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# Potential of the projective Colour Association Method to reflect physiological responses to stimuli with a different emotional charge (PARC study) – a study protocol

## Potenciál projektivní Metody asociace barev odrážet fyziologické reakce na podněty s jiným emočním nábojem (studie PARC) – protokol studie

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### Abstract
Measurement of many important psychological concepts, such as health-related quality of life, satisfaction with different types of public and private services, personality, or attitudes, is most often based on various questionnaires. Nevertheless, this method is based on the subjective testimony of an individual about his/her features, feelings, attitudes, etc. It provides relevant responses only when respondents have well developed introspection, a reason to open up, express themselves, and provide adequate and relevant answers. Projective methods represent an alternative option to measure psychological concepts with the potential reduction of conscious or unconscious bias. The Colour Association Method (CA method) is an advanced projective technique, based on principles of the Lüscher colour test and word associations, which uses an objective computer data evaluation and thus overcomes one of the main weaknesses of projective methods. The study aims to investigate the ability of standard questionnaire and CA method to capture the responses of humans to different stimuli with emotional charge. A sample of 101 individuals will be used for this purpose, where each of them will be exposed to 145 different predefined stimuli. Comparison of physiological responses (cerebral blood flow, electrical activity of the brain, heart rate variability, and skin conductance response) to these stimuli and the perception of the stimuli measured by the questionnaire and CA method will be performed to determine which method provides a more accurate information on the real perception of the stimuli. These findings may have important consequences for the use of the questionnaire and the CA method to measure different psychological concepts.

### Key words
colour association method – questionnaire – emotion – cerebral blood flow – heart rate – skin conductance – study

### Klíčová slova
metoda asociace barev – dotazník – emoce – mozkový krevní průtok – srdeční frekvence – kožní impedance – studie

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## Souhrn

Měření mnoha důležitých psychologických konceptů jako jsou kvalita života související se zdravím, spokojenost s různými typy veřejných a soukromých služeb, osobnost nebo postoje, se nejčastěji opírá o různé dotazníky. Tyto dotazníkové metody jsou však založeny na subjektivním svědectví jednotlivce o jeho vlastnostech, pocitech, postojích atd. Relevantní odpovědi poskytují pouze tehdy, mají-li respondenti dobře vyvinutou introspekci, důvod otevřít se, vyjádřit se a poskytnout adekvátní a relevantní odpovědi. Projektivní metody představují alternativní možnost měření psychologických konceptů s potenciálním snížením vědomé či nevědomé zaujatosti. Metoda asociace barev (The Colour Association Method; CA) je pokročilá projektivní technika, inspirovaná Lüscherovým testem barev a slovních asociací, která využívá objektivního vyhodnocení počítačových dat a překonává tak jednu z hlavních slabin projektivních metod. Cílem studie je prozkoumat schopnost standardního dotazníku a metody CA v zachycení reakce lidí na různé podněty s emočním nábojem. K tomuto účelu bude do studie zařazen vzorek 101 jedinců, kdy každý z nich bude vystaven 145 různým předem definovaným podnětům. Bude provedeno srovnání fyziologických odpovědí (průtok krve mozkem, elektrická aktivita mozku, variabilita srdeční frekvence a kožní impedance) na tyto podněty a vnímání podnětů měřených dotazníkem a metodou CA s cílem zjistit, která metoda poskytuje přesnější informace o skutečném emočním vnímání podnětů. Tato zjištění mohou mít důležité důsledky pro použití dotazníku a metody CA k měření různých psychologických konceptů.


## Introduction

Measurement of many important psychological concepts, including health-related quality of life, satisfaction with different types of public and private services, personality, or attitudes, most often relies on a questionnaire. The questionnaire method is based on the subjective testimony of an individual about the individual's features, feelings, attitudes, reactions to different situations, etc. The measured constructs are thus derived from introspection, as proband responses are tied to his/her internal knowledge. The advantage of a questionnaire is its easy and fast administration, processing, and evaluation, which allows a large amount of information to be obtained from many individuals in a short time. The use of a questionnaire is beneficial in situations where respondents have a reason to open up, express themselves, and provide appropriate and relevant responses. On the other hand, the possibility of intentional result distortion by a respondent in order to bias the results in a desired way represents the most important disadvantage of questionnaires, as even a person of average intellect can quickly find the principles on which the questionnaire items are built and how to influence the results [1–5].

This may lead to low validity of many questionnaires, as validity scales often do not represent a sufficient assessment of a truthful answer to the questionnaire. These arguments sometimes lead to questioning the use of this method, especially in the cases of individuals who are not fully cognitively, socially, or medically eligible, and in individuals who do not cooperate or have some reason to influence the results [6–12]. Therefore, the combination of the questionnaire with other methods is suggested.

Projective methods represent an alternative measurement method. They identify personality, patterns of behaviour and experience, features, abilities, and other characteristics of an individual via undifferentiated and unstructured situations in which an individual reacts freely and expresses the individual's uniqueness. The ambiguity of situations gives rise to the possibility of a wide range of responses, allowing for the higher individualisation of the findings. Therefore, projective methods provide a deeper understanding of the mental processes of individuals and their dynamics [13,14].

The undisputed advantage of projective methods is that the examined person usually has little or even no chance to influence the results of the evaluation, because he/she does not know the principles of the examination and cannot anticipate 'suitable' answers. On the other hand, ambiguity in answers results in difficulties in score quantification and standardization. It also places a high demand on psychologists who use these methods, especially on their knowledge and experience. Therefore, the validity of these methods represents their most common criticism, especially since traditional psychometric approaches and external validity criteria are mostly not accurate enough and thus hardly applicable [2,15–18]. Despite these difficulties, the use of projective methods for measuring increases [19–21], especially in the case of children [17,18] and other target groups facing difficulties in using questionnaires.

The Colour Association Method (CA method), employed along with the questionnaire in this study, represents an advanced projective technique based on principles of Lüscher's colour test [22] and word associations. As a projective technique, it is more sensitive towards human diversity allowing for a more complex interpretation. It can be used in small children and illiterate individuals as it is based on colours, and it is more difficult to bias the results as the link between colour choice and the results is not straightforward. Moreover, it overcomes one of the main weaknesses of the projective methods, as it applies objective computer data evaluation based on a clearly defined algorithm and thus removes the risk of biased results caused by inexperienced users. Despite the fact that the CA method represents a promising measurement method, a rigorous evaluation of its validity is still lacking [23].

The Questionnaire and Projective Colour Association in Physiological Responses to a Different Emotional Charge (PARC) study aims to reveal how well the questionnaire and the CA method are able to capture the human responses, approximated by their physiological reactions, to different stimuli with emotional charge.

## Methods

### Participants

Healthy volunteers will be consecutively selected for the study. They will be recruited by a market and media research agency Nielsen Admosphere, a.s. (Prague, Czech Republic). The inclusion criteria are: 1) male or female; 2) aged 18–64 years; and 3) signing Informed consent. The exclusion criteria for participation in the study are: 1) contraindication to MRI examination, i.e., presence of metal fragments or implants, implanted pacemaker, and claustrophobia; 2) pregnancy or no possibility to exclude pregnancy; 3) head injury in the medical history; 4) seizures (photophobia, epilepsy) or a history of dizziness; 5) significant neurological, psychiatric, or other clinical difficulties, especially depression, anxiety, panic

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attacks, claustrophobia, AD(H)D, stroke, and cardiovascular problems; and 6) regular medication including hypnotics, analgesics, or other substances that affect brain function (antihistamines, medicines to reduce mucosal swelling, etc.).

### Sample size calculation

An estimate for the minimum sample size needed to reach significant differences between emotionally positive and negative or neutral stimuli evaluated using the CA method compared to the questionnaire was calculated for the 50% difference in skin conductance response (0.25 ± 0.1 vs. 0.5 ± 0.1) with an alpha level of 5% and power of 80%. Pre-study statistical calculations determined that a minimum sample size of 101 subjects is required when it is assumed that 20% of the subjects' arteries would not be able to finish the entire procedure.

### Procedure

Firstly, the focus and procedure of the study will be explained to all participants. After Informed consent signing, the physiological responses including cerebral blood flow, brain electrical activity, heart rate variability, and skin conductance response to visual word and picture stimuli will be measured in all participants in the Multimodal and Functional Imaging Laboratory (MAFIL) at Masaryk University, Czech Republic. The measurement takes about 35 min and the total time spent in MAFIL is approximately 60 min. After the measurement of physiological responses, participants will be asked to complete the online CA method application and online questionnaire within the next 24 h. Both the CA method application and the online questionnaire are focused on capturing the participants' perception of word and picture stimuli using different measurement methods, i.e., the colour association and questioning method. On average, this task takes approximately 20 min for each method. It should be noted that the different time periods for measuring physiological reactions to and the perception of stimuli by participants is given by the necessity to capture only brain activation connected with the perception of the stimuli, and not with answering the online questionnaire and CA method application. It poses higher demands on the robustness of the relationship between them, as both measurements must be highly reliable in the short term.

In the case of all kinds of measurements, i.e., physiological responses, CA method, and questionnaire, the respondents are exposed to the same 145 stimuli. The set consists of 115 words (three sets of words with positive, neutral, and negative content) and 30 pictures (two sets of 15 photos with positive and negative content). Combining stimuli with different content ensures greater variability of reactions, which supports the robustness and relevancy of the results. The content of the stimuli will be evaluated according to the national norm of the CA method for words and the International Affective Picture System [24] for pictures. It should be noted that participants are exposed to 21 stimuli twice during the measurement of physiological responses to check the stability of the physiological reactions. The order of the stimuli is identical for all participants, and the stimuli combine words and pictures regardless of their content. Repetition of the entire procedure will be performed in at least 20% of participants to check the robustness of intra-individual and inter-individual reliability.

### Measurements

#### Physiological responses

In our study, physiological responses to word and pictorial stimuli will be measured and statistically analysed. Four measurement modalities will be used to record data: functional MRI (fMRI), surface skull EEG, heart rate activity, and galvanic skin response (GSR) representing electrodermal activity of the body.

Functional MRI is used for the quantification of cerebral blood flow. Measurement is carried out using a Siemens Prisma 3T MRI scanner (Siemens Medical Solutions, Princeton, NJ, USA) with a 64-channel head-neck coil, Syngo version VE11c, accompanied by a standard Anatomical T1 MPRAGE scan. Multi-echo MB-EPI fMRI acquisition based on CMRR EPI sequences with TR = 700 ms and TE = 15/34/53 ms at 1,570 scans. GRAPPA PAT factor 2 and slice acceleration (MB factor) set to 6 are used. The dimension of the recorded voxel is 3 × 3 × 3 mm with a slice thickness of 3 mm. The number of axial slices is 48 (transverse) in the in plane FoV = 192 mm and the setting of the flip angle is 45°.

Time-frequency analysis is performed from indirect measurements of neural EEG activity. The measured parameter is the power of oscillations in each frequency band in the scalp EEG measured in each sensor. Non-invasive brain electrical activity is measured by high density EGI using a Net Amps GES400 series amplifier (Magstim EGI, Eugene, OR, USA) and 256 HydroCel Geodesic Sensor Net (GSN) (Magstim EGI, Eugene, OR, USA) on the skull surface with a 1 kHz sampling frequency.

Heart rate variability (HRV) is measured from an indirect measurement of the response of the autonomic nervous system. The measured parameter is the period of the cardiac cycle read from the ECG curve. ECG data are acquired as a supplementary channel during EEG data acquisition (EGI system) with a 1 kHz sampling frequency. The ECG channel is measured as a bipolar lead with one electrode placed under the left clavicle and the other electrode on the left side of the chest.

Electrodermal activity is measured as an indirect measurement of the response of the sympathetic autonomic nervous system. The measured parameter is the galvanic skin response (GSR). GSR is measured between the index and middle fingers of the dominant hand using the bipolar BrainAmp ExG MR (Brain Products GmbH, Gilching, Germany) with a sampling frequency of 5 kHz.

An MRI compatible LCD monitor (BOLD screen MR 24 from the Cambridge research system) [Cambridge Research Systems Ltd, Rochester, UK] is used as an interface to display word and pictorial stimuli during the measurement of physiological responses to them. The subject sees the image on the MRI scanner due to a mirror mounted above the head coil holes supplied by the MRI scanner manufacturer. Each of the 145 stimuli is displayed for 5 s to the subject under investigation.

### Pre-processing of physiological data

For subsequent statistical processing, the measured data will be adjusted and pre-processed. It makes it possible to use them for the evaluation of the potential of the questionnaire and the CA method to reflect the physiological and neurophysiological responses of the organism to stimuli.

The measured fMRI data will be realigned (motion correction) according to the middle echo signal. Optimally, multi-echo (ME) images using the weighted average based on the temporal signal-to-noise ratio (tSNR) and echo time (TE) will be combined. Spatial normalization will be adopted in the standard anatomical template (MNI space) and spatial smoothing will be performed with the Gaussian kernel, FWHM = 5 mm. The quality check will be performed by movement analysis based on the frame-wise displacement metric. In addition, a positive valid data check will be performed by spatial cover-

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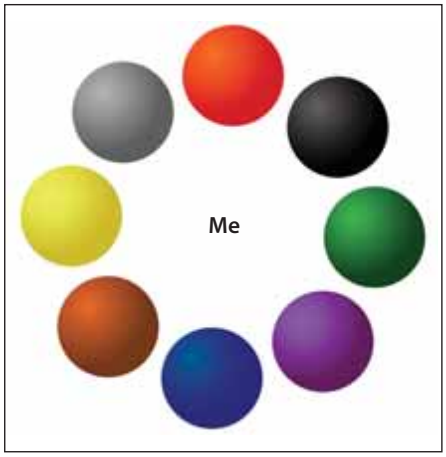




Fig. 1. On-line sensor.
Obr. 1. On-line senzor.

age of the brain based on the mask explorer 2.12 tool. Pre-processing will be performed in SPM12 software.

The EEG data represent a time-frequency decomposition of the data (time-frequency analysis) separately for each electrode. The data are 3-dimensional, where the time dimension has boundaries of 0–5 s, the frequency dimension has boundaries of 4–40 Hz, and the third dimension is represented by individual sensors. First, considering the amount of data, compression of the EEG recordings will be used. The frequency dimension will be divided into 33 bins (logarithmic) and the time axis will be divided into 5 bins (1 s each). The third dimension will not be compressed. Thus, each cell will contain a number that is indicative of a particular 1 s segment in a given electrode for a given narrow frequency band. EEG pre-processing will be performed in MATLAB software Brain Vision Analyzer 2.0 toolbox. Gradient and pulse artifacts will be removed using the IIR Butterworth bandpass at 1–40 Hz. Additionally, ocular artifacts will be removed using ICA decomposition corresponding to the ocular artifact. Subsequently, back-reconstruction of the signal without artifact components will be performed. The recordings will be segmented according to the moment of stimulus appearance with a segment length of 5 s (segment 0 to 5 s relative to the stimulus). Fifty two sensors will be cut from the analysis because the sensors are located on the cheeks, just above the eyes, around the ears, and the last row on the neck. These sensors often contain too many artifacts, and the electrodes are not used in the analyses by default. For each segment, the time frequency analysis will be calculated using the cwt function in MATLAB R2017a (The MathWorks, Natick, MA, USA) and the absolute value of the Cont coefficients is taken. The total signal power (Ptot) will be calculated for each segment. Segments that have Ptot > 1.5*median (Ptot) will be marked as artifacts. The threshold will be set arbitrarily to reasonably filter out segments with a poor signal. The average will be calculated over each second, which corresponds to matrix compression in the time domain. The result will be a matrix for each segment that is $33 \times 5 \times 204 \times 166$, where there are 33 frequency bins, 5-time bins, 204 electrodes, and 166 stimuli.

The ECG data will be pre-processed in Brain Vision Analyzer 2.0 (Brain Products GmbH, Gilching, Germany) for MATLAB software. Gradient artifacts will be denoised using IIR 1-20Hz bandpass filtering with semi-automatic R-wave detection. Data will be segmented according to the onset of stimulus occurrence. The segment length is 6 s. The average length of the R-R interval (hereafter R-R) will be calculated for each segment. For each proband separately, a correction for the R-R drift will be performed during the task. A third-order polynomial function will be modelled on the R-R waveform, and the estimated slow change trend will be subtracted from it. Subsequently, R-R will be converted to heart rate (HR) in units of pulses per minute.

Galvanic skin response data will also be pre-processed in MATLAB software LEDALAB Toolbox. Gradient artifacts and residual gradient artifacts will be removed using a median filter with a complementary IIR pass filter with a cut-off frequency of 1 Hz (median over 20 samples).

## Colour Association Method

The CA method is a combined projective technique based on the principles of two recognized psychological concepts: the Lüscher's colour test and word associations. Lüscher [22] studied the connection between colours, defined by their different wavelengths, and the psychological state. He found perception of colours to be independent of volition, universal, and common to all people, which is why it is objectively measurable. The theory of association stems from the pioneering works of James [25] and Wundt [26], who described and explained the principles of associations in the human brain. The CA method links the knowledge of the basic principles of associations in human consciousness with the measurement of colour preferences and combines their benefits.

The CA method uses calibrated sets of words or visual objects (hereafter stimuli) and a palette of eight colours. A stimulus provokes an immediate association, i.e., activation of particular neuronal junctions and synapses, to which the person is instructed to react via colours. As associations cannot be disrupted or influenced rationally, the CA method is focused on measuring and evaluating 'authentic uncensored associations' (see DAP Services 2014 for details on the computation). The process of measurement is stimulated by automated and intuitive patterns of feeling, thinking and experiencing stemming from the four autonomous subsystems of consciousness – physical, emotional, intellectual and relational subsystems. Therefore, the CA method enables one to capture the social psychology attitudes of individuals or groups, including not only what they think and prefer, but also whether they will project their attitudes in their behaviour, how successful they will be, and in what timeline. It also measures the dynamics of functional processes of consciousness, attitudes, and consequently complex units within consciousness. Furthermore, the use of colours to capture associations makes the CA method one of the so-called ‚blind techniques', since an individual has little chance to consciously or unconsciously bias his/her responses according to the situation, social norms, or expectations of others. Focusing on association mechanisms that are almost identical across individuals allows its use in all individuals regardless of the level of their knowledge or rational thinking.

Stimuli-colour associations will be captured through an online sensor (Fig. 1; an explanation of the principles on which it is based is described at [27]). Data gathering through an online sensor has three phases: initial colour selection (ordering colours from the most to the least pleasant), capturing stimuli-colour associations (selecting three colours that are the most convenient for each stimulus) and final colour selection (ordering colours from the most to the least pleasant). Subsequently, data are automatically processed and evaluated using the Object-Communication Analysis of Consciousness (OCAC) method, resulting in a structured consciousness map, i.e., hierarchy of attitudes and values. The evaluation can be based on the selected colour combination, where different colour combinations are connected with different attitudes and values (hereafter Scheme A), the preferential

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scale of colours defined in the initial selection of colours (hereafter Scheme B), the preferential scale of colours defined in the final selection of colours (hereafter Scheme C), and the preferential scale of colours computed from the choices of the respondents during a measurement (hereafter Scheme D). These schemes differ not only in computation, but also in the interpretation of the results. In this study, all these schemes can be used to evaluate the stimuli into five categories: Destructors – Stressors – Operators – Motivators – Idols (ordered from the most negatively perceived categories to the most positive ones), and evaluation of the correlation between physiological and psychological responses to selected word and visual stimuli.

### Questionnaire

Finally, participants will be asked to express their perception of word and pictorial stimuli using a questionnaire. For each of the 145 stimuli, they will be asked to evaluate how the word/image (displayed) affects them using the numerical scale 1–7, where the number 1 represents a completely negative perception, and the number 7 represents a completely positive perception.

### Data analysis

In this study, two main approaches to the statistical analysis will be applied. General linear models will be used to analyse the MRI data (using SPM12 software). The models explore brain activity depending on the positivity of the stimuli approximated by questionnaire responses and CA method categories. Group analyses will be calculated with SPM12 random effect models controlling for sex and age. One sample t-test, paired t-test, and flexible factorial model (ANOVA) will be used to evaluate the data according to individual goals. A threshold level of $p = 0.05$ (FEW corrected) will be used for all results.

Statistical analysis of the relationship between the CA method, questionnaire categories, heart rate, and skin conductance will be performed on aggregated data. First, the mean heart rate and skin conductance will be calculated for the categories of the most negative, neutral, and positive stimuli for each individual. Subsequently, these aggregated values will be analysed by the nonparametric Kruskal-Wallis rank test and Dunn's test of multiple comparison in order to reveal whether/which categories embody statistically significant differences compared to others. These tests will be used in the case of the ordinal character of the data or the violation of assumptions for parametric tests; otherwise, ANOVA and Tukey test will be used. The same procedure will be used for the analysis of the mutual relationship between the CA method and the questionnaire. Moreover, this analysis will be further replenished by a correlation analysis employing the Pearson's or Spearman's correlation coefficients depending on the characteristics of the analysed data.

## Ethical aspects

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Ostrava (reference number 10/2021), approved on 12/10/2021 and is carried out according to national and international law. All participants will give their written consent, consent to personal data processing according to the EU General Data Protection Regulation (GDPR) and they will receive financial compensation for their participation. The study was registered at ClinicalTrials.gov (ID: NCT05363852) prior to enrollment of the first participant.

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## Conflict of interest

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

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