condition) represented the alpha TRPI. Noteworthy, we preferred the term “TRPD/TRPI” index to the standard term “ERD/ERS”, since we investigated EEG power changes associated with different conditions maintained along time (resting state vs music performance) rather than phasic EEG power changes associated with a transient and discrete “events” compared to “pre-stimulus baseline” across EEG single trials.

3. Results

The quality of the EMG data was evaluated by a visual inspection of the signals during the music performance (Fig. 3). The quality of the EEG data was evaluated by the rate of artifact-free EEG epochs and by the features of dominant

alpha rhythms (about 8–12 Hz) during both resting state and music performance. The mean across subjects of artifact-free 2-sec EEG epochs was 36.7 (± 2 SE), namely 79% of the recorded 2 sec-lasting EEG epochs. Furthermore, the analysis of EEG power density spectrum revealed typical features of human cortical EEG oscillatory activity during resting state and engaging events. During the resting state, dominant EEG power density values were observed at alpha band (8–12 Hz) in the posterior cortical regions. Furthermore, values of EEG power density at lower frequency bands were maximum in the anterior cortical regions (delta, 1–4 Hz; theta, 4–8 Hz). Finally, values of EEG power density at high-frequency bands were globally negligible (beta, 14–30 Hz; gamma, >30 Hz). During the music performance, alpha power density values decreased in amplitude in several cortical regions, whereas power density values within narrow frequencies of high-frequency beta and gamma were enhanced. These results are illustrated by Fig. 4, which plots the grand average of EEG spectral power density values computed in the four subjects for three electrodes of interest (Fz, Cz, and Pz) and for the frequencies from 1 to 40 Hz (frequency resolution = 1 Hz). The EEG spectra refer to the resting state condition (plotted in gray) and the condition relative to the music performance (plotted in black).

Fig. 5 maps the sLORETA showing the performance-related desynchronization/synchronization of alpha sources (TRPD/TRPI) at low- (about 8–10 Hz) and high (about 10–12 Hz) frequency sub-bands in the four musicians. It can be observed that performance-related desynchronization of alpha sources (TRPD; index of cortical activity) was greatest in the alto saxophone player, at both low- and high-frequency alpha sub-bands. This effect was observed in several cortical regions of interest in both hemispheres. With respect to the alto saxophone player, the desynchronization of alpha sources (i.e., TRPD) was gradually lower in the remaining musicians in the following sequence: tenor, baritone, and soprano (from the highest to lowest alpha desynchronization). Table 1 shows the score to the EQT empathy indices in the four musicians (see Methods).

Noteworthy, the level of the empathy quotient test (EQT) appeared to be related across subjects to the global amount of alpha desynchronization during the music performance (i.e., TRPD), namely the higher the EQT, the higher the alpha

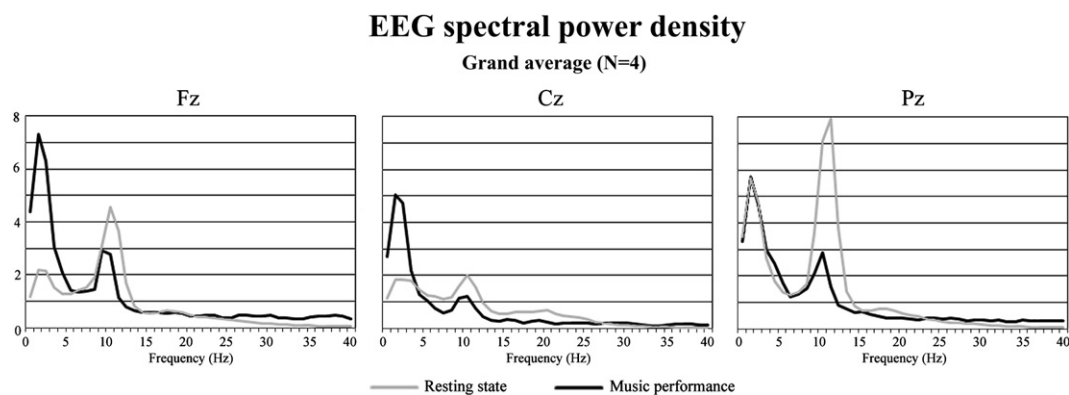


Fig. 4 – Grand average of EEG spectral power density values computed in the four subjects for three electrodes of interest (Fz, Cz, and Pz) and for the frequencies from 1 to 40 Hz (frequency resolution = 1 Hz). The EEG spectra refer to the resting state condition (plotted in gray) and the condition relative to the music performance (plotted in black).

sLORETA MAPS OF ALPHA TRPD/TRPI

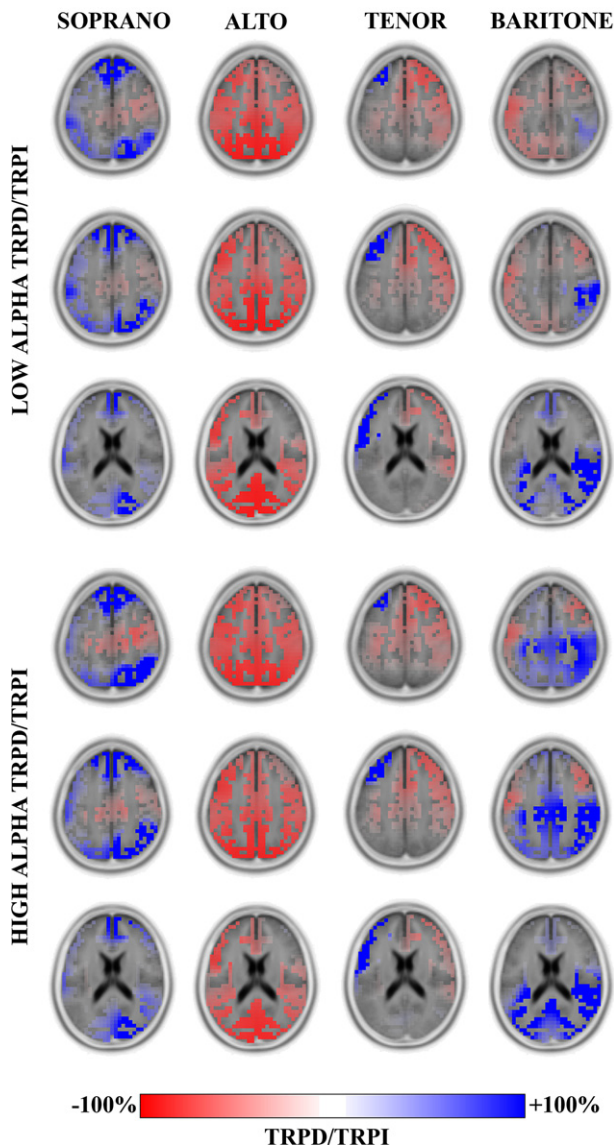


Fig. 5 – sLORETA solutions of the four musicians (i.e., soprano, alto, tenor and baritone saxophone players). The maps model distributed EEG sources of the low- (about 8–10 Hz) and high-alpha (about 10–12 Hz) frequency TRPD/TRPI in three slices.

desynchronization (i.e., TRPD). The present limited number of observations did not allow statistical analysis or final conclusions, and should be considered as a preliminary descriptive finding to be quantitatively evaluated in future studies using larger data sample (more quartets) with a proper data analysis design. This is beyond the aim of the present methodological study.

4. Discussion

In the present study, we propose a system for the simultaneous EEG–EOG–EMG recording in musicians playing in ensemble.

Table 1 – Score to two empathy indices obtained in the four musicians, namely the EQT and a confidential ad hoc questionnaire rating empathy feeling experienced during the music performance.

	Soprano	Alto	Tenor	Baritone
EQT	50	64	43	51
Empathy for the colleagues	73.3	95	80	95
Empathy during the performance	78.3	95	76.6	95

This system was designed for obtaining safe recording conditions, high-quality EEG–EOG–EMG data, triggering signals to track the task and to align EEG segments of the musicians, high-fidelity audio sounds, friendly visualization and management of the EEG–EOG–EMG–control data during the signal acquisition and subsequent analysis. These EMG data can be used to identify “on” and “off” states of the musicians’ motor performance, thus potentially allowing the investigation of the relationships between EEG dynamics and different modes of that performance. Noteworthy, the proposed system consists of commercial hardware and software items whose technical features and quality are well known (i.e., they were licensed by European community regulatory agencies). The novelty of the present methodological approach is their inter-connection for a new scientific purpose. For this reason, we did not get into deep technical details about these items but focused on the quality of EEG signals recorded in multiple musicians during music performance.

The quality of the EEG data was evaluated by the rate of artifact-free EEG epochs and by the features of dominant alpha rhythms (about 8–12 Hz) during both resting state and music performance. Results showed high-quality EEG data recorded in the four musicians with about 80% of artifact-free EEG epochs during music performance (i.e., the musicians were trained to minimize head, neck, forelimb, and trunk movements during their performance). Globally, the relatively high percentage of artifact-free EEG epochs represents a good first index of the quality of the EEG recordings. Furthermore, the analysis of EEG power density spectrum revealed typical features of human cortical EEG oscillatory activity during resting state and engaging events. During the resting state, dominant EEG power density values were observed at alpha band (8–12 Hz) in the posterior cortical regions. Furthermore, values of EEG power density at lower frequency bands were maximal in the anterior cortical regions (delta, 1–4 Hz; theta, 4–8 Hz). Finally, values of EEG power density at high-frequency bands were globally negligible (beta, 14–30 Hz; gamma, >30 Hz). During the music performance, alpha power density values decreased in amplitude in several cortical regions, whereas power density values within narrow frequencies of high-frequency beta and gamma were enhanced.

To illustrate possible future neurophysiological applications, performance-related power decrease (i.e., TRPD) of alpha sources was shown in the four musicians experiencing different levels of empathy during the music performance. It was observed that performance-related power decrease (i.e., TRPD) of alpha sources differed in the musicians across

several cortical regions. It can be speculated that the individual differences in cortical activity are related to some features of the music performance and/or specific features of the subjects' brain architecture and functional connectivity at the basis of psychological traits associated to music "sense" or "feeling", etc. Just as an example of the scientific opportunities opened by the present methodological approach, we reported empathy indices in the four musicians that seemed to be roughly related to alpha desynchronization during the music performance (i.e., TRPD). Future studies using more quartets and a proper statistical design will allow the quantification of the relationships between cortical activity and features of music performance, emotional experience, and psychological traits in musicians playing in ensemble. In conclusion, the present methodological approach appeared to be suitable for simultaneous EEG–EOG–EMG recordings in musicians playing in ensemble, thus opening a new avenue to the study of neurophysiological mechanisms at the basis of social communication in multiple individuals.

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