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Topics are announced at least three months in advance of each issue with the submission date. Up to five articles, limited to 8000 words, will be published with as wide a range of theoretical views as possible on a given topic. Peer review of papers will be done in a month following the specified submission date. For those that are accepted, on-line publication of the uncorrected article occurs within seven days with the final corrected version done in six weeks. During the next month following acceptance, the authors of the articles will write a commentary paper of no more than 2000 words evaluating the other simultaneously published articles vis-á-vis their own, with the goals of synthesis, integration, and expansion where possible. For any obviously incompatible points, authors are asked to provide specific suggestions on ways to empirically address the points to clarify which is likely correct. These commentaries will be published in the next issue. At the time the original articles are published, a general call for additional commentary papers will occur, targeting submission within a month. These will be subjected to peer review and up to five new commentaries will be published the third month. The overall process is designed to foster productive interaction, leading to the generation of hybrid theories and new ideas.

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Contents

Classification of Spike Wave Propagations in a Cultured Neuronal Network: Investigating a Brain Communication Mechanism

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ABSTRACT

In brain information science, it is still unclear how multiple data can be stored and transmitted in ambiguously behaving neuronal networks. In the present study, we analyze the spatiotemporal propagation of spike trains in neuronal networks. Recently, spike propagation was observed functioning as a cluster of excitation waves (spike wave propagation) in cultured neuronal networks. We now assume that spike wave propagations are just events of communications in the brain. However, in reality, various spike wave propagations are generated in neuronal networks. Thus, there should be some mechanism to classify these spike wave propagations so that multiple communications in brain can be distinguished. To prove this assumption, we attempt to classify various spike wave propagations generated from different stimulated neurons using our original spatiotemporal pattern matching method for spike temporal patterns at each neuron in spike wave propagation in the cultured neuronal network. Based on the experimental results, it became clear that spike wave propagations have various temporal patterns from stimulated neurons. Therefore these stimulated neurons could be classified at several neurons away from the stimulated neurons. These are the classifiable neurons. Moreover, distribution of classifiable neurons in a network is also different when stimulated neurons generating spike wave propagations are different. These results suggest that distinct communications occur via multiple communication links and that classifiable neurons serve this function.

Keywords: cultured neuronal network; spike wave propagation; spatiotemporal form; classifying; multiple communications

1. Introduction

The brain is an intellectual information processing system [1–5]. How a neuronal network of ambiguously behaving neurons establishes a highly reliable information processing system, distinct communication, and organized communication links is an unanswered question. Despite many

researchers attempting to solve this question, it remains a mystery. In previous studies, factors such as spatiotemporal coding, the Synfire chain, and the spatiotemporal form of spike activity were considered the fundamental generators of natural intelligence in the brain [6–11]. However, basic communication functions between neurons have not been elucidated in these studies. Therefore, the abovementioned question still remains unsolved. Recently, we focused on distinct and different communication to investigate the previously mentioned question [12–15]. In previous work [16], spike propagation as a cluster of excitation waves, termed as spike wave propagation, was observed in cultured neuronal networks. However, in those experiments, it was only observed that various spike wave propagations were generated in neuronal networks. The details of these mechanisms were still unclear. To investigate these mechanisms, we simulated a 9 × 9 2D mesh neural network consisting of an integrate-and-fire model without leak. Resulting from this method, multiplex communication is possible at a success rate of 99% [17]. This result suggested that distinction of the spike wave propagation spatiotemporal form was the clue to classifying multiple communications in the brain. Here, we assume spike wave propagations are just communication events in the brain and attempt to prove this assumption. However, physiological experiments, analysis, and discussions about these events have yet to be reported [17].

In this study, we attempt to classify various spike wave propagation from different stimulated neurons in cultured neuronal networks, as well as discuss the implications of these classifying results in a view of brain communication. The authors' research group is presently studying the functions of neuronal networks by combining experiments with cultured neuronal networks with artificial neural network simulations. This paper corresponds to previous work on the ability of remote receiving neurons to identify two transmitting neuron groups stimulated in a neuronal network, i.e., 2 to 1 communication [17]. These mechanisms may be the basis of higher cortical functions. The aim of this study is to investigate the most essential question in our study: to identify what the spatiotemporal form of spike wave propagation suggests in view of communication in brain physiologically.

2. Methods

2.1. Cell cultures

Cell cultures of hippocampal neurons were dissected from Wistar rats on embryonic day 18. The procedure conformed to the protocols approved by the Institutional Animal Care and Use Committee of the National Institute of Advanced Industrial Science and Technology. Hippocampi were dissociated with 0.1% trypsin (Invitrogen; Tokyo, Japan) in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline at 37°C for 15 min. The dissociated neurons were planted at a density of 3.3 × 105 cells/mm^2 in

polyethylentimine-coated microelectrode array (MEA) dishes (MED-P515A, Alpha MED Scientific; Kadoma, Japan) with 8 × 8 planar microelectrodes. The size and spacing of the electrodes were 50 × 50 μm^2 and 150 or 450 μm, respectively. To position the neuronal networks in the central area of each MEA dish, a cloning ring with an inner diameter of 7 mm was used. The ring was removed the following day. Neurons adhered to the substrate of the MEAs, covering all electrodes. Neurons were maintained at 37°C in a humidified atmosphere of 5% CO_2 and cultured for 21–40 days in Dulbecco's modified Eagle's medium (Invitrogen), which contained 5% horse serum and 5% fetal calf serum with supplements of 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml insulin. Half of the culture medium was renewed twice per week. In this study, four cultured cell samples at 22–50 days in vitro were prepared and are referred to as Cultures 1, 2, 3, and 4. Figure 1 shows a micrograph of the cultured neurons in an MEA.

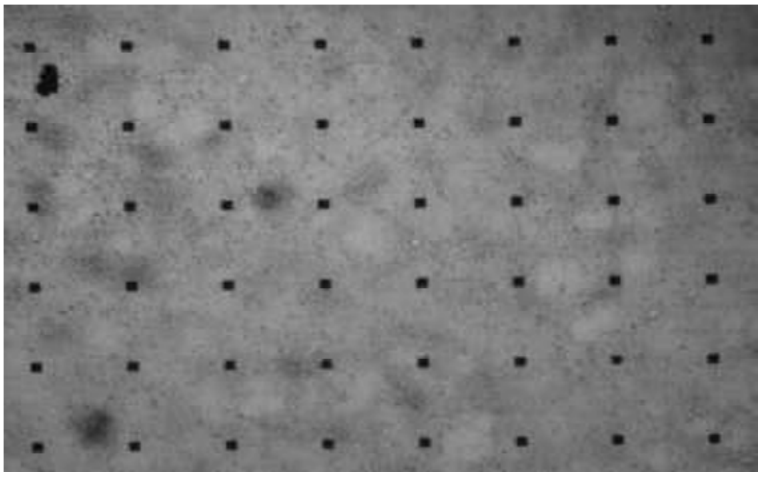


Figure 1. Micrograph of cultured neurons in an MEA (×20).

2.2. Stimulated spike recording

Stimulated spikes were recorded using MED64 (Alpha MED Scientific; Osaka Japan), an extracellular recording system with 64 electrodes (channels). The size of each electrode is approximately the size of a neuron. The recording was performed for 3 s at a sampling rate of 20 kHz.A selected channel was stimulated at 5 ms after the start of the recording. The stimulation signal was a current-controlled bipolar pulse (positive, then negative) with a strength of 10 uA and a duration of 100 us.

Two to three channels in each culture were selected as the stimulation channels, and they were subjected to 10–15 recordings. In this study, the stimulated channels are referred to as StimA, StimB, and StimC. Incidentally, this study investigates whether the original stimulated channels (StimA, B, or C) can be identified from spike train at each channel (including multi-neurons), rather than by single neurons. Therefore, spike sorting was not performed.

2.3. Coding spike trains

The recorded spike trains were coded as follows: first, raster plots were generated by detecting peaks above a pre-specified threshold on each channel in the recorded spike responses [18]. Then, spike interval trains were calculated from the raster plot data.

2.4. Classifying procedure

Previously [16], effort was made to analyze the differences in the spike spatiotemporal pattern corresponding to the stimulated neuron using the dynamic time warping (DTW) method. This method uses a dynamic programming technique to find the minimum distance by stretching or shrinking the linearly or non-linearly warped time series and is thus useful for finding the optimal alignment between two non-uniform time series [19]. However, the DTW method does not offer an adequate resolution [20]. Therefore, the qualities of the analysis results were not enough to clarify whether multiple spike waves are classifiable. The brain must have some physiological learning mechanism for classifying spike wave propagations with various temporal patterns. Considering previous experimental results, we used an analytical method with a learning algorithm instead of DTW. In the field of machine learning, back propagation, deep learning, etc. are well known. Though these methods, which imitate the behavior of physiological neuronal networks, are very effective for classifying various and complex data, the learning algorithm seems to be better suited for arranging physiological behavior to fit machine learning. Therefore, in this study, we use a simpler learning algorithm based on the arithmetical average method, which seems to have more compatibility with natural recognition (See Supplementary S-1).

The outline of classifying procedure is as follows.

- Repeat for each 64 channel on MED64
  - (1) Spike train is learned by 5–10 spike temporal patterns with the same stimulated neuron (called neuron *A* temporarily). This spike train form is termed *Learning pattern A*.
  - (2) *Learning pattern B* (stimulated neuron is neuron *B*) are created by the same method as *Learning pattern A.*
  - (3) To find *classifiable neurons*, the resemblance of spike train (before learning) on trial (named *Trial Data*) and *learning pattern A or B* was estimated by the procedure described in Supplementary S-2.

3. Results

**To explain the detection method of classifiable neurons, the results of Cultures 1 and 2 are in described in detail.*

3.1. Culture 1

In Culture 1, 15 spike responses were recorded when channel 4 was stimulated. Five spike responses from the 15 were used for Trial Data named Tr401, Tr402, . . . Tr405, while the other 10 spike responses were used for Learning Pattern 4. Next, five Trial Data named Tr2801, Tr2802, . . . Tr2805, and Learning Pattern 28 (channel 28 is stimulated) were created by the same procedure as Tr401-Tr405 and Learning Pattern 4.

Figure 2 shows the result of the resemblance test for Tr2801. In Fig. 2b, which focused on channel 16, the mean value of SpsetTrial was significantly greater than that of SpsetLocal (see Supplementary S-2), when the stimulated neuron of the trial was different than that in the learning pattern. No significant difference was observed when the stimulated neuron of Trial Data was the same as in learning pattern (Figure 2a). This result suggested that the stimulated neuron of these Trial Data was not neuron 4. In other words, these Trial Data can be extracted from Leaning Pattern 4 and the stimulated neuron 28 can be classified successfully as a neuron on channel 16. Therefore, this neuron was a classifiable neuron. In this trial, there were 14 classifiable neurons. Table 1a shows the number of classifiable neuron in each trial in Culture 1.

3.2. Culture 2

In Culture 2, Tr1301, Tr1302, . . . Tr1305, Learning Pattern 13, Tr3001, Tr3002, . . . Tr3005, Learning Pattern 30, Tr5401, Tr5402, . . . Tr5405, and Learning Pattern 54 (the stimulated neurons were channels 13, 30, and 54, respectively) were prepared for experiments and learning patterns were created by 5-spike responses. Figure 3 shows the estimation result of the comparison for Tr1304. Sixteen classifiable neurons were observed through comparison with Learning Pattern 54 and 10 through comparison with Learning Pattern 30. Table 1b shows the number of classifiable neurons for each trial.

(a)

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64

(b)

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64

Figure 2. Estimation results of the comparisons for Tr2801. (a) Comparison with Learning pattern 28 (b) Comparison with Learning pattern 4. Green cells indicate that the mean value of SpsetTrial was significantly greater than that of SpsetLocal (see Supplementary S-2). Blue cells indicate that SpsetTrial was not significantly greater than SpsetLocal. Gray cells indicate no spikes or that the number of spike was less than eight in the recording.

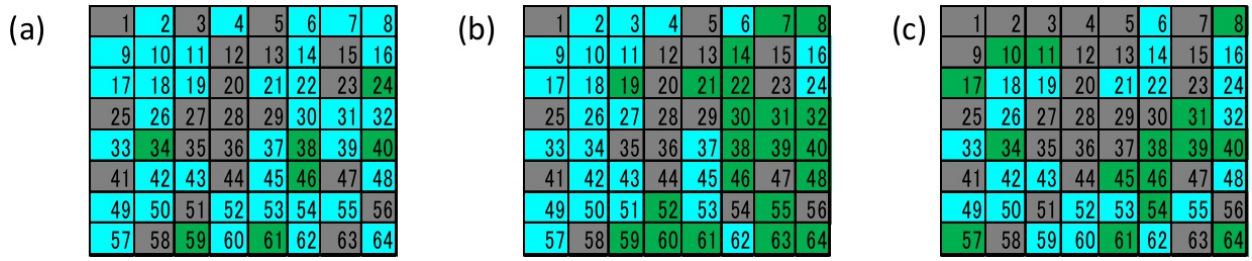


Figure 3. Estimation results of the comparisons for Tr1304. (a) Comparison with Learning Pattern 13 (b) Comparison with Learning Pattern 54 (c) Comparison with Learning Pattern 30. Green cells indicate the mean value of SpsetTrial was significantly greater than that of SpsetLocal (see Supplementary S-2). Blue cells indicate that SpsetTrial was not significantly greater than SpsetLocal. Gray cells indicate no spikes or that the number of spikes was less than eight in this recording.

3. 3. Cultures 3 and 4

For Culture 3, channels 4 and 38 were stimulated. For Culture 4, channels 8, 10, and 57 were stimulated. The detection method of classifiable neurons in these cultures was similar to Culture 1 and 2. Therefore, in Culture 3 and 4, only the number of classifiable neurons for each Trial Data in Table 1c and 1d is shown.

3.4. Comparing with Spike Interval Shuffling data

As shown in Figure 2, Figure 3, and Table 1, classifiable neurons were observed in particular areas of neuronal networks. However, there was indication that these classifiable neurons were detected accidentally and purpose of the number of experiments performed was not to dispel this doubt. Therefore, we attempted to detect classifiable neurons from shuffled spike-interval sequence, called Interval Shuffle (Int. Shuf) [21], in parts of the trial data in Cultures 2 and 3. The numbers of classifiable neurons from Interval Shuffle data were less than from original (non-Interval Shuffle) spike-interval data. In Culture 2, the difference between the two was significant ($p < 0.05$, as result of t-test). These results show that the detected classifiable neurons from the original spike data were not accidental.

Table 1. The number of classifiable neurons for each Trial Data.

a. Culture 1

Trial	Classification	
	vs ch 4 stim	vs ch 28 stim
Tr401	–	10
Tr402	–	9
Tr403	–	10
Tr404	–	17
Tr405	–	13
Tr2801	14	–
Tr2802	12	–
Tr2803	13	–
Tr2804	14	–
Tr2805	14	–

b. Culture 2

Trial	Classification		
	vs ch 13 stim	vs ch 54 stim	vs ch 30 stim
Tr1301	–	10	10
Tr1302	–	17	19
Tr1303	–	16	15
Tr1304	–	16	10
Tr1305	–	11	6
Tr5401	21	–	3
Tr5402	18	–	7
Tr5403	16	–	2
Tr5404	19	–	5
Tr5405	19	–	6
Tr3001	16	9	–
Tr3002	21	6	–
Tr3003	8	7	–
Tr3004	15	3	–
Tr3005	10	10	–

c. Culture 3

Trial	Classification	
	vs ch4 stim	vs ch 38 stim
Tr401	–	19
Tr402	–	25
Tr403	–	21
Tr404	–	24
Tr405	–	10
Tr3801	18	–
Tr3802	0	–
Tr3803	19	–
Tr3804	20	–
Tr3805	17	–

d. Culture 4

Trial	Classification		
	vs ch57 stim	vs ch08 stim	vs ch 10 stim
Tr5701	–	9	0
Tr5702	–	2	0
Tr5703	–	8	2
Tr0801	0	–	0
Tr0802	0	–	0
Tr0803	0	–	0
Tr1001	10	7	–

e. Culture 2 (*Int. Shuf*)

Trial	Classification		
	vs ch13 stim	vs ch54 stim	vs ch 30 stim
Tr1301	–	0	0
Tr1302	–	5	1
Tr1303	–	0	0
Tr1304	–	0	2
Tr1305	–	0	3
Tr5401	6	–	0
Tr5402	0	–	0
Tr5403	2	–	0
Tr5404	6	–	3
Tr5405	0	–	0

f. Culture 3(*Int. Shuf*)

Trial	Classification	
	vs ch4 stim	vs ch 38 stim
Tr401	–	3
Tr3805	0	–

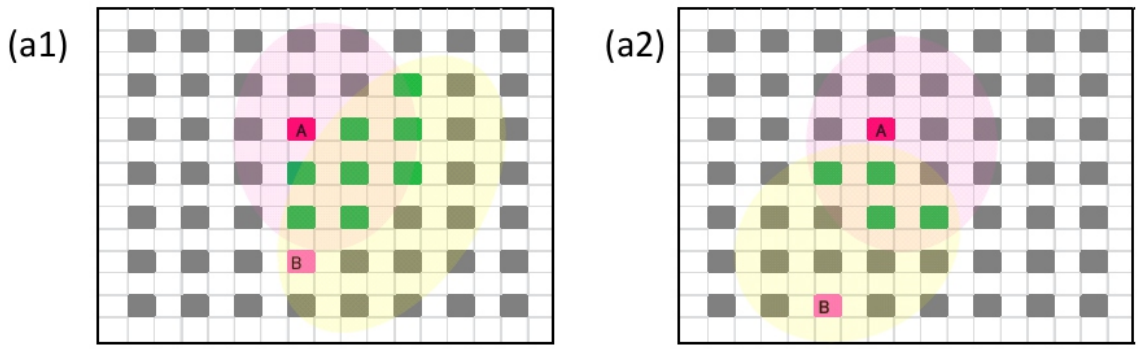
4. Discussion

4.1. Discussion on the analysis results

Based on the experimental results, several classifiable neurons were observed in particular areas of neuronal networks. In detail, multiplexed spike wave propagation share several neurons and some may be used to classify different spike wave propagations. Accordingly, questions arose considering the distribution of classifiable neurons: do both classifiable and non-classifiable neurons exist in the same neuronal network? The distribution of classifiable neurons is influenced by the distribution of synaptic weights in the neuronal network. It is well known that each neuron has an individually specific (intrinsic) synaptic weight and each neuron is considered classifiable neuron or not depending on conditions such as synaptic weights. In the physiological experiments, unlike the simulation experiments [17], it is difficult to determine weight distributions intentionally and only a limited number of realized weight distributions were observed. Therefore, distributions of classifiable neurons varied between different

cultures. In attempt to understand why non-classifiable neurons are intermingled with classifiable neurons are intermingled in the same neuronal network, three conditions of spike wave propagation scheme were presumed, as shown in Figure 4. For simplicity, it was assumed that all neurons were connected to neighboring neurons and spike waves spread radially from stimulated neurons. Due to the influence of the synaptic weight distribution in neuronal networks, each spike wave propagates with its own individual spatiotemporal pattern. Therefore, neurons sharing multiple spike wave propagations could be used to classify different spike wave propagations if a spike wave does not spread to neurons stimulated another spike wave each other (Figure 4 a1-2). However, if one spike wave spreads to neurons stimulated by another spike wave, as shown in Figure 4b, some neurons fire the same temporal patterns, even when a different neuron is stimulated. Results shown in Figure 2, Figure 3, and Table 1 suggest that this condition was realized in neuronal network used in these experiments.

Moreover, it was difficult to classify the stimulations of channel 54 and channel 30 in Culture 2, as fewer classifiable neurons were observed. The reason for this result was that spike waves spread to neurons that were stimulated by other spike waves, as shown in Figure 4c. Under this condition, some neurons fire the same temporal patterns, even when a different neuron is stimulated. Additionally, although we assume in this discussion that the spike waves spread in a simple radial direction, neurons are connected randomly in reality. Therefore, both classifiable neurons and non- classifiable neurons observed (Figures 2 and 3). From Figures 3b and 3c, the distribution of classifiable neurons in Learning Pattern 54 (stimulated neuron was ch54) was different from the distribution of classifiable neurons based on Learning Pattern 30. This phenomenon provides explanation for how spikes wave spread, as shown in Figure 4. If a pair of naturally stimulated neurons generate two different spike waves, the distribution of these spike waves and the overlap area are different, thus reflecting the distribution of classifiable neurons. Consequently, the spatial distribution of classifiable neurons in the network varies when there are multiple targets for spike waves.



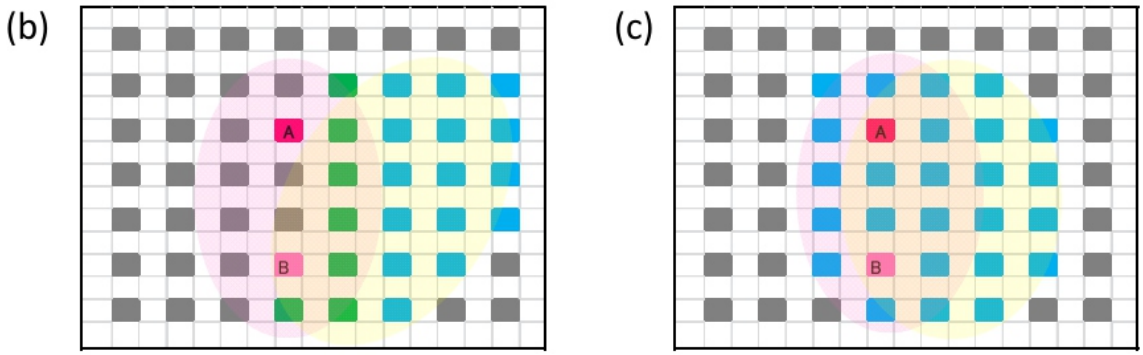


Figure 4 Condition of spike wave propagation scheme. (a1-a2) The spike wave generated from neuron A did not cover neuron B and spike wave generated from neuron B did not cover neuron A. In this condition, each spike wave was generated independently when neuron A or B was stimulated. Neurons overlapping both spike waves (green) generate different temporal patterns when the stimulated neuron was different Therefore, two stimulated neurons were classifiable in this area. If a pair of stimulated neurons generated two different spike waves, the distribution of these spike waves and the overlap area were different, thus reflecting the distribution of classifiable neurons. (**a2**) If the location of neuron B was different from **a1**, the spread and distribution of "green neurons," corresponding to the different overlapping areas. (**b**) Spike waves generated from neuron A covered neuron B; neuron B fired and spike wave were generated from neuron B. Under this condition, neurons indicated in blue fired in the same temporal pattern both when neuron A was stimulated and when neuron B was stimulated. Therefore, no difference was observed in the temporal pattern in this area. However, two stimulated neurons were classifiable (green). (**c**) Spike waves generated from neuron A covered neuron B and spike wave generated from neuron B covered neuron A. Both spike wave were generated from either stimulated neuron A or B. Hence, the temporal pattern was observed.

Furthermore, we investigated how multiplexed communication affects the processing of intellectual information in the brain. A simple multiplexed communication in the brain was modeled, as shown in Figure 5. The establishment of a virtual communication link from stimulated neurons to a particular area in the neuronal network was observed. Consequently, specific information was received in a particular area (Figure 5). We consider these processes as the fundamental mechanisms of intelligence in the brain. In fact, we hypothesize that the present model is valid not only for simple situations, but also for more complex similar situations.

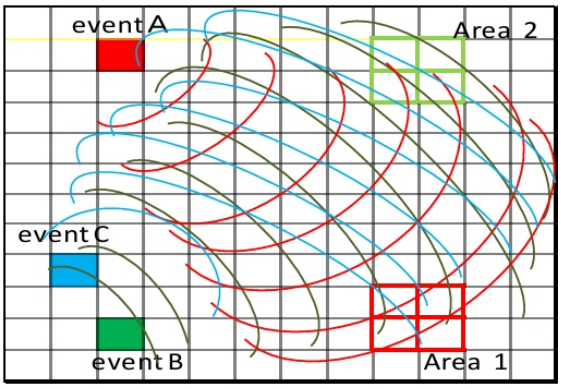


Figure 5. A sample of the multiplexed communication field in the brain. The figure shows events corresponding to stimulated neurons and spike wave propagations. In Area 1, event A was distinguishable from event C and in Area 2, event B was distinguishable from event C because classifiable neurons were concentrated in these areas. From a broad perspective, information for event A was receivable in Area 1 and information for event B was receivable in Area 2. Thus, two communication links from event A to Area 1 and from event B to Area 2 were extracted. In this case, event C was the comparison criterion of the spike spatiotemporal pattern of events A and B (if another event, such as event A or B, was the comparison criterion, the communication link for event C could also be extracted).

In contrast, for a few neurons, the mean value of SpsetTrial was greater than SpsetLocal. The mean value was significantly greater when both the trial pattern and the learning pattern were generated from the same stimulated neurons (Figures 2a and 3a). The results of these experiments suggest the possibility of the incorrect classification of some spike wave propagations. However, such neurons are fewer in number than classifiable neurons (when the stimulated neurons are different between the trial and the learning pattern). Therefore, the activities of such neurons may be masked by classifiable neurons. In brief, the trials successfully classified the entire neuronal network in a broad way and the experimental results reflect the distribution of synaptic weight in neuronal networks.

4.2. Function of classifiable neurons in the brain

The function of classifiable neurons was investigated in the brain. It was considered that classifiable neurons may participate in distinguishing different communications in the brain and that multiplexed spike wave propagations correspond to multiplexed communications in the brain. Some communications use the same neurons, as shown in Figure 4. In this case, the function of classifiable neurons was to classify multiple communications and recognize individual information. This function is similar to the multiplexed communication mechanism in artificial communication systems, such as mobile phones.

5. Conclusion

In this study, we classified various spike wave propagations individually generated from different stimulated neurons using an original spatiotemporal pattern matching the method of spikes in a cultured neuronal network. Based on the experimental results, classifiable neurons were observed in the neuronal network. We also confirmed that the spatial pattern of classifiable neurons within the neuronal network depended on stimulated neurons generating different spike wave propagations. These results suggest that distinct communications occur via multiple communication links in the brain and classifiable neurons play a significant role in this process. Moreover, multiplexed communication scheme in the neuronal network were modeled in order to discuss the meaning of the multiplexed communication mechanism with regard to the management of intellectual information in the brain. The results of this study suggest that communication in the neuronal network is the basis of brain activity. This research provides a significant clue to solving one of the deepest mysteries of neuronal networks, namely, how seemingly ambiguous behavior among neurons leads to a reliable information processing system. In this study, multiplexed communication is only modeled for one simple situation in a neuronal network. Because the comparable spatiotemporal patterns in the present analytical program are limited to two (events A vs B, A vs C, or B vs C), the resulting multiple analyzed spike spatiotemporal pattern includes only a pair of events (events A vs B, A vs C, or B vs C). Thus, the present multiplexed communication scheme is incomplete and further research is required to investigate situations with more than three events. Although the present scheme may be adequate for more complex situations as well, it is necessary to clarify these situations of multiple communications in the brain in future studies.

Lastly, the features of this paper are summarized as follows:

(1) To our current knowledge this study is the first attempt to investigate multiplex communication in a cultured neuronal network.

(2) Experiments and analysis correspond to a simulation experiment in 9 × 9 2D mesh neural network and sought to identify two transmitting neuron groups stimulated in a simulated neuronal network, i.e., 2:1 communication [17].

(3) The results of this study show a signal transmission principle in neuronal networks which provides a possible solution to the mystery of the manner of reliable neuronal communication, which is thought to be the basis of brain activity.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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The Magnetic Acoustic Change Complex and Mismatch Field: A Comparison of Neurophysiological Measures of Auditory Discrimination

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ABSTRACT

The Acoustic Change Complex (ACC), a P1-N1-P2-like event-related response to changes in a continuous sound, has been suggested as a reliable, objective, and efficient test of auditory discrimination. We used magnetoencephalography to compare the magnetic ACC (mACC) to the more widely used mismatch field (MMF). Brain responses of 14 adults were recorded during mACC and MMF paradigms involving the same pitch and vowel changes in a synthetic vowel sound. Analyses of peak amplitudes revealed a significant interaction between stimulus and paradigm: for the MMF, the response was greater for vowel changes than for pitch changes, whereas, for the mACC, the pattern was reversed. A similar interaction was observed for the signal to noise ratio and single-trial analysis of individual participants' responses showed that the MMF to Pitch changes was elicited less consistently than the other three responses. Results support the view that the ACC/mACC is a robust and efficient measure of simple auditory discrimination, particularly when researchers or clinicians are interested in the responses of individual listeners. However, the differential sensitivity of the two paradigms to the same acoustic changes indicates that the mACC and MMF are indices of different aspects of auditory processing and should, therefore, be seen as complementary rather than competing neurophysiological measures.

Keywords: Acoustic change complex; auditory discrimination; magnetoencephalography; mismatch

1. Introduction

The ability to discriminate between different sounds is a basic prerequisite for spoken language perception [1]. However, poor performance on behavioural tests of auditory discrimination need not necessarily indicate a perceptual impairment. This is especially true in populations such as young children, or individuals with neurodevelopmental or degenerative conditions, for whom poor attention or task understanding might impact adversely upon performance. For this reason, researchers have increasingly made use of electroencephalography (EEG) and magnetoencephalography (MEG) to passively measure event-related cortical responses to changes in auditory stimuli, taking the presence

and magnitude of the elicited brain response as an index of perceptual discrimination. The majority of these studies have employed an oddball paradigm, in which participants hear a sequence of discrete sounds composed of frequent "standards" and rare "deviants" that differ along a single stimulus dimension. The Mismatch Negativity (MMN) or its magnetic counterpart, the Mismatch Field (MMF), is calculated by subtracting the brain response to the standard from the response to the deviant sound [2,3]. The amplitude of the MMN has been found to correlate with performance on behavioural discrimination tasks [4–7]. However, a number of researchers have questioned the reliability of the paradigm, noting that the MMN response is not always elicited, even for easily discriminable stimuli [6,8–12]. A less commonly used alternative is the acoustic change paradigm in which participants hear a continuous auditory stimulus containing a discrete change in, for example, pitch. This elicits a P1-N1-P2 evoked potential referred to as the Acoustic Change Complex (ACC) [13–15]. Like the MMN, the ACC is correlated with behavioural measures of intensity and frequency change [13,16,17] and has good test-retest reliability [15,16]. Surprisingly, only one study to date has directly compared the MMN and the ACC. Using EEG, Martin and Boothroyd [14] elicited an ACC response by presenting participants with 790 ms stimuli that transitioned at their midpoint from a complex tone to spectrally matched noise (or back again). The MMN was elicited using 150 ms tones and noise bursts as standards and deviants. Martin and Boothroyd reported that the ACC response was 2.5 times larger than the equivalent MMN. Moreover, every participant produced an ACC response that was clearly visible and identifiable.

The current investigation extended Martin and Boothroyd's study in two directions. First, rather than tones and noises, we employed linguistically relevant stimuli. Specifically, participants heard semi-synthesized vowel sounds with changes in either pitch (fundamental frequency) or vowel identity (formant frequencies). Second, rather than EEG, we used MEG to measure the MMF and the magnetic ACC (henceforth mACC). As a research tool, MEG has a number of advantages over EEG, particularly for studies of child and clinical populations. Set-up is quick and straightforward and does not involve scalp-scratching or physical contact with the sensors [18,19]. Moreover, MEG has higher spatial resolution, allowing for more accurate source reconstruction and clearer resolution of hemispheric differences [20,21]. However, because MEG is mostly sensitive to cortical sources oriented tangentially to the surface, it does not always provide a superior signal to EEG. Participants were tested in two 15-minute sessions, once with an MMF paradigm using pitch- and vowel-changes in semi-synthesized speech, and once using a mACC paradigm with the same stimulus changes. We compared the amplitude and signal to noise ratio (SNR) of the MMF and mACC, and objectively determined whether a reliable response could be obtained for each individual participant. In this way, we aimed to determine whether the ACC advantage identified by Martin and Boothroyd extended to MEG and to linguistically-relevant acoustic changes.

2. Materials and Method

2.1. Subjects

Seventeen participants were tested, but three were excluded due to (a) movement of the head-position cap during recording; (b) missing event triggers; and (c) excessive noise in the source waveforms. The final sample included 14 participants, aged 19–40 years (mean = 29.02, SD = 8.71). Thirteen of the fourteen participants were right-handed according to the Edinburgh Handedness Inventory [22]. Participants had a mean score of 11.9 (SD = 2.9) on the Matrices subtest of the Wechsler Adult Intelligence Scale [23]—a measure of nonverbal IQ (population mean = 10, SD = 3). None of the participants reported any history of neurological abnormalities or hearing impairment and hearing threshold was in normal range (<20 dB HL) at frequencies 0.25, 0.5, 1, 2, 4, 8 kHz for all subjects. Written consent was obtained from all participants and procedures were approved by the Macquarie University Human Research Ethics Committee. Participants received monetary compensation for taking part in the study.

2.2. Stimuli

Three synthesized speech vowels were generated in Praat [24] based on source-filter theory. The standard sound (e_{low}) was a synthesized /e/ vowel sound. The pitch deviant ($e_{high)}$ differed from the standard in its fundamental frequency, whereas the vowel deviant ($u_{low)}$ had the same fundamental frequency as e_{low}, but differed in the second and third formats, making an /u/ sound. Table 1 shows the frequency composition of the three sounds.

Table 1. Formant Frequencies (in Hertz) for the three stimuli.

	e_{high}	e_{low}	u_{low}
F0	138	125	125
F1	280	280	280
F2	2620	2620	920
F3	3380	3380	2200

For the MMF paradigm (Figure 1, upper panel), the stimuli were each 75 ms in duration (including 10 ms ramp on and off). Each sequence contained 86% standards (e_{low}), 7% pitch deviants (eh_{igh}) and 7% vowel deviants (u_{low}) in a pseudo-random order. Within each sequence, at least the first ten sounds were standard sounds in order to create a memory trace and at least two standard sounds were presented between

deviants. Stimulus onset asynchrony (SOA) was jittered uniformly between 450–550 ms. Stimuli were presented in three blocks, each lasting 5 minutes, resulting in 1600 trials including 112 pitch deviants and 112 vowel deviants. For the mACC paradigm (Figure 1, lower panel), a single continuous sound sequence was created, consisting of five units of sound, each of 1500 ms. To prevent audible clicks whilst maintaining as much as possible a constant stimulus amplitude, each stimulus was windowed with a 10 ms rise-fall ramp and the stimuli were concatenated with 5 ms of overlap. Each sound sequence (total duration 7500 ms) was separated by a 1500 ms silence. The order of the sounds in each sequence was: e_{low}, e_{high}, e_{low}, u_{low}, e_{low}. A total of 96 sequences were presented across three 5 minute blocks, so participants heard 96 onset responses, 96 pitch changes (e_{low} to e_{high}), and 96 vowel changes (e_{low} to u_{low}). mACC responses were also elicited by the e_{high} to e_{low} and u_{low} to e_{low} changes but these were not analysed as corresponding changes were not present in the MMF paradigm.

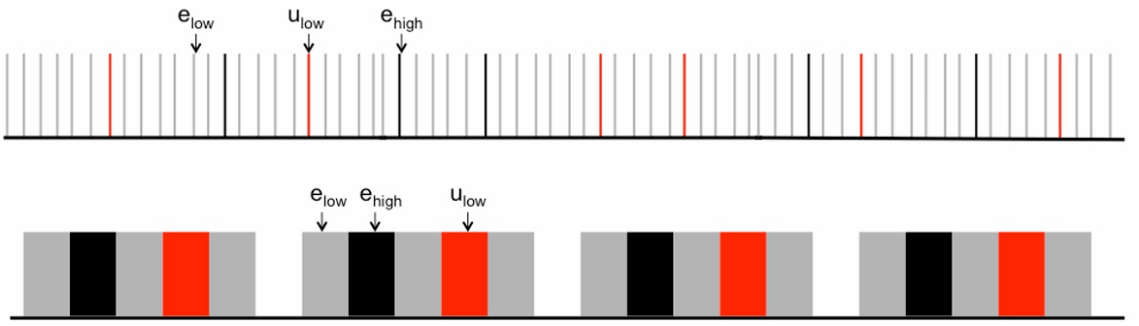

Figure 1. Schematic representation of the MMF (above) and mACC (below) paradigms.

2.3. MEG recording

All MEG testing was performed at the KIT-Macquarie Brain Research Laboratory. Neuromagnetic data were recorded at 1000 Hz using 160-channel whole cortex MEG (Model PQ1160R-N2, KIT, Kanazawa, Japan). The MEG system consists of 160 coaxial first-order gradiometers with a 50 mm baseline [25,26]. During the recording, participants lay on a comfortable bed inside the magnetically shielded room and watched a silent DVD of their choice projected on the ceiling to keep them occupied and awake. They were told to ignore the sounds, give their full attention to the movie, and keep still throughout.

Prior to MEG recording, five marker coils were placed on an elasticised cap on the participant's head, and their positions and the participant's head shape were measured with a pen digitiser (Polhemus Fastrack, Colchester, VT). Head position was measured with the marker coils before and after each MEG recording. Participants were monitored for head movements via a video camera placed inside the magnetically shielded room. Participants who exceeded head-movement of 5 mm (pre- and post-marker coil measurement, as pre-processed in MEG160) were excluded from further analyses.

The mACC and MMF were acquired in two separate acquisition blocks, each with 3 blocks of sounds. Order of testing was randomized across participants. All sound sequences were presented binaurally using MATLAB software at 75dB SPL via pneumatic tubes and custom insert earphones. The stimulus delivery system has a relatively flat frequency response between 500 and 8 kHz and an approximate 10 dB/octave roll-off for frequencies below 500 Hz [27].

2.4. MEG data analysis

MEG data were analysed using the SPM12 M/EEG analysis suite [28] and custom MATLAB scripts. The initial processing steps were as follows: (i) downsample to 250 Hz; (ii) high pass filter at 0.1 Hz; (iii) bandstop filter between 49 and 51 Hz; (iv) low pass filter at 30 Hz; (v) epoch between −100 and 400 ms (from either the onset of the response or the change in the stimulus); (vi) baseline correct between −100 and 0 ms. At this point, we computed the "single trial" MMF by subtracting the response to the preceding standard (predeviant) from the deviant response [29]. Next, for all conditions, we performed robust averaging [30], which down-weights extreme values, thereby minimizing the influence of artefacts. We then re-applied the 30Hz low pass filter to remove any high frequency noise introduced by robust averaging and calculated the global field power (see Figure 2) which provides an overall measure of scalp field strength at each time point [31].

The mACC and MMF responses were extracted from virtual sensors placed in the vicinity of bilateral auditory cortex using a single sphere forward model. Bilateral dipoles were fitted to the M100 response to the onset of the sequence (mACC) or the standard stimulus (MMF), operationalized as the peak in the global field power between 52 and 152 milliseconds. Dipoles were placed in left and right auditory cortex (MNI coordinates: [40 −21 9]; [−40 −21 9]) then fitted by allowing them to orient freely and move within a gaussian centred at this location with standard deviation 10 mm. Next we used the dipole solution as a spatial filter to extract single-trial epoched "virtual sensor" data from the bilateral auditory cortices. Source waveforms for each hemisphere and condition were calculated by robust averaging of the single-trial waveforms followed by 30 Hz low-pass filtering (see Figure 3).

Statistical analyses of the waveforms were performed in R (R Markdown detailing each step of the analyses is available at http://rpubs.com/JonBrock/242837). From each hemisphere, paradigm, and condition, we determined the peak amplitude (maximum for the mACC and minimum for the MMF as these have opposite polarities) within a 100 ms window centred on the peak in the corresponding grand mean global field power. We calculated the SNR by dividing the root mean square of the response post stimulus onset (i.e., 0 to 400 ms) by the root mean square of the response during the baseline period

(−100 to 0 ms). Amplitude and SNR measures were subjected to analysis of variance (ANOVA) with Paradigm (mACC vs MMF), Stimulus (Pitch vs Vowel) and Hemisphere (Left vs Right) as repeated measures. We also performed single-trial analysis of each waveform to determine whether there was a statistically reliable response for each participant. We conducted a one-sample (mACC or MMF vs zero) non-parametric test implemented using the "std_stat" function of the EEGlab toolbox [32] applied to the single trial data for the epoch −100–400 ms. We ran 1000 permutations and set statistical significance at a p-value of 0.01, false discovery rate corrected.

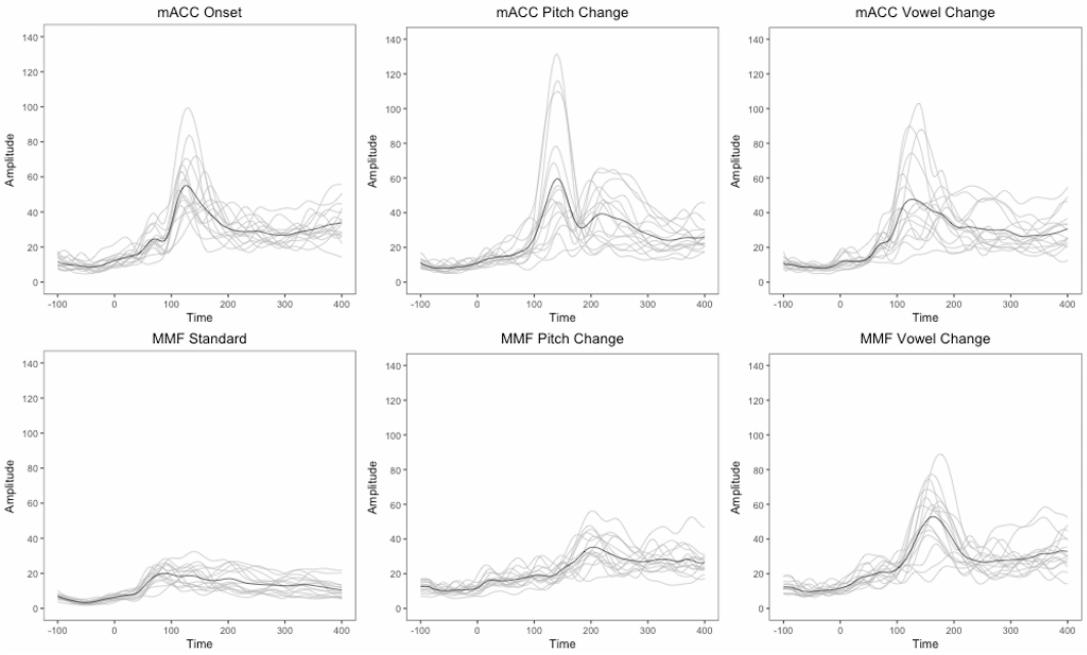

Figure 2. Global field power waveforms for the mACC and MMF paradigms. Grey lines show the responses for individual subjects. Black lines show the mean of all subjects.

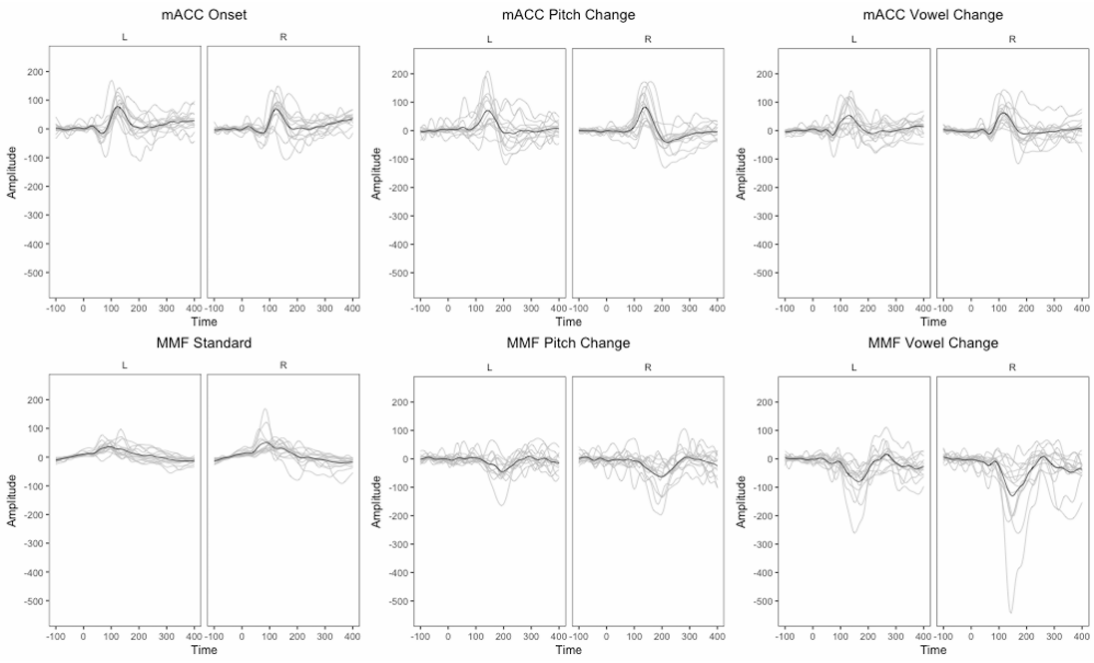

Figure 3. Left (L) and right (R) source waveforms for the mACC and MMF paradigms.
Grey lines show the responses for individual subjects. Black lines show the mean of all subjects.

3. Results

Figure 4 shows the peak amplitude and SNR of the mACC and MMF responses. Consistent with the global field power and source waveforms (Figures 2 and 3), it suggests differential sensitivity of the two responses to the Pitch and Vowel stimuli—an impression confirmed by the analyses of variance.

ANOVA on the Peak Amplitude showed no overall effect of Paradigm ($F[1,13] = 0.54$, $p = 0.474$, $\eta_G^2 = 0.008$), but we did find a significant effect of Stimulus ($F[1,13] = 6.10$, $p = 0.028$, $\eta_G^2 = 0.017$), which was qualified by a Paradigm by Stimulus interaction ($F[1,13] = 23.03$, $p < 0.001$, $\eta_G^2 = 0.061$). ANOVAs conducted for each Paradigm separately both showed a main effect of Stimulus, but in opposite directions. For the MMF, the Vowel response was larger than the Pitch response ($F[1,13] = 15.87$, $p = 0.002$, $\eta_G^2 = 0.094$). For the mACC, the Pitch response was larger than the Vowel response ($F[1,13] = 8.30$, $p = 0.013$, $\eta_G^2 = 0.027$). No effects or interactions involving Hemisphere were significant.

For SNR, the results were similar. We again found no significant effect of Paradigm ($F[1,13] = 2.64$, $p = 0.128$, $\eta_G^2 = 0.048$], a significant effect of Stimulus ($F[1,13] = 4.71$, $p = 0.049$, $\eta_G^2 = 0.036$), and a significant Paradigm by Stimulus interaction ($F[1,13] = 7.82$, $p = 0.015$, $\eta_G^2 = 0.039$). Again, no effects of Hemisphere and no interactions involving Hemisphere were found to be significant. The Vowel MMF was larger than the Pitch MMF ($F[1,13] = 9.39$, $p = 0.009$, $\eta_G^2 = 0.192$). However, in contrast to the Peak Amplitude analysis, we found no difference between the Pitch and Vowel mACCs ($F[1,13] = 0.01$, $p = 0.937$, $\eta_G^2 = 0.000$). For Pitch stimuli, the SNR was higher for the mACC than for the MMF ($F[1,13] = 9.45$, $p = 0.009$, $\eta_G^2 = 0.209$), but there was no Paradigm effect for Vowel stimuli ($F[1,13] = 0.02$, $p = 0.894$, $\eta_G^2 = 0.000$).

Figure 4 shows one participant with a particularly large MMF response. We re-analyzed the Peak Amplitude and SNR data excluding this outlier. In both cases, the interaction between Paradigm and Stimulus remained significant, indicating that it could not be attributed to that outlying participant. Finally, we determined whether each source waveform for each participant was reliably different to a null response (see Table 2). For the mACC, the Pitch and Vowel changes both elicited a significant response in at least one hemisphere for 12/14 and 13/14 of the participants respectively. The vowel-change MMF was present for 11/14 participants, but only 8/14 produced a reliable pitch-change MMF.

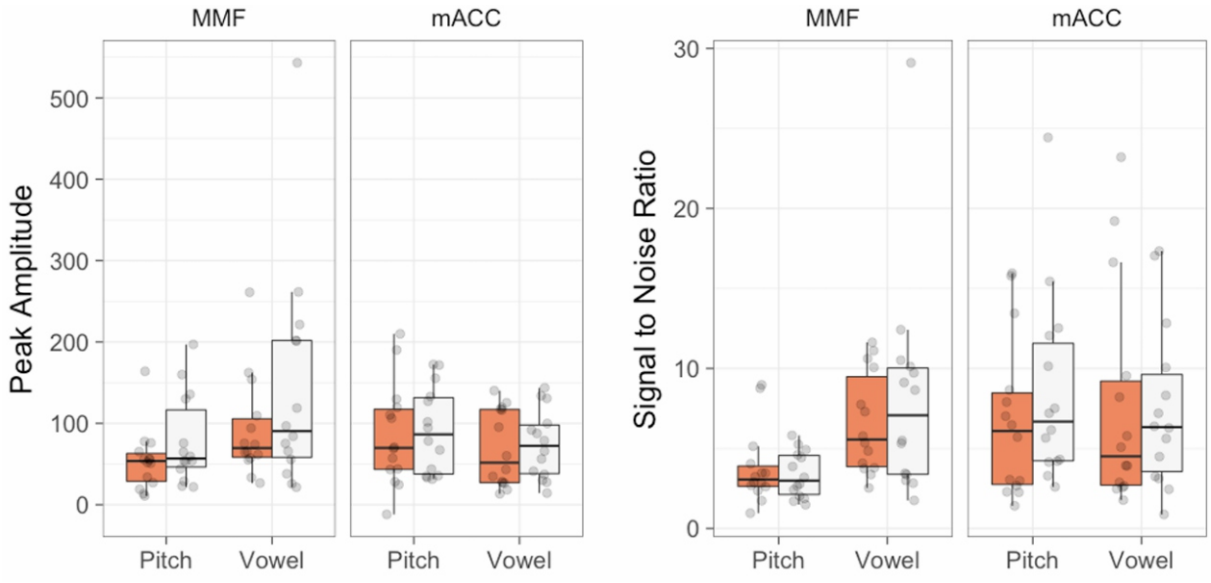

Figure 4. Boxplots showing peak amplitude (left panel) and signal-to-noise ratio (right panel) for mACC and MMF. Dots represent individual participants. Left and right hemisphere sources are in orange and white respectively.

Table 2. Presence of mACC and MMF for individual subjects in the left (L) and right (R) hemisphere. X indicates that the waveform contains at least one cluster of time points that was significantly different to zero (cluster-corrected).

	mACC				**MMF**			
	Pitch		**Vowel**		**Pitch**		**Vowel**	
Subject	L	R	L	R	L	R	L	R
1	X	X	X	X		X	X	X
2	X		X		X			
3	X	X	X	X	X	X	X	X
4		X		X		X	X	X
5	X	X						
6	X	X	X	X			X	
7	X	X	X	X	X	X	X	X
8	X	X	X	X				X
9		X		X			X	X
10		X		X		X		X
11				X			X	X
12		X	X	X		X	X	X
13	X	X	X	X	X	X	X	X
14				X				
Total	8	11	8	12	4	7	9	10

4. Discussion

The current study directly contrasted two complementary MEG paradigms for investigating auditory change detection, the MMF and the less commonly employed mACC. Although the MMN/MMF is much more widely used, the only previous study directly comparing the two responses found that the ACC is a larger and more robust response than the MMN elicited by the same auditory change [14]. The results of the current study indicate that the relative merits of the two paradigms may in fact be contingent on the stimuli used. For pitch changes, the mACC had a significantly higher SNR than the MMF and reliable responses were obtained more consistently. However, for vowel changes, the MMF had similar SNR to the mACC, and similar reliability at the individual subject level. Importantly, this interaction between Paradigm and Stimuli was also apparent in the global field power, which provides an assumption-free measure of brain responses across the sensors. As such, it would appear to reflect genuine differences in the relative sensitivity of the MMF and mACC to pitch and vowel changes, as opposed to differences in the quality of source model fit across conditions.

In seeking an explanation for this interaction, it is worth considering the putative mechanisms responsible for the mACC and MMF. The ACC/mACC is thought to arise due to the activation and deactivation of neural populations within the tonotopically organised auditory cortex [14,33,34]. The MMN/MMF on the other hand has sources in prefrontal areas [2,35–37] as well as auditory cortex [38–40] and, in addition to change detection, is thought to index processes of memory, attention switching, and the adjustment of internal models of the auditory environment [41–47]. The differential MMF response to vowel and pitch changes (in the presence of similar mACC responses) may, therefore, reflect the influence of one or more of these higher order functions, perhaps involving differences in frontal activation or fronto-temporal connectivity.

Whatever the precise explanation, the current results provide partial support for Martin and Boothroyd's contention that the ACC paradigm is the more efficient measure of auditory change detection. The mACC/ACC has now been found to have superior SNR for both pitch changes and complex tone to noise changes, with equivalent SNR for vowel changes. For the MMF procedure, there appears little scope to improve SNR without making the testing session considerably longer. In contrast, the efficiency of our mACC procedure could be further increased by eliminating the redundant elow at the end of the stimulus, decreasing the duration preceding acoustic changes that are not of interest (e_{high} to e_{low} and u_{low} to e_{low}), and potentially, decreasing all durations and using deconvolution techniques to separate overlapping responses [48].

This is not to say, however, that MMN/MMF should be abandoned. The ACC paradigm is restricted to the study of discrete changes in steady state stimuli such as tones and vowel sounds. In contrast, the MMN can be used to index discrimination of consonant sounds (e.g., /ba/ vs /da/) and is also sensitive to more abstract representations of complex rules such as the conjunction of two different acoustic features [44,49–52]. Moreover, studies that use both paradigms may prove particularly informative. For example, in clinical populations, the profile of response across the mACC/ACC and the MMF/MMN may allow a distinction to be made between individuals with basic auditory discrimination deficits, and those with higher-order auditory processing difficulties. Ultimately, the choice of paradigm depends on the question at hand and, as the current results indicate, the precise nature of the acoustic change under investigation.

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Conflict of Interest

The authors report no conflict of interest.

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Interaction between Neural and Cardiac Systems during the Execution of the Stroop Task by Young Adults: Electroencephalographic Activity and Heart Rate Variability

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A B S T R A C T

Executive processes and heart rate variability (HRV) are supposedly regulated by an integrated inhibitory neurovisceral network mainly coordinated by the prefrontal cortex. Inhibitory control, a core executive function, is demanded by the Stroop task. This study aimed to assess the interaction between electroencephalographic activity and HRV of 50 healthy undergraduate students while performing a computerized version of the Stroop task with three stages (paradigmatic congruent – CS – and incongruent – IS – stages in addition to a stage in which words were phonetically similar to color names – PSS). Behavioral results suggested a Stroop interference effect among the stages, with greater difficulty in IS followed by PSS. A pattern of cortical activation in a frontoparietal gradient with left lateralization and involvement of the prefrontal, temporal and occipital cortices was found especially in IS and PSS, which might be correlated to executive control of behavior, inhibitory control, mental representation of words, preparation of the verbal response, and processing of visual stimuli. Mean power of brain activity (μV) was higher for IS and PSS for all tested frequency oscillations. HRV parameters of SDNN and pNN50 were smaller in PSS compared to the other stages, while rMSSD was higher for CS, suggesting higher mental stress for IS and PSS. During PSS, LF/HF ratio was negatively correlated with EEG power in frontal, central and temporal regions whilst rMSSD was positively correlated with activity in frontal and parietal regions. Therefore, marked prefrontal cortex activity was associated with parasympathetic dominance, which is in line with the integrated inhibitory neural network model. In summation, the execution of the Stroop task required increased recruitment of prefrontal cortical areas and led to high mental stress, but, as it was associated with parasympathetic dominance of HRV control, conflict was solved and subjects behaved successfully.

Keywords: Autonomic nervous system (ANS); Central nervous system (CNS); EEG; electrophysiology; executive functions; HRV; inhibitory control; neuropsychology; Prefrontal cortex (PFC); selective attention

Abbreviations:

ACC: Anterior cingulate cortex; ANS: Autonomic Nervous System (ANS); CS: Congruent stage of the Stroop task; DLPFC: Dorsolateral prefrontal cortex; EEG: Electroencephalogram; EKG: Electrocardiogram; EMG: Electromyogram; FDR: False discovery rate method; GSR: Galvanic skin

response; HR: Heart rate; HRV: Heart rate variability; ICA: Independent components analysis; IS: Incongruent stage of the Stroop task; LF/HF ratio: Ratio of low (0.04–0.15 Hz) and high (0.15–0.4 Hz) frequencies; OFPFC: Orbitofrontal prefrontal cortex; pNN50: Percentage of differences between adjacent normal RR intervals that are greater than 50 milliseconds; PSS: Phonetic similarity stage of the Stroop task; rMSSD: Root mean square of the difference of successive RR interval; RR intervals: Intervals between successive R peaks on the heartbeat; SDNN: Standard deviation of all normal RR intervals; VLPFC: Ventrolateral prefrontal cortex; VMPFC: Ventromedial prefrontal cortex

1. Introduction

Executive functions cover a wide range of complex cognitive processes responsible for the coordination of neural activity in order to produce goal-oriented behaviors in a consistent manner over time [1]. Recently, working memory, cognitive flexibility and inhibitory control were regarded as central executive processes [2], which together enable individuals to plan and monitor their actions, as well as to evaluate the consequences of these, make decisions, solve problems, resist to interference, sustain attention over a task, deal with novelty, anticipate the consequences of the actions of others and regulate their behavior according to what is socially accepted [2–5]. The concept of cognitive control relates to the dichotomy between automatic and controlled processing. Therefore executive functions involve shifting the focus towards controlled processes that are influenced by individuals' goals and compete with automatic and more usual processes [1]. Most times, these activities also comprise social rules and/or emotional states, resulting in the so-called emotional/motivational executive functions.

Inhibitory control can be considered as an emotional/motivational executive function, essential for successfully living in society [6], responsible for inhibiting inappropriate behaviors, thoughts or emotions in response to certain stimulus [7]. It allows the interference control through the inhibition at the level of attention, which requires selectively attending to a given attribute of a stimulus while ignoring other [2], e.g., attending to the color of a word and not to its meaning, as in the case of the Stroop task. This may also lead to the inhibitory control at the level of behavior [2], which allows reporting the color of the word instead of reading it. Inhibitory control is frequently assessed by the Stroop task [8]. In its classical version, the test is divided into two stages. In both stages, words that represent color names are presented to the subject. In the first stage, the word and the color of the word match (the word ―blue‖ is written in blue, for example). In the second stage, an interference factor is added, since these attributes may or may not match ("blue" written in red, for example). Participants are instructed to report the color in which the words are written, inhibiting the automatic response to read them. Stroop interference effect

is defined as the resultant increase in the number of errors and in the amount of time necessary to respond to incongruent stimuli, supposedly due to the conflict between the two attributes of the word (its meaning and its color) in this condition [9]. Currently there are variations to this version, such as the protocol followed in this study, which includes a stage with words that are phonetically similar to color names. Other studies also included stages composed of words that did not represent colors and found that those stages are generally easier than incongruent stages but more difficult than the congruent ones [10–13]. Considering the notorious relationship between brain and behavior, it is important to study not only behavioral performance on a test but also its neural and physiological correlates. Thayer, Hansen, Saus-Rose et al. [14] argued in favor of an integrated inhibitory neural network responsible for mediating executive functions, emotional regulation and heart rate (HR) through an integrated neurovisceral mechanism. The role of the prefrontal cortex both in the executive control and in the inhibition of the heart rate acceleration is essential for such integration. Thus, the aim of this study was to assess the electroencephalographic activity and heart rate variability (HRV) of young adults during the execution of the Stroop task. Such a broad study is especially important considering the hypothesis presented by Thayer, Hansen, Saus-Rose et al. [14] addressed above. The electrophysiological activity was assessed using the electroencephalogram (EEG). This technique allows establishing correlations between the patterns of cortical activity and the behavior displayed by individuals, without attributing, however, a causal relationship between them. The brain oscillations obtained can be decomposed using the Fourier Fast Transformation into frequency oscillations, the most commonly studied being Theta, Alpha, Beta and Gamma. The dominance of each frequency of brain oscillations can be interpreted according to its associated physiological and psychological states.

These oscillations can be subdivided into two types of processing modes that act together in dynamic interactions: global modes which are composed of Delta, Theta and Alpha oscillations and span relatively large brain regions promoting its integration, and local modes distributed across more limited topographical areas and composed of Beta and Gamma oscillations [15]. A review of studies using the Stroop task and other executive tests indicates that they are associated to a complex brain system composed of circuits that connect several cortical and subcortical structures, including the prefrontal cortex [16] and its main subdivisions, such as the dorsolateral (DLPFC), ventrolateral (VLPFC), orbitofrontal (OFPFC), and ventromedial (VMPFC) prefrontal cortices, and the anterior cingulate cortex (ACC).

This is in line with a neural network model that helps explaining the brain dynamics during the Stroop task [17,18]. This model stated that mental representation of the color of the word is attributed to the occipital cortex while the superior temporal cortex is concerned with the representation of the word

itself. The automatic process of reading the word usually overcomes the process of naming the color, generating a verbal response mediated by the motor cortex. However, individuals are able to give the correct response due both to a conflict monitor, supposedly mediated by the ACC, and to an attentional control system mediated by the DLPFC. The former monitors the conflict between two possible responses (the color and the word itself) which is solved by enhancing the attention using the latter system. Thus, the attentional control system takes into account the purpose of the task (naming the color of the word) and increases the contribution of the mental representation of the color for the generation of the verbal response. Other studies using EEG during the Stroop task also highlighted the involvement of the frontal lobe [10], left occipital lobe [19], temporal lobe [20], and ACC [21], especially during incongruent conditions. Several studies also addressed the physiological aspects of the Stroop task by examining the subjects' heart rate variability (HRV). HRV measures are based on changes in the duration of RR intervals, i.e. duration of intervals between successive R (or N) peaks of typical heartbeat oscillations. HRV analysis can be conducted considering time or frequency domains [22]. In the time domain, RR intervals can be considered individually for obtaining the standard deviation of all normal RR intervals (SDNN), or as adjacent intervals for the root mean square of the difference of successive RR intervals (rMSSD) and the percentage of differences between adjacent normal RR intervals that are greater than 50 milliseconds (pNN50). Additionally, the ratio of low (0.04–0.15 Hz) and high (0.15–0.4 Hz) frequencies (LF/HF ratio) can be calculated on the frequency domain. HRV is modulated by the combined action of sympathetic and parasympathetic divisions of the Autonomic Nervous System (ANS) and therefore is responsive to stress. When an individual is exposed to acute stress, the sympathetic division of the ANS becomes more active increasing the LF/HF ratio and decreasing SDNN and rMSSD parameters [23].

SDNN, rMSSD as well as pNN50 reflect the parasympathetic activity in short term variations on the heart rate at each heartbeat [24], thus they decrease in mental stress situations. According to Thayer, Hansen, Saus-Rose et al. [14], HRV can be considered as an index of the functional capacity of brain structures that support executive success. Such statement is based on their results in which higher vagally mediated HRV values are associated with better performance of individuals during executive tasks [14]. Some studies have assessed HRV during the execution of the Stroop task, but they favored the comparison of the parameters during this test and a baseline or between two groups of subjects and so did not emphasize the possible HRV parameter differences between the stages of the task [23–28]. We consider that this study is original and relevant as it investigates the possible interaction between nervous and cardiac systems of young adults while performing a version of the Stroop task that includes a stage with words phonetically similar to color names.

2. . Materials and Methods

2.1.Subjects

The subjects were 50 young adults (24 women) with an age range from 17 to 28 years old and mean age of 21.6 (SD = 2.8). They were healthy undergraduate students recruited by written announcement at the University of Brasilia. All subjects were right-handed according to the Edinburgh Inventory [29], and reported no personal or family history of neurological or psychiatric disorders. The participants declared that they had not used drugs or drank alcoholic beverages within the 24 hours that preceded the research. All subjects gave informed consent to a protocol approved by the Ethics Committee of the Health Sciences Faculty, University of Brasilia, Brazil (CAAE 24418013.2.0000.0030).

The performance on the Stroop task was analyzed using the data from 22 participants (10 females) with an age range from 18 to 27 years old and mean age 21.6 (SD = 2.9). The behavioral assessment was methodologically designed as a manipulation check in order to assure that the version of the test worked properly and demanded executive functions.

2.2. Data acquisition and processing

The electrophysiological recordings were taken using the Neuron-Spectrum-4/EPM device (Neurosoft®, Ivanovo, Russia) with A/D conversion and sampling rate of 2000 Hz. For the electrocardiographic registration (EKG), a standard bandpass filter (0.5–75 Hz) and a common-mode rejection ratio of 100 mV were used. The device allows the simultaneous registration of EEG and EKG and other electrophysiological signals using 29 channels. 28 channels were used: 24 for the EEG registration, 1 for the EKG, 1 for the galvanic skin response (GSR) measure and 2 for the electromyography (EMG). The GSR and EMG data were not analyzed in this study.

EEG was recorded using the 10:20 International Electrode System [30] and reference electrodes positioned on the mastoids. Ag/AgCl individual electrodes were attached by a conductive paste (Ten20, Weaver and Company, Aurora, USA) on sites previously cleaned using an abrasive gel (Nuprep®, Weaver and Company, Aurora, USA) and the impedances were kept under 5 kΩ during the entire session. For electrocardiography, one self-adhesive electrode was allocated on the neck, above the right external jugular vein, and another on the cubital fossa over the radial artery [31].

Neuron-Spectrum-4/EPM were connected to a computer (portable, Satellite INTR®, AMD Athlon processor of 1,1 GHz, 256 MB of RAM, and 15‖ screen) and used the Neuron-Spectrum software (Neurosoft®, Ivanovo, Russia), version 2.3.56.0, for the display of the registration in real-time. This computer was also used to run the cognitive tests, but a keyboard and a mouse were connected to it for the participant use.

EEG data were analyzed using the open source EEGLab toolbox, version 9.0.4.5 [32], considering the epochs corresponding to the test execution (64s intervals between the onset and end of each stage of the Stroop task). The data were resampled to 500 Hz and subjected to an Infomax algorithm in order to be decomposed into their independent components [33]. The components related to artifacts presented standard attributes and were then removed using the independent components analysis (ICA). Afterwards, EEG data were recalculated with the remaining components. A study was generated and was pre-computed for the calculation of the spectral power (in μV). Data were analyzed according to the standard frequency oscillations: Theta (4–8 Hz), Alpha (8–13 Hz), Beta (13–30 Hz) and Gamma (30–70 Hz). Topographical maps were generated for the stages of the Stroop task on the abovementioned frequency oscillation using the power registered on each electrode and the smoothing technique of spherical interpolation around the channels [31,34]. HR data corresponding to the task execution (64s intervals between the onset and end of each stage of the Stroop task) were digitally isolated using scripts developed in our laboratory in MatLab, v. 7.8.0 (R2009a) and were later processed using the EKG module of the software Protolize! [35] for the detection of R peaks and for the calculation of the heart rate variability (HRV) parameters in time (SDNN, rMSSD, pNN50) and frequency (LF/HF ratio, i.e. ratio of low (0.04 to 0.15 Hz) and high (0.18 to 0.4 Hz) frequency components) domains calculated according to international standard guidelines [36] and as in the study developed by Garcia, Uribe, Tavares et al. [31].

2.3. Procedure

The experiment was conducted in the laboratory of Neuroscience and Behavior of the University of Brasilia, inside a Faraday cage (WxHxD: 259 × 223 × 396 cm), in order to reduce electromagnetic interferences on the electrophysiological registration. The session began by marking and cleaning the sites for electrode placement with an abrasive gel (Nuprep®, Weaver and Company, Aurora, USA) for the EEG and with alcohol for the EKG. After positioning the electrodes, the electrophysiological signals were taken continuously as the task was performed by the subjects. At the end of the session, the electrodes were removed and the scalp of the subjects cleaned using gauze soaked in alcohol and water.

2.4. Stroop task

A computerized version of the Stroop task resembling the Victoria version [37], developed in our laboratory, was used (STROOP software, Borland Delphi, v. 7.0) in which a word at a time was presented to the participants during 800 milliseconds over a gray background (Figure 1). The subjects were instructed to name the color of the word aloud as quickly and accurately as possible. The task had three stages with 32 trials, each. The interval between the stages was solely used to change the stage and repeat the instructions, and the subjects were told to keep their eyes closed. The words used were names of colors on the first two stages (red, blue, yellow and green, in Portuguese, vermelho, azul, amarelo, and verde) and phonetically similar to those words (in Portuguese, velho, cabul, marmelo, and verdade) on the last stage. At the first stage (congruent, CS) the two attributes of the stimuli (the word itself, and its color) matched, while at the second (incongruent, IS) and third (phonetic similarity, PSS) stages, those attributes could be different. The words were ordered pseudo-randomly in all stages. The audio was recorded using the MSWindows® Sound Recorded and later the responses given by the subjects were noted by the experimenters and compared to the words presented. The possible results were: a) hits, when the subjects' responses matched the color of the word presented; b) errors, when the responses differed from the color; and c) omission errors, when no responses were given.

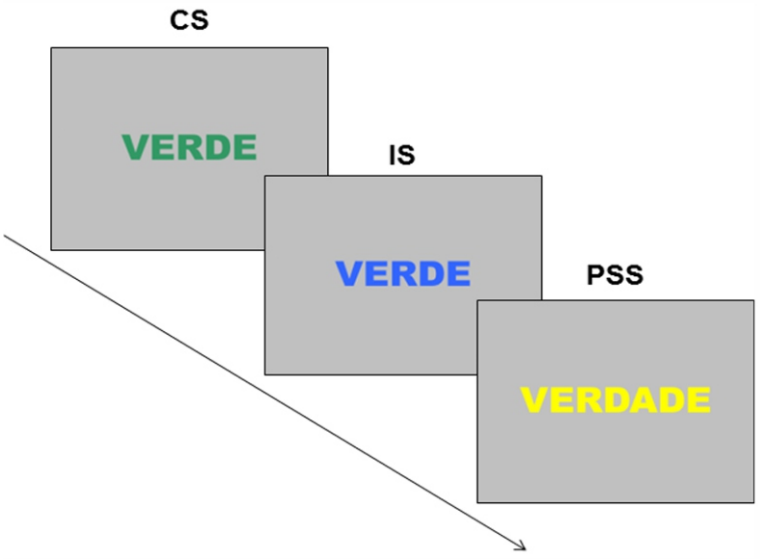

Figure 1. Stroop task sequence with exemplifying images of each stage.

2.5. Statistical analysis

The data was normally distributed according to visual inspections of QQ plots and interquartile range (IQR) test. Outliers were removed from data. One-way within subjects ANOVA (repeated measures)

using the PSAW Statistics software (v. 18.0 for Windows) was used to compare the performance (hit rate and omission error rate) and HRV measures (SDNN, rMSSD, pNN50 and LF/HF ratio) between the stages (3). Post-hoc pairwise comparisons were performed using t-tests. The level of statistical significance was set at 5% ($p \leq 0.05$) for all tests and adjusted for the post hoc tests by the Bonferroni method ($p \leq 0.0167$ i.e. 0.05/3). Bonferroni adjustment for multiple comparisons was used on the reported p-values (LSD p-values × 3) and therefore these are significant at the .05 level. The results are presented as mean ± standard deviation (SD). Pearson bivariate correlations were also conducted using PSAW Statistics software and the same level of statistical significance ($p \leq 0.05$).

The statistical analysis of the EEG data was performed using the parametric statistical tools of the open source EEGLAB platform, version 9.0.4.5 [32]. The statistical significance was 5% corrected by the false discovery rate method (FDR). The EEG results are presented in topographical maps for each frequency oscillation (Theta, Alpha, Beta and Gamma) and stage of the Stroop task. The cortical activity of each electrode was compared between the maps of the stages, two at a time, using paired samples t-tests (CS vs. IS, CS vs. PSS and IS vs. PSS). Pearson bivariate correlation coefficients (r) and bootstrap bias-corrected and accelerated confidence intervals (BCa 95% CI) were calculated between the mean power (μV) measured in each EEG electrode and the HRV parameters. Correlations were considered significant and reliable for coefficients (r) that did not occur in the interval between −0.40 and 0.40, for p-values of less than or equal to 0.05 and confidence intervals that did not cross zero.

3. Results

3.1. Behavioral results

A significant difference was observed between the hit rate for all stages ($F2,42 = 18.673$, $p < 0.001$, $\eta p^2 = 0.471$, pairwise comparisons shown in Figure 2A). Analysis of variance also showed a main effect of stage for the omission error rate (Mauchly's test of sphericity: $\chi^2(2) = 6.133$, $p = 0.047$; Huynh-Feldt correction: $\varepsilon = 0.845$; ANOVA: $F_{1.69,35.48} = 16.318$, $p < 0.001$, $\eta p^2 = 0.437$), Figure 2B), but the pairwise comparison of omission errors between CS and PSS showed only a marginal effect after the Bonferroni adjustment ($p = 0.051$).

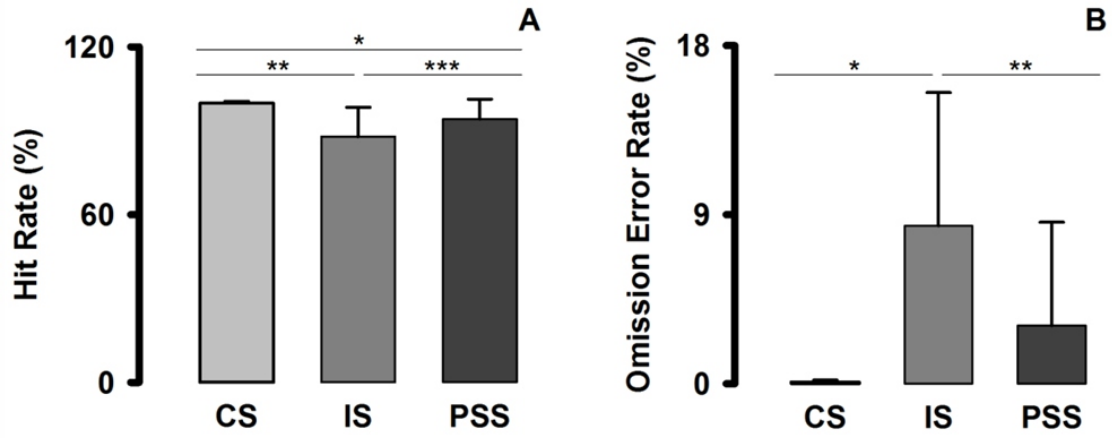

Figure 2. Mean (± SD) of hit rate (A; percentage) and omission error rate (B; percentage) of young adults (n = 22) at each stage of the Stroop task. A: ⋆ CS > PSS, $p = 0.005$; ⋆⋆ CS > IS, $p < 0.001$; ⋆⋆⋆ IS < PSS, $p = 0.012$; B: ⋆ CS < IS, $p < 0.001$; ⋆⋆ IS > PSS, $p = 0.003$; repeated measures ANOVA. Bonferroni adjustment for multiple comparisons was used on the reported p-values (LSD p-values × 3).

3.2. EEG Results

Electroencephalographic data were filtered and divided into traditional frequency oscillations: Theta (4–8 Hz), Alpha (8–13 Hz), Beta (13–30 Hz), and Gamma (30–70 Hz). Figure 3 shows the topographic maps of activity for each stage of the Stroop task. The colored bar on the topographical map indicates the power (μV) measured in each electrode in a gradient, where high power is represented by dark red color and low power by dark blue. The power ―estimates the magnitude of oscillatory amplitude within a defined time window‖ [38]. Paired samples t-tests with correction by the FDR method were used to compare power in each electrode between two conditions. Therefore, comparison maps on the right in Figure 3 depicts significant differences, represented by red dots, between CS vs. IS, CS vs. PSS, and IS vs. PSS, respectively. T and p-values comparing each electrode across stages of the Stroop task are presented in Table 1.

Theta activity (Figure 3A) at all stages was more evident among Fz and Cz electrode, but there was also activation from frontopolar to parietal regions in the midline with attenuation around. For IS and PSS, there was also higher activation in F3 electrode. For all conditions it was possible to notice left lateralization towards the left occipital region (O1 electrode). Only comparisons between IS and PSS did not show significant differences.

Topographic maps of IS and PSS in Alpha oscillation (Figure 3B) showed high activity from the left frontal pole to the ipsilateral occipital region through the midline. Noticeable activity occurred in F3, Fz, Cz. Although congruent stage is topographically similar to the other two, activity in frontopolar and F3 region are not as marked as observed in other stages. For all electrodes significant differences were found, except in the comparison between CS and PSS for C3, P3, P4, Oz, and O2 electrodes. Beta activity (Figure 3C) at all stages was high in the superior temporal region in both hemispheres, and also activity in Fp1, Fp2, F3, F4 and C3 electrodes with decreased power in the other regions. Only O1 and T4 electrodes did not differ in the comparison between IS and PSS. At all stages, Gamma activity (Figure 3D) was high in superior temporal regions of both hemispheres. Left lateralization for the frontopolar region, F3 and O1 electrodes was observed as well. Attenuation towards the other brain regions was more pronounced in CS but also present in IS and PSS. Power in all electrodes was significantly different for the comparisons between stages, except for the one between IS and PSS.

Power in all oscillations was lower in CS when compared to the other two.

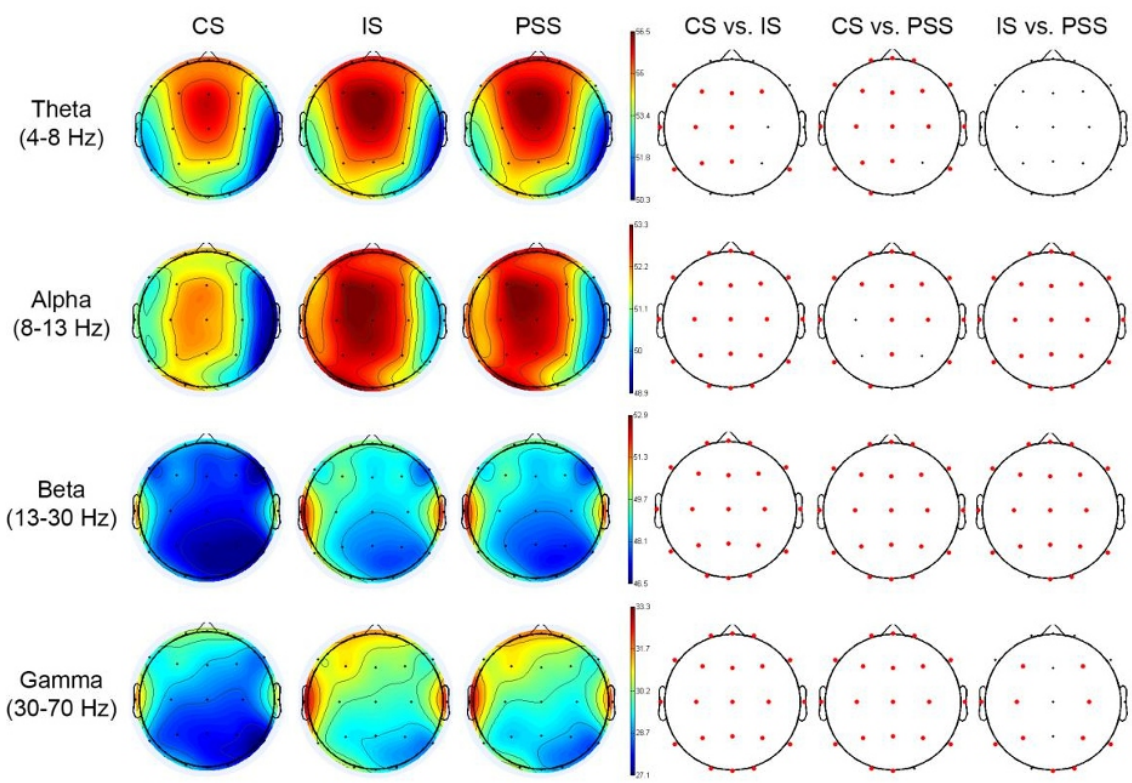

Figure 3. Relative topographic power spectrum distribution for specific oscillations in each stage of the Stroop task—congruent stage (CS), incongruent stage (IS) and phonetic similarity stage (PSS)—performed by young adults (n = 50). Red dots indicate significant differences ($p \leq 0.05$) in each electrode site according to the paired samples t-tests with correction by the FDR method.

Table 1. Paired samples t-test results, t and p values, for comparisons of mean power at EEG electrode sites between the stages of the Stroop task, two at a time, for each frequency oscillation. Degrees of freedom were equal to 49 for all the comparisons.

EEG Electrode Site	CS vs. IS				CS vs. PSS				IS vs. PSS			
	θ	α	β	γ	θ	α	β	γ	θ	α	β	γ
Fp1	2.13^{*}	4.16^{***}	4.26^{ns}	3.61^{***}	2.85^{**}	2.68^{*}	3.98^{***}	3.56^{***}	-0.70^{ns}	3.36^{**}	2.87^{**}	1.34^{ns}
F3	2.75^{**}	4.54^{***}	4.77^{*}	4.02^{***}	2.87^{**}	3.08^{**}	3.74^{***}	2.98^{**}	-0.17^{ns}	3.20^{**}	3.29^{**}	2.59^{*}
C3	2.83^{**}	4.10^{***}	5.32^{**}	4.12^{***}	2.88^{**}	2.09^{*}	3.89^{***}	2.90^{**}	0.67^{ns}	3.71^{***}	3.99^{***}	3.17^{**}
P3	2.61^{*}	3.61^{***}	5.23^{**}	3.81^{***}	2.81^{**}	1.77^{ns}	3.98^{***}	2.97^{**}	0.47^{ns}	3.21^{**}	4.41^{***}	2.52^{*}
O1	1.99^{ns}	4.17^{***}	5.22^{**}	3.81^{***}	2.57^{*}	2.97^{**}	4.26^{***}	2.93^{**}	-0.74^{ns}	2.56^{*}	1.78^{ns}	1.15^{ns}
F7	2.32^{*}	4.28^{***}	4.51^{*}	3.86^{***}	3.05^{**}	3.37^{**}	4.49^{***}	3.42^{**}	-0.82^{ns}	3.21^{**}	2.38^{*}	1.71^{ns}
T3	2.42^{*}	4.67^{***}	4.90^{*}	4.68^{***}	3.13^{**}	4.04^{***}	4.84^{***}	4.04^{***}	-0.37^{ns}	2.81^{**}	2.53^{*}	3.02^{**}
T5	2.91^{**}	5.13^{***}	5.14^{**}	4.48^{***}	2.79^{**}	3.65^{***}	4.30^{***}	3.15^{**}	1.57^{ns}	4.08^{***}	3.55^{***}	3.84^{***}
Fp2	1.71^{ns}	3.60^{***}	4.01^{ns}	3.36^{**}	2.59^{*}	2.20^{*}	3.78^{***}	3.15^{**}	-1.11^{ns}	3.10^{**}	2.44^{*}	1.41^{ns}
F4	2.38^{*}	4.11^{***}	4.99^{**}	3.99^{***}	2.93^{**}	2.46^{*}	4.36^{***}	3.23^{**}	-0.30^{ns}	3.60^{***}	3.42^{**}	2.47^{*}
C4	2.21^{*}	3.90^{***}	5.44^{**}	4.10^{***}	2.59^{*}	2.21^{*}	3.96^{***}	2.97^{**}	-0.05^{ns}	3.29^{**}	3.87^{***}	3.02^{**}
P4	2.01^{ns}	3.75^{***}	5.11^{**}	3.86^{***}	2.05^{*}	1.73^{ns}	3.68^{***}	3.21^{**}	0.70^{ns}	3.19^{**}	4.25^{***}	2.45^{*}
O2	1.17^{ns}	3.87^{***}	4.08^{ns}	2.97^{**}	1.17^{ns}	1.87^{ns}	3.22^{**}	1.82^{ns}	0.35^{ns}	3.47^{**}	2.95^{**}	2.57^{*}
F8	1.55^{ns}	3.48^{**}	4.23^{ns}	2.97^{**}	2.42^{*}	2.36^{*}	3.73^{***}	2.50^{*}	-1.26^{ns}	3.28^{**}	2.89^{**}	1.77^{ns}
T4	1.63^{ns}	4.42^{***}	5.48^{**}	4.08^{***}	2.12^{*}	2.91^{**}	3.00^{**}	2.49^{*}	-0.20^{ns}	3.14^{**}	1.51^{ns}	1.55^{ns}
T6	2.36^{*}	5.31^{***}	5.54^{**}	4.44^{***}	2.62^{*}	3.65^{***}	4.80^{***}	3.95^{***}	0.46^{ns}	3.95^{***}	3.35^{**}	2.44^{*}
Fz	2.31^{*}	4.13^{***}	4.80^{*}	3.72^{***}	2.92^{**}	2.41^{*}	4.15^{***}	3.25^{**}	-0.45^{ns}	3.30^{**}	3.68^{***}	1.78^{ns}
Cz	2.34^{*}	4.65^{***}	5.05^{**}	4.54^{***}	2.54^{*}	2.70^{**}	3.79^{***}	3.93^{***}	0.31^{ns}	3.57^{***}	4.54^{***}	1.96^{ns}
Pz	2.43^{*}	4.39^{***}	5.09^{**}	4.44^{***}	2.76^{**}	2.59^{*}	3.92^{***}	4.09^{***}	0.16^{ns}	3.14^{**}	4.34^{***}	1.45^{ns}
Fpz	1.77^{ns}	3.83^{***}	3.85^{ns}	3.51^{**}	2.64^{*}	2.37^{*}	3.76^{***}	3.41^{**}	-1.23^{ns}	3.28^{**}	2.38^{*}	1.09^{ns}
Oz	1.95^{ns}	4.18^{***}	4.57^{*}	3.43^{**}	1.42^{ns}	2.07^{*}	3.30^{**}	2.04^{*}	1.06^{ns}	3.32^{**}	3.42^{**}	2.50^{*}

CS: Congruent stage, IS: Incongruent stage, PSS: Phonetic similarity stage, θ: Theta oscillations, α: Alpha oscillations, β: Beta oscillations, γ: Gamma oscillations, ns: non:significant *p*-values, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

3.3. HRV Results

Heart rate variability (HRV) was analyzed in the time domain for SDNN, rMSSD, and pNN50 parameters and in the frequency domain for the LF/HF ratio. Main effect of stage was observed for pNN50 ($F_{2,96} = 6.211$, $p < 0.003$, $\eta_p^2 = 0.115$, Figure 4A). Pairwise comparisons indicated that pNN50 was lower for PSS when compared with CS ($p = 0.007$) and IS ($p = 0.031$). The same pattern was found for rMSSD (Figure 4C), i.e. main effect of stage ($F_{2,96} = 12.631$, $p < 0.001$, $\eta_p^2 = 0.208$) and significantly lower values for PSS when compared to CS ($p = 0.006$) and IS ($p < 0.001$). SDNN was also mainly influenced by the stage (Mauchly's test of sphericity: $\chi^2(2) = 6.889$, $p = 0.032$; Huynh-Feldt correction: $\varepsilon = 0.911$; ANOVA: $F_{1.82,87.45} = 8.586$, $p = 0.001$, $\eta_p^2 = 0.152$; Figure 4B), with significant pairwise comparisons for CS versus IS ($p = 0.029$) and PSS ($p = 0.003$). There was no significant main effect of stage on the LF/HF ratio (Mauchly's test of sphericity: $\chi^2(2) = 18.578$,

$p < 0.001$; Huynh:Feldt correction: $\varepsilon = 0.773$; ANOVA: $F_{1.55,74.21} = 0.252$, $p = 0.72$, $\eta_p^2 = 0.005$), Figure 4D).

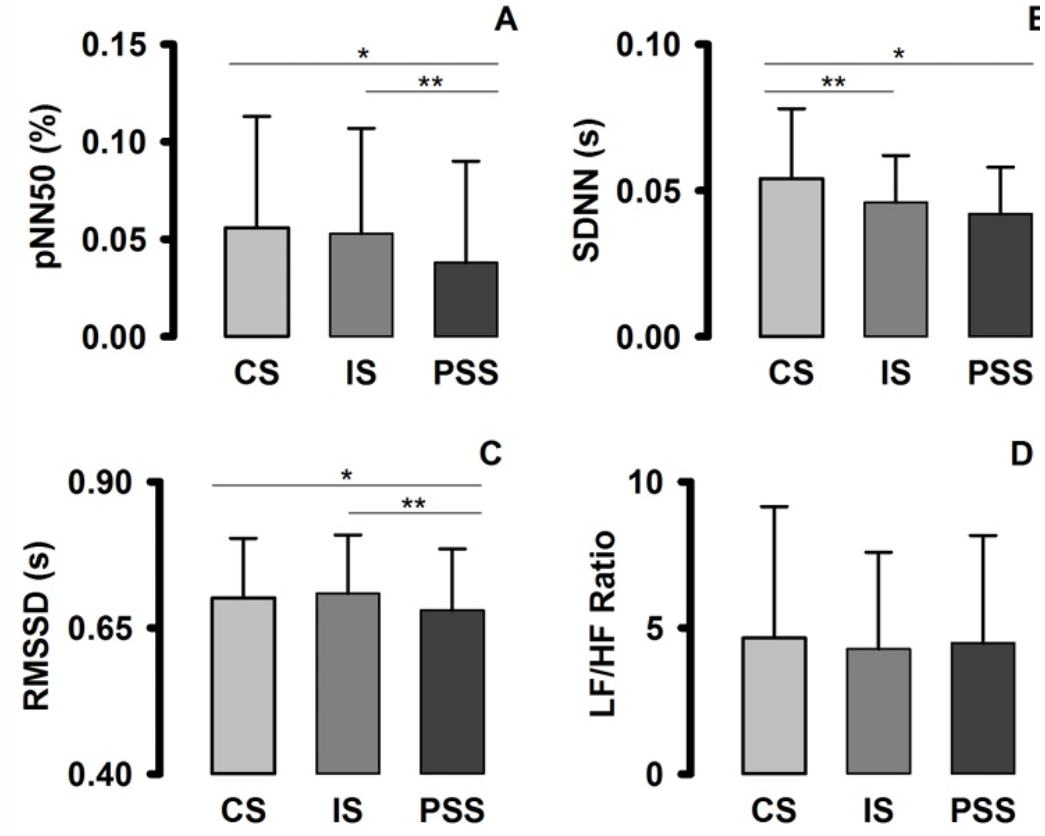

Figure 4. Young adult (n = 49) HRV parameter means (± SD) at each stage of the Stroop task. Parameters in the time domain were pNN50 (A; percentage), SDNN (B; s), and rMSSD (C; s); and in the frequency domain, the LF/HF ratio (D). A: ✱ CS > PSS, $p = 0.007$; ✱✱ IS > PSS, $p = 0.031$. B: ✱ CS > PSS, $p = 0.003$; ✱✱ CS > IS, $p = 0.029$. C: ✱ CS > PSS, $p = 0.006$; ✱✱ IS > PSS, $p < 0.001$. Repeated measures ANOVA. Bonferroni adjustment for multiple comparisons was used on the reported p-values (LSD p-values × 3). Attention to y-axis scale variation among figures.

3.4. Correlation results

Pearson bivariate correlation coefficients were calculated between mean power (μV) measured at each EEG electrode and mean HRV parameters at Stroop stages. Correlations were considered significant and reliable for coefficients (r) that did not occur in the interval between −0.40 and 0.40, for p-values of less than or equal to 0.05 and confidence intervals that did not cross zero.

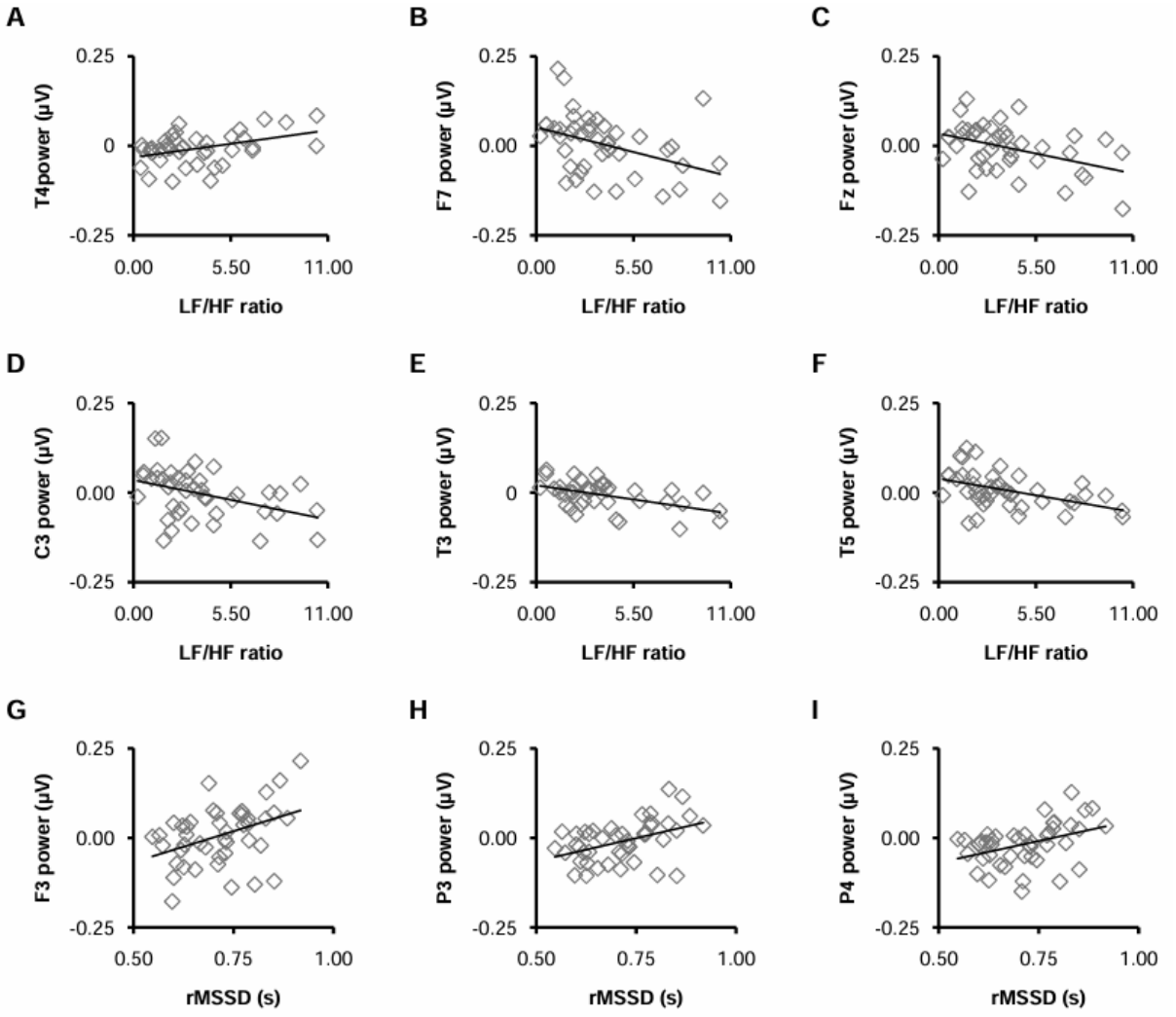

Figure 5. Scatterplots showing significant correlations for CS (A) and PSS stages (B-I) between mean power (µV) measured at each electrode and mean HRV parameters.

A significant positive correlation was found between LF/HF ratio and power in T4 electrode for CS stage (r = 0.41, BCa 95% CI [0.12, 0.64], p = 0.01). For PSS stage, there were negative correlations between LF/HF and F7 (r = −0.40 [−0.66, −0.05]), Fz (r = −0.42 [−0.69, −0.09]), C3 (r = −0.40 [−0.62, −0.15]), T3 (r= −0.50 [−0.70, −0.24]), and T5 (r = −0.46 [−0.64, −0.24], all ps ≤ 0.01). Positive correlations were also found in PSS between rMSSD and F3 (r = 0.40 [0.10, 0.65]), P3 (r = 0.44 [0.11, 0.67]), and P4 (r = 0.40 [0.14, 0.63], all ps ≤ 0.01). Scatterplots in Figure 5 summarize these results. No significant or reliable correlations were observed for IS, neither in the remaining variables for CS, nor PSS. Table 2 shows correlations found for all stages (ps ≤ 0.05), including those with coefficients occurring in the interval between −0.40 and 0.40.

Table 2. Correlation coefficient (r) and bootstrap bias-corrected and accelerated confidence intervals (BCa 95% CI, reported in square brackets) resulting from significant (p ≤ 0.05) Pearson

correlations between HRV parameters and EEG electrodes at each Stroop stage. Only underlined correlations were considered for discussion, as their coefficients are higher than 0.40 or lower than -0.40 and their confidence intervals did not cross zero.

Stroop stage	HRV parameter	EEG electrode site	*r* [BCa 95% CI]
CS	SDNN	O2	0.35* [0.12, 0.57]
CS	LF/HF	T4	0.41** [0.12, 0.64]
IS	rMSSD	Oz	−0.33* [−0.58, −0.03]
IS	LF/HF	F3	−0.33* [−0.63, −0.08]
PSS	SDNN	C4	−0.32* [−0.52, −0.08]
PSS	SDNN	O2	−0.37* [−0.57, −0.13]
PSS	SDNN	F8	−0.39** [−0.59, −0.15]
PSS	SDNN	Cz	−0.35* [−0.63, −0.01]
PSS	rMSSD	F3	0.40** [0.10, 0.65]
PSS	rMSSD	C3	0.37** [0.00, 0.67]
PSS	rMSSD	P3	0.44*** [0.11, 0.67]
PSS	rMSSD	C4	0.32* [−0.01, 0.58]
PSS	rMSSD	P4	0.40** [0.14, 0.63]
PSS	rMSSD	T6	0.33* [0.03, 0.56]
PSS	rMSSD	Fz	0.37** [0.01, 0.66]

PSS	rMSSD	Cz	0.35* [0.04, 0.61]
PSS	rMSSD	Pz	0.31* [−0.01, 0.59]
PSS	LF/HF	F3	−0.31* [−0.63, 0.04]
PSS	LF/HF	C3	−0.40** [−0.62, −0.15]
PSS	LF/HF	P3	−0.35* [−0.61, −0.05]
PSS	LF/HF	F7	:0.40** [−0.66, −0.05]
PSS	LF/HF	T3	−0.50*** [−0.70, −0.24]
PSS	LF/HF	T5	−0.46*** [−0.64, −0.24]
PSS	LF/HF	T4	−0.30* [−0.52, −0.05]
PSS	LF/HF	Fz	−0.42** [−0.69, −0.09]
PSS	LF/HF	Cz	−0.34* [−0.67, 0.05]
PSS	LF/HF	Pz	−0.33* [−0.56, −0.09]

CS: Congruent stage, IS: Incongruent stage, PSS: Phonetic similarity stage, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

4. Discussion

4.1. Discussion of behavioral results

Significant differences for the hit rate were found between all stages with increasing difficulty in congruent, phonetic similarity and incongruent stages, in this order. This suggests that there was a Stroop interference effect on the accuracy of the subjects' responses. Therefore, we assumed that the test was working properly and demanded executive functions.

PSS may be regarded as a stage of intermediate difficulty between CS and IS based both on the hit rate and on the omission error rate as for the last one it does not differ from CS. This is in line with other studies, in which the accuracy and reaction times in stages using strings of letters or words that are not color names are intermediate compared to the congruent and incongruent stages [10–12]. Another study that used words phonetically similar to color names showed the same pattern of difficulty between stages as ours, based on reaction times [13]. According to the neural network model of the Stroop task mentioned in the Introduction session, the congruent stage does not induce conflict between the two attributes of the stimulus (word itself and color) and consequently the subject is able to present a correct response based on any of those attributes [18]. Therefore, although it is expected that the subjects present baseline levels of focused attention because they keep attending to the rule of the task as it is performed, their behavior does not necessarily demand high levels of selective attention and inhibitory control, which makes CS less complex. As the word in the PSS does not refer to a color, but phonetically resembles the name of a color, it is expected a higher conflict than when both attributes are coincident (CS), but less than when they are incongruent (IS) [10].

This hypothesis was supported by the results of the hit rate and the omission error rate presented by the participants in this study. In the study by Salo, Henik and Robertson [12], the reaction time for neutral stimuli consisting of words unrelated to color names was higher than for non-lexicons neutral stimuli (e.g., "XXXX") in the Stroop task. This indicates that there is conflict when neutral words are presented to the subjects, since the subjects are also more prone to reading them than to naming its color. However, the conflict is reduced because there cannot be incongruity between the two attributes of the stimulus since the word is not a color name. Milham, Erickson, Banich et al. [20] noted that the congruent stage of the Stroop task actually involves less conflict than a neutral one and possibly demands less inhibitory control; but the congruent and incongruent stages demands more attentional control than the neutral stage because there are two sources of information about color, the meaning of the word itself and the color in which it is written, and if this information are not coincident, the demand for attention increases further. Both hit ratio and omission error rate were significantly different between incongruent stage and the others. Therefore, this stage possibly increased the demand for inhibitory control and attention. The increase in omission error rate indicates that for this stage, the conflict generated was enough to impair the performance of subjects, possibly because of the failure in inhibiting the tendency to read the word.

4.2. Discussion of EEG results

EEG results showed a difference in the pattern of cortical activation when comparing the lower (Theta and Alpha) to the higher oscillations (Beta and Gamma), in line with the evidence of the existence of two

processing modes in the human EEG, respectively global and local modes [15]. Moreover, the power in the congruent stage was lower than in the other two stages for all the oscillations analyzed, which highlights the evidence of reduced demand for executive resources in CS. The topographic distribution of Theta and Alpha oscillations occurred in a frontoparietal gradient. There is evidence that executive functions do not depend on the isolated activity of the prefrontal cortex, but also on the activity of the posterior cortex [16] in a synchronized frontoparietal network between Theta and Alpha oscillations, especially when there is greater demand of the central executive [39].

Although there was activation of both hemispheres, there was marked activity on the left hemisphere in all stages of the Stroop task. This result is in line with the traditional notion of specialization of the left hemisphere for language [40]. This might relate to the verbal response required from the subjects what could have led to the activation of regions on the left hemisphere involved in spoken language and in cases in which the subjects were not able to inhibit reading the word, even with regions involved in written language, also in the left hemisphere. Regarding Theta oscillation, the marked activity in Fz, Cz and F3 electrode sites, corresponding to prefrontal and motor cortices, during IS and PSS may indicate that these stages require more cognitive resources related to the PFC such as attention, mental effort, and inhibitory control than CS. This is consistent with the behavioral results, for which the incongruent stage was regarded as the most difficult, followed by PSS. However, cortical activity in IS and PSS did not show significant differences for the Theta oscillation. The marked activity on the F3 electrode site during the incongruent stage is of particular interest, since this site is related to the region of the dorsolateral prefrontal cortex (DLPFC), and the activation of this region is related to the manipulation of data stored in working memory [41], which might induce to the behavioral success on the inhibition of inappropriate responses [42].

The activation in the left occipital region (O1 electrode) may be related to the mental representation and the processing of the color of the word, as both occur in the visual cortex [41]. The involvement of the left occipital region at Theta oscillation was also observed in the study by Ghimire, Paudel, Khadka et al. [19] and was more evident in the incongruent condition. This can be interpreted as evidence of greater influence of the irrelevant attribute of the stimulus (the word itself) at the incongruent stage, which may have contributed to the performance cost when compared to the congruent stage. The topographic distribution in Alpha oscillation was similar to Theta. However, the activity in the frontal poles was even less marked in Alpha during the congruent stage, indicating again that this stage demands less activity in areas related to the PFC and is less difficult than the other two. In another study, activity in the frontal poles (Fp electrode sites) in IS and PSS, near the orbitofrontal prefrontal cortex (OFPFC) region, was positively correlated with success in behavioral performance in a Go/No-Go task [18], indicating that

this region is important for inhibitory mechanisms. Increased activity of the frontal poles was also observed during the incongruent stage on the study by Hanslmayr, Pastotter, Bauml et al. [10]. The neural network model for the Stroop task [17] contributes for the comprehension of the topographical power spectrum distribution pattern found in this study, with involvement of the DLPFC (F3) during IS and PSS. This region is supposedly associated with the activation of an attentional control system in response to the detection of a conflict by the anterior cingulate cortex (even knowing that the conflict is smaller in PSS), leading to increased attentional demand. The cingulate cortex activity, important in tasks that require sustained attention, is depicted on the frontal midline activity in the Theta oscillation [21,43] and was evident in incongruent and phonetic similarity stages in this study.

Activation at T3 electrode, corresponding to the superior and middle temporal gyri [44], shown in Beta and Gamma oscillations might relate to the mental representation of the word itself, according to the neural network model for the Stroop task [17,18]. It could also be related to the verbal processing of word, which also occurs in temporal regions, as shown in the fMRI study by Milham, Erickson, Banich et al. [20] while subjects were performing the Stroop task. Activity on the left temporal lobe has also been observed when nouns are being processed [45]. A study with a task in which subjects had to generate color names showed bilateral activity of the temporal lobe more marked in the left hemisphere and also activation of the left DLPFC, which was interpreted by the authors as related to the evocation of the word [46]. This pattern is similar to the one found in our study. The color processing was evidenced by the activity of the left occipital lobe (O1 electrode), similar to that observed in Theta and Alpha oscillations. Also, similarly to those oscillations, there was activity in the frontal poles and in the left DLPFC.

In Beta oscillation, there was a significant increase in the power of the temporal activity during IS and PSS, especially on the left side. In Gamma, no significant differences between IS and PSS were observed in frontal regions (except for F3 and F4), in the left occipital region, as well as in central midline region, which is similar to the comparison of the power spectrum distribution between these two stages in Theta oscillation. The electroencephalographic evidence of the color processing (occipital region) and of the word processing (temporal region) indicates that both of the stimuli's attributes were processed. The fact that the activity on those regions was higher during the incongruent and phonetic similarity stages indicates that there really existed a conflict, which detection and resolution demanded higher activity of prefrontal regions.

4.3. Discussion of HRV results

Time-domain HRV parameters results indicated the existence of an interference effect for the Stroop

task as it was observed for the behavioral results. However, only the comparison between CS and PSS was significant for all measures in the time domain. This indicates that there was a dominance of parasympathetic activity during the congruent stage, suggesting that this stage demanded less executive abilities from the subjects. rMSSD and pNN50 results differ between PSS and IS, whereas SDNN differed between CS and IS. The results in studies that assess the HRV during the Stroop task are controversial regarding these time domain measures. Sometimes they are lower during the execution of this task in comparison with a baseline [24,25], indicating sympathetic dominance during the test. In another study, SDNN and rMSSD tended to be lower during a neutral stage, and comparable between the congruent and incongruent stages [26].

When the version of the Stroop task had only one stage, similar to the incongruent stage of this study, rMSSD was significantly lower during the task than in a resting condition [23]. Similarly, rMSSD correlated positively with the high frequency content (HF: 0.15 to 0.40 Hz, modulated by the parasympathetic division of the ANS), which was in turn positively associated with the behavioral performance in this stage [27]. Despite being difficult to interpret, it is noticeable that in most studies, time-domain parameters are lower during difficult and cognitively loaded conditions. In the frequency domain, for the LF/HF ratio, there were no significant differences between the stages. These results are consistent with the comparison between the Stroop task and a baseline performed by [25], in which there were no significant differences in the LF/HF ratio. However they contrast with other studies where this measure was higher in an interference condition of the task compared to the baseline [24,28]. Dupuy, Lussier, Fraser et al. [26] also did not find significant differences in LF/HF ratio among incongruent, congruent and neutral stages, which is in line with our study. Taken together, the results of the HRV time domain measures indicate that there was a decrease in the autonomic parasympathetic activity during the Stroop task for PSS and IS compared to CS, which indicates an increase in mental stress in the former stages.

4.4. Discussion of correlation results

Interaction between neural and cardiac systems during the execution of the Stroop task (shown in Figure 5 and Table 2) was found at CS stage solely between LF/HF ratio and electrode T4. In the PSS stage, correlations were found among LF/HF ratio and F7, Fz, C3, T3, and T5 electrodes, and also among rMSSD and F3, P3, and P4 electrodes. No significant and reliable correlations were found for the IS stage.

In view of all the variables that influence HRV—such as those related to subcortical activity, ANS activity, endocrine system, HR itself, among others—correlations between cortical activity and HRV are impressive, despite of being moderate. Considering the possible existence of an integrated inhibitory neural network responsible for mediating HRV and executive functions, Thayer, Hansen, Saus-Rose et al. [14] suggested that decreased activity in the prefrontal cortex would lead to a concomitant sympathetic dominance on HR control. This would be indexed by decreased time-domain HRV and increased LF/HF ratio, for example. These authors also state that adaptation to each specific behavioral situation, i.e., different executive tests, is complex and can recruit additional central nervous system structures. However the relation between these other regions and HRV was not clear in their revision. There was a moderate, positive correlation between power in T4 electrode and LF/HF ratio during CS stage. Scatterplot shows a distribution mainly towards low electrode power associations with low LF/HF ratio (Figure 5A).

Therefore, the majority of subjects presented parasympathetic dominance associated with recruitment of temporal regions, possibly for word processing. This is in line with EEG results showing lower power in CS and also with HRV results indicating vagal dominance for this stage. However, as the relation between HRV and the activity of neural regions outside the integrated inhibitory neural network is not clear, it was not possible to state whether this finding goes in line with the theory by Thayer, Hansen, Saus-Rose et al. [14]. During PSS, LF/HF ratio was negatively correlated with frontal, central and temporal region activity (Figure 5B-F). This indicates a somewhat consistent relationship between prefrontal cortical activity and distributed posterior cortical activation. Increases in EEG power were correlated with decreases in LF/HF ratio. This suggests that the higher executive demand during PSS stage recruited prefrontal cortex, as well as in central and temporal cortical regions, was associated with higher levels of parasympathetic dominance. This is in line with the integrated inhibitory neural network model suggested by Thayer, Hansen, Saus-Rose et al. [14]. Also during PSS, rMSDD was positively correlated with mean power in F3, P3, and P4 electrodes (Figure 5G-I). Therefore, increased cortical activity was correlated with an increased time-domain HRV parameter. High EEG power was therefore associated with parasympathetic dominance, which again is in line with the network model of Thayer, Hansen, Saus-Rose et al. [14].

4.5. Final discussion

Considering the results together we may infer lower difficulty and absence of conflict on the congruent stage as evidenced by the behavioral (higher hit rate compared to the other two stages, lower errors of omission rate when compared to IS), electrophysiological (decrease in the power of cortical activation,

in the OFPFC activity for the Alpha oscillation and increased time-domain HRV parameters) and interaction results (showing parasympathetic dominance associated with temporal cortical activity). Behavioral results suggested an intermediate difficulty for PSS. However, EEG results showed no differences in cortical activation between IS and PSS for Theta oscillation. In Alpha and Beta, the differences observed between IS and PSS are apparently related more to the decrease on the power spectrum than to the topographic distribution of the cortical activity. Decreased pNN50 and rMSSD parameters during PSS compared to CS and IS, and decreased SDNN compared to CS indicated that mental stress for PSS was higher than in the other stages. Considering that HRV is an index of the success of executive brain mechanisms as suggested by Thayer, Hansen, Saus-Rose et al. [14], HRV results for the PSS should be associated with a greater behavioral collapse in this stage when compared to the others, as they indicate high mental stress. Three factors help understanding why the impairment in PSS was not as great as in IS. Firstly, the interaction results among LF/HF ratio, rMSSD, and EEG power suggested that at PSS, higher recruitment of prefrontal cortex was associated with parasympathetic dominance. Hansen, Johnsen and Thayer [47] showed that a group of subjects with high HRV (i.e. parasympathetic dominance) performed better in terms of speed and accuracy on executive tests than the low HRV group. Secondly, there was conflict but not incongruity between the two attributes of the word in PSS. Finally, cortical activation in PSS stage was very similar to IS. These are important factors to consider when explaining the intermediate difficulty described for PSS. Nevertheless, stating only that PSS presented intermediate difficulty is probably insufficient to describe the results found since there is evidence that it resembled IS at the electroencephalographic level but generated higher mental stress.

Although HRV results indicated higher mental stress in PSS and IS in comparison with CS, correlations and scatterplots showed overall parasympathetic dominance due to the increased prefrontal cortex activity observed in EEG topographical maps. Both recruitment of areas associated with executive functions and high levels of time-domain HRV (and therefore low levels of LF/HF ratio) were important for the general behavioral success. That is, although IS and PSS were more difficult for the subjects, their behavior was not as impaired as it would be for patients with dysexecutive syndrome and/or older subjects [16], for instance. Two factors can be highlighted as limitations of the present study as an attempt to improve future protocols: a) the absence of reaction time measures in the Stroop task, and b) the difficulty in separating the contributions of the topographic distribution and the power spectrum values to account for the significant differences observed in the topographic EEG maps.

5. Conclusion

This study reinforces the importance of proper PFC functioning and related success in executive

functions for real-world and everyday activities. These activities commonly demand inhibition and therefore need adequate physiological and behavioral responses in order to allow appropriate adaptation of individuals to environment and society.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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Nucleus Accumbens and Its Role in Reward and Emotional Circuitry: A Potential Hot Mess in Substance Use and Emotional Disorders

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ABSTRACT

Nucleus accumbens (NAc) is a key region in the brain that is integral to both the reward and the emotional systems. The aim of the current paper is to synthesize the basic and the clinical neuroscience discoveries relevant to the NAc for the purpose of two-way translation. Selected literature on the structure and the functionality of the NAc is reviewed across animal and human studies. Dopamine, gamma-aminobutyric acid (GABA) and glutamate are the three key neurotransmitters that modulate the reward function and the motor activity. Dissociative roles of the core and the shell of the NAc include getting to the reward and staying on task with discretion, respectively. NAc shows decreased activation to reward in the individuals with major depressive disorder and the bipolar disorder, relative to that healthy controls (HC). The "difficult to please" or insatiability in response to reward in the emotional disorders may possibly be explained by such a neural pattern. Furthermore, it is likely that the increased amygdala activity reported in mood disorders could be accentuating the "wanting" of the reward by the virtue of its connections with the NAc, explaining the potential "hot mess". In contrast, the NAc shows increased reward response in substance use disorders, relative to HC, in response to reward and emotional tasks. Accurate characterization of the NAc and its functionality in the human imaging studies of mood and substance use has important treatment implications.

Keywords: Nucleus accumbens; bipolar; substance abuse; emotion; reward; brain circuitry; fMRI

Abbreviations:

The brain regions engaged in reward and emotional circuitry overlap and are interconnected in daily operations [1]. It is, therefore, only natural to hypothesize that any malfunction in the regions of either circuit is likely to impact both circuits and underlie the comorbidity of emotional disorders and drug addiction [2]. Nucleus accumbens (NAc) is one such key region in the brain that is integral to both the reward and the emotional systems involving functions such as motivation, reinforcement learning, pleasure seeking, processing fear or aversive stimuli and initiating motor activity. The aim of the current paper is to provide an in-depth and foundational description of the NAc's structure, connections, and functional role in emotional and substance abuse disorders.

This description provides potential explanations for common clinical questions that arise in relation to reward seeking, emotion regulation, and the child development and the impact of associated stimuli. In this regard it is important to understand the structure of the Nac, in the context of the emotional and the reward neural circuitry. This includes the relevant neurochemicals which are dopamine (DA), gamma-aminobutyric acid (GABA), glutamate (Glu), serotonin and noradrenaline, as well as the related neural activity to explain the crucial link between the emotional and substance abuse disorders [3].

2. Basic Neuroscience of Nac

2.1. NAc connectivity

The connectivity between various parts of the prefrontal cortex, dorsal striatum, ventral striatum, pallidum, amygdala, insula, hippocampus and hypothalamus is depicted in Figure 1. As seen, the NAc is shown in cartoon form to depict the hedonic hotspot (orange) in the rostral region that is responsible for "liking" of rewards based on animal studies. The NAc shell also contains a caudal hedonic coldspot (blue) responsible for "not liking". Similarly, the orange region depicted in the pallidum in the caudal area is responsible for the hedonic hot spot with opioid activity, and suppression in the rostral blue spot. The amygdala is responsible for "wanting", and hypothalamic stimulation leads to an increase in both the "liking" and the "wanting". Dopamine (DA) and glutamate (Glu) are motivating neurotransmitters while gamma amino-butyric acid (GABA) has the effect on lowering the activity. DA is transmitted from ventral tegmental area (VTA) to the NAc and the ventral (V) pallidum. DA is also directly transmitted to the dorsal striatum from the VTA. GABA is transmitted from the NAc to the V. pallidum, VTA, and lateral hypothalamus. Orexin is transmitted from the lateral hypothalamus to the V. pallidum. Glu is transmitted to the NAc from the basolateral nucleus of the amygdala, orbitofrontal cortex, and hippocampus in synchrony with "wanting", valuing, and memories, respectively. The NAc's strong connectivity to insula underlies the visceral sensation of arousal and excitability corresponding to increase in DA and decrease in $GABA_A$.

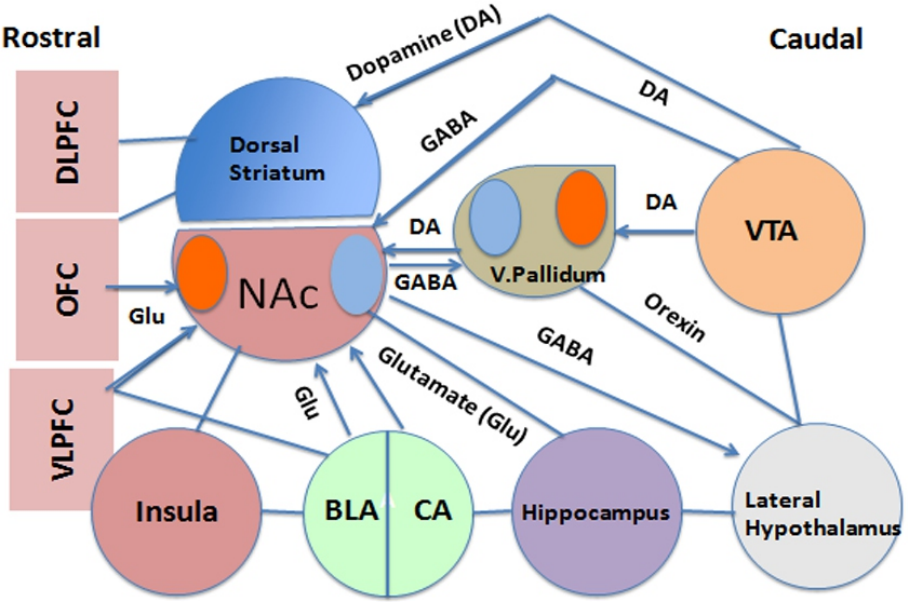

Figure 1. Basic Neuroscience: Nucleus Accumbens Connectivity. The connectivity between various parts of the prefrontal cortex, dorsal striatum, ventral striatum, pallidum, amygdala, insula, hippocampus and hypothalamus is depicted in the sagittal view. The NAc is shown in cartoon form to depict the hedonic hotspot (orange) in the rostral region that is responsible for "liking" of rewards based on animal studies. The NAc shell also contains a caudal hedonic coldspot (blue) responsible for "not liking". Similarly, the orange region depicted in the pallidum in the caudal area is responsible for the hedonic hot spot with opioid activity, and suppression in the rostral blue spot. The amygdala is responsible for "wanting", and hypothalamic stimulation leads to an increase in both the "liking" and the "wanting". Dopamine (DA) and glutamate (Glu) are motivating neurotransmitters while gamma amino-butyric acid (GABA) has the effect on lowering the activity. DA is transmitted from ventral tegmental area (VTA) to the NAc and the ventral (V) pallidum. DA is also directly transmitted to the dorsal striatum from the VTA. GABA is transmitted from the NAc to the V. pallidum, VTA, and lateral hypothalamus. Orexin is transmitted from the lateral hypothalamus to the V. pallidum. Glu is transmitted to the NAc from the basolateral nucleus of the amygdala, orbitofrontal cortex, and hippocampus in synchrony with "wanting", valuing, and memories, respectively. The Nac's strong connectivity to insula underlies the visceral sensation of arousal and excitability corresponding to increase in DA and decrease in GABAA. This figure is adapted in part from Castro et al., 2015, Frontiers in Systems Neuroscience. [63]

2.2. The structure within the NAc of the ventral striatum

The accumbens nucleus or the nucleus accumbens septi (Latin for nucleus adjacent to the septum) is part of the basal ganglia, and is located between the caudate and putamen with no specific demarcation from

either caudate or putamen [4]. The NAc and the olfactory tubercle together comprise the ventral striatum. It is round in shape with the top portion being flat. The NAc is longer in its rostro-caudal length relative to its dorso-ventral length. It has two components—shell and the core [5,6]. The two parts of the NAc share connections and serve distinct and complementary functions.

2.3. Complementary cellular operations and neurochemical differentiation between the shell and the core

2.3.1. Shell of the NAc

The outer portion (i.e., the shell) of the NAc is like a hammock on the ventral, lateral and medial sides of the core [7,8]. It is part of the extended amygdala, with the amygdala being located rostral to the shell, and sends afferents to the basolateral amygdala. It is a transition zone between the amygdala and the dorsal striatum. The shell also sends afferents to the lateral hypothalamus [8]. Neurons in the shell include medium spiny neurons (MSNs). They contain the D1-type or D2-type dopamine (DA) receptors [9,10]. In the shell, around 40% of the MSNs express both types of neurons. Furthermore, these neurons have lower density of dendritic spines and less branching and terminal segments compared to the core MSNs. Additionally, serotonin receptors are predominantly located in the shell [11,12].

2.3.2. Core of the NAc

Neurons in the core (i.e., inner part of the NAc) consist of densely placed, highly branched outer cells that are either the D1-type or D2-type dopamine receptors [10]. These cells project to the globus pallidus and the substantia nigra. Enkephalin receptors, which are opioid receptors with enkephalins as ligand responsible for nociception, and GABAA receptors, which bind the GABA molecules to open chloride channels and increase chloride conductance to inhibit new action potentials, are predominantly present in the core [13,14].

2.4. Neurotransmitters underlying the reward, excitement and habituation dopamine-motivation and reward function

Both in the shell and the core, DA action is greater than that in the dorsal striatum [15]. NAc is specifically involved in the acquisition of fear response through instrumental conditioning during which animals freeze in the context of aversive stimuli [16–18]. The NAc core is different from the shell in that it is involved in learning to identify the cues of aversive stimuli in order to avoid them, generalizing to the

temporally discrete stimuli. NAc shell is known to define or signal safety periods between aversive cues [19,20]. Therefore, when external stimuli are ambiguous or unpredictable, NAc with its dissociable functionality, can aid in avoidance and approach towards intended goal. Therefore, lesions, DA receptor antagonism in the NAc core, or disconnecting inputs from the anterior cingulate cortex to the core, reduce approach toward incentive stimuli [21–23]. This finding supports the concept that the core plays a key role to "get to the reward". Complementary to this finding, NAc shell is the key region responsible for suppressing irrelevant, non-rewarding, and less profitable actions to help "stay on task". Evidence points to the fact that any lesion to the NAc shell leads to uninhibited approach to the reward with less discretion [24]. Also, while high density of transporters renders greater utility of DA in the core, drug induced serotonin and DA antagonism (e.g., clozapine, a treatment for psychosis) leads to greater DA turnover in the shell. Indeed, the shell is the main region of the anti-psychotic action based on corresponding mRNA activity within the shell [25,26]. Appetitive, addictive, excitable, and psychotic behaviors are associated with high levels of DA. High levels of amphetamine will increase DA to equal levels in the extracellular space of the shell and the core [27].

Such an increase in DA due to psychostimulant administration for attention deficit hyperactivity (ADHD) can lead to excitability and mania, psychosis, or more intense drug seeking among vulnerable individuals prone to these illnesses [28,29]. While we understand the clinical phenomena of such occurrences, it remains unclear as to what makes subgroups of individuals prone to such instability with DA administration. Non-drug rewards are also known to increase DA, specifically in the NAc shell, leading to habituation [30,31]. Furthermore, repeated drug induced stimuli and corresponding increase in DA lead to more pernicious habituation in those individuals relative to repeated non-drug related rewards and DA spikes [32]. The possibility that non-drug related rewards could cause DA spikes and habituation may explain the concept of video game addiction, establishing the neural correlates of addiction.

Furthermore, the NAc is a key structure in motivation, emotion regulation and impulse control. With regards to reward seeking and impulsive judgments, both the lesion studies of the NAc in animals and functional imaging studies in gambling have implicated ventral striatum abnormalities as leading to impaired intertemporal choice, risk-taking, or impulsive behaviors in tasks involving options with probability differences. Impulsivity may have many causes, but the NAc is one such channel implicated in reward and emotion regulation [33].

2.5. Dopamine and glucocorticoid receptors- role in mental excitability and potential psychosis

DA and glucocorticoid receptors are present in the NAc shell [34,35]. Excessive steroids or DA in the NAc, lead to psychosis. Glucocorticoid receptors enhance the DA release and related activity [35,36], potentially inciting psychosis. Additionally, epigenetic changes, such as DNA methylation of the glucocorticoid receptor gene (NR3C1) due to traumatic events, are particularly present in adolescence [37,38]. Therefore, stress, as well as dopamine increase associated with psychostimulants or drugs of abuse, can precipitate psychosis through interrelated mechanisms in the NAc. Additionally, the Nac receives direct projections from the hippocampus and the basolateral amygdala. When there is a lesion in NAc and/or the stria terminalis pathway that connects to the amygdala, glucocorticoid agonists cannot enhance and modulate memory consolidation [39]. Therefore, dopamine abnormalities leading to psychosis or early adversity may lead to co-occurring cognitive problems, such as those related to memory.

2.6. GABA and glutamate- moderate motoric excitability

2.6.1. GABA

If GABAA is low in the NAc, it leads to hyperactivity or excitability, and the reverse is true for hypoactivity [12,40,41]. This may have pharmacological value where DA induced hyperactivity can be reduced by GABAA by way of the NAc connections to V. pallidum (i.e., external segment of the globus pallidus of the basal ganglia in the subcortex) that influences motor activity [42]. Based on the insula's role in processing visceral sensation of arousal [43,44], the NAc's strong connectivity to the insula can explain the physiological arousal associated with DA increase and GABAA decrease or vice versa [45,46]. The GABAB receptors also inhibit locomotion, but are mediated by acetylcholine (ACh) [45,47].

2.6.2. Glutamate

This neurotransmitter has parallel, but the opposite effect, of GABAA via the NAc [48]. It has been shown that locomotor activity or motoric excitability is not contingent on DA activity alone, but is also based on the NAc activity involving GABA and glutamate [49,50]. It was recently demonstrated through animal studies that the motoric decision to reach for reward is not initiated in the NAc, but is facilitated through efficiency in motor action selection while approaching the reward [51].

2.7. Acetylcholine (ACh) and its role in reward system

Striatal muscarinic ACh interneurons include M_1, M_2, and M_4; M_1 is post-synaptic and excitatory,

whereas M_2 and M_4 are pre-synaptic and inhibitory. These interneurons synapse with GABA mediated spiny output neurons. The NAc, central to the motivations and reward behaviors that underlie drug addiction, projects ACh output neurons to the V. pallidium. Preclinical studiesshowed that ACh from the NAc mediates reinforcement through its effect on reward, satiation, and aversion, and chronic cocaine administration has shown neuroadaptive changes in the NAc. ACh is further involved in the acquisition of conditional associations and drug seeking behavior through its effects on arousal and attention. Long-term drug use was shown to cause neuronal alterations in the brain that affect the ACh system and impair executive functions. As such, it may contribute to impaired decision making that characterize this population and may exacerbate the risk of relapse during recovery [52]. In addition to its interface with the GABAB receptors in inhibiting locomotion, ACh is also responsible for satiety after feeding, and reduced levels are associated with bulimia like feed-purge cycles [53]. Therefore, ACh has a role in indirectly moderating the reward circuit.

2.8. Connective dynamics of the interfacing reward and emotional circuitry regions involving the NAc: The basis for emotion regulation and habit formation

Disorders involving mood and substance abuse often coexist. Factors that appear to be involved include those related to overt affective processing, motivation, and impaired decision-making. To understand the habit formation, to the first step begins with the reward system's modus operandi. The dorsal and ventral regions of the striatum work in complementary fashion. The dorsal striatum is central to learning the contingencies of the reward stimulus, and entraining the instrumental conditioning [54,55]. In other words, the dorsal striatum optimizes the reward related action-choice. Subsequently, it is the NAc in the ventral striatum that is responsible for the subsequent outcome based predictions [56]. The NAc predicts the error-based outcome and updates the predictions of reward or punishment [57,58]. The mesolimbic neurons of the ventral tegmental area (VTA) synthesize DA and the substantia nigra sends the DA predominantly to the shell and the core of the NAc, to allow it to perform its functions [59,60]. It is the incoming signals from the frontal lobe and the amygdala, modulated by DA, that biases the behavior towards reward [61,62]. Search behavior is facilitated by the connections between the hippocampus and the NAc shell, especially if there is ambiguity and lack of clear direction towards reward [1].

Additionally, the lateral hypothalamus, that is involved in regulatory activities (e.g., the "feeding center") sends signals through mesocorticolimbic projections to NAc and the V. pallidum [63]. It appears the NAc and the V. pallidum serve as hedonic hotspots for "liking" and motivational function of "wanting" rewards [64,65]. The mu opioids and the DA receptors in the shell of NAc and the V. pallidum specifically serve in "liking" and "wanting" functions [66,67]. The DA levels in the NAc and the

norepinephrine released at locus coeruleus in the brain stem play a critical role in addiction, specifically in drug seeking when deprived of the habituated drug [68,69]. Additionally, the dopaminergic neurons from the VTA that innervate the olfactory tubercle, part of the striatum next to the NAc [69], and are involved in mediating the rewarding effects of drugs such as amphetamine by generating arousal. Therefore, while the initial learning of pleasure and associated contingencies occur through dorsal fronto-striatal circuitry, it is the ventral reward system

of the orbitofrontal cortex (OFC), striatum, and pallidum that maintains the cycle of habituation [70]. Furthermore, input from the glutamatergic neurons of the amygdala, hippocampus, thalamus and prefrontal cortex (PFC) to the NAc facilitate the synchrony between the "liking" and the "wanting" [71]. More specifically, glutametergic projections from the OFC and ventromedial PFC to the NAc shell are known to strengthen the reward seeking [72,73]. Therefore, the amygdala and the OFC can be viewed as conveying the "want and need" or the opposite state of "not wanting or aversion". It is the NAc that sets the tone for the motivational significance or appreciation in the case of feeding or any other pleasurable activity (i.e., "liking" or "not liking"). The amygdala sends the affective signals that are conducive to the desire for the drug [74,75]. The hippocampus is responsible for storing memories associated with past drug use and associated pleasure [75,76]. The insula provides the aspect of the bodily experiences of pleasure and arousal state related to the drug intake [77]. Relative value of the reward and associated outcome-guided behavior is determined by the OFC, both in relation to the rewarding stimulus or, in the case of devaluation of the stimulus, cessation of the seeking behaviors [61].

Overall, output from the NAc extends to the regions of the basal ganglia, amygdala, hypothalamus and the PFC regions. Based on neuroimaging studies involving healthy controls (HC), mood disordered subjects, and substance abuse subjects, medial prefrontal cortex (MPFC), anterior cingulate cortex (ACC), ventrolateral prefrontal cortex (VLPFC) and precuneus emerged as hubs in the interlinked reward and emotion circuitries. Impulsive and compulsive drug seeking behaviors aremoderated both by nature and nurture. The genetics behind disorders of impulse control and addiction serves to explain the physiological predisposition, while the environmental influencing factors (e.g., parental restrictions or peer pressure in drug usage) may limit or expand the exposure and actively contribute to entraining the habit circuitry.

3. Clinical Neuroscience of Nac

3.1. Nucleus Accumbens' role in the hot mess of emotion dysregulation and addiction

The predominant activation pattern is depicted in Figure 2. This shows patient groups in each of the disorders in comparison to healthy controls with tasks probing either reward or emotion neural circuitry. The arrows represent an increase or a decrease in activation in the key regions of the reward and the emotion circuitry that are intricately connected. In the case of bipolar disorder (BD), the NAc shows increased activation in response to emotional stimuli and decreased activation in response to rewards, the latter pattern being similar to that seen in major depressive disorder (MDD). In MDD, the NAc shows decreased activation to both emotional stimuli and reward, opposite to that observed in substance abuse disorder.

Figure 2. Pattern of Activation in Patients vs. Healthy Individuals

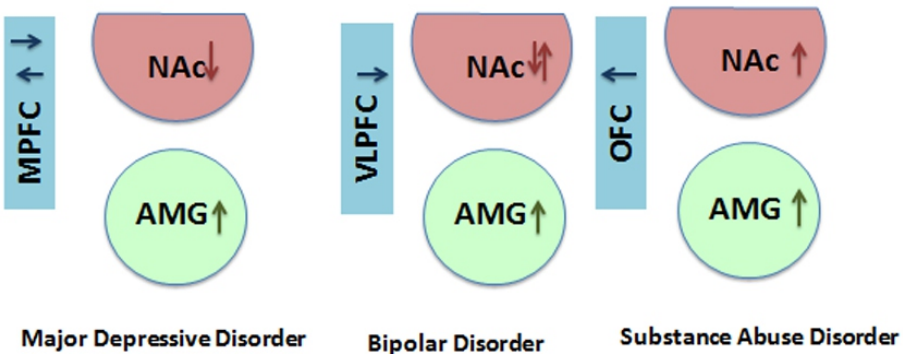

Figure 2. Clinical Neuroscience: Nucleus Accumbens' Role in the Hot Mess of Emotion Dysregulation and Addiction. The predominant activation pattern is depicted in this figure in which patient groups in each of the disorders were directly compared to healthy controls with tasks probing either reward or emotion neural circuitry. The arrows represent an increase or a decrease in activation in the key regions of the reward and the emotion circuitry that are intricately connected. In the case of bipolar disorder, the Nucleus Accumbens (NAc) shows increased activation in response to emotional stimuli and decreased activation in response to rewards, the latter pattern being similar to that seen in major depressive disorder (MDD). In MDD, the NAc shows decreased activation to both emotional stimuli and reward, opposite to that observed in substance abuse disorder. VLPFC: ventrolateral prefrontal cortex; MPFC: medial prefrontal cortex; AMG: amygdala; OFC: orbitofrontal cortex.

3.2. Neural pattern of activation in the NAc in substance abuse and mood disorders: human imaging studies of emotional and reward stimuli

Most of the human studies that extended the knowledge on the role of the NAc are based on fMRI studies probing the reward and/or emotional circuitry. In relation to the NAc, the most accurate view is obtained as T2 images and in the coronal section where it is the longest and shows the most detail [3]. A consistent

pattern of brain activation has emerged in identifying the interfacing circuitry dysfunction across the disorders. In the interpretation of these experiments, both increased activity and the absence of activity must be considered. When there is stimulus of moderate intensity, brain region that is partially operating even if impaired, shows increased activation. If the same brain region is probed with stimulus of severe intensity (also mediated by the type of the disorder where perceptions vary, such as patients with bipolar disorder react to angry faces more than fearful faces), it would show no activation or decreased activation relative to healthy population. This phenomenon has been noted on careful examination of the patterns over multiple studies to make sense of the variability in brain activation in response to varying probes.

3.2.1. Major depressive disorder (MDD)

Relative to that of HC, the individuals with MDD showed decreased activation in the NAc in response to any rewarding stimuli, but increased activation to implicit emotional stimuli (e.g., covert face processing or cognitive generation of positive affect) [78]. In other words, in MDD, the NAc is underactive with reward and this may explain why this population appears to need larger reward to attain the same level of activation as HC (i.e., "not easily pleased") An alternative physiological explanation is that the reward stimuli may serve as explicit emotional triggers in depression, with lower impact on activating the NAc. Hence, it may be that incidental or implicit emotional stimuli trigger the excessive reactivity in the NAc. Corresponding to the NAc activity, the amygdala also shows increased activation in the MDD patients, relative to HC, in response to negative or implicit emotional stimuli [79]. The various prefrontal regions show variable patterns of either increased or decreased activation, unlike the consistent pattern noted in the subcortical areas [80,81]. Within our clinical experience excessive use of substances appears to have the purpose of self-medicating to subdue negative emotional states associated with a lowered threshold for reactivity to negative triggers. This corresponds with the physiological experiments we have summarized.

3.2.2. Bipolar disorder (BD)

In response to reward task and regardless of comorbid substance abuse, relative to HC patients with BD show lower activation of the VLPFC and increased activation of the amygdala for implicit or explicit negative emotions, in addition to compensatory over activation of the ACC [82]. A fascinating observation is that the NAc behaves in the exact manner as the VLPFC; implicit negative affective processing leads to decreased activation, while both implicit and explicit happy or fearful faces lead to increased activation [83]. One notable point is that, in BD, sad or angry emotions tend to be more directly

relevant than fear as negative emotional stimuli, which can explain the increased activation associated with fear. Therefore, when emotional tasks are used to activate the emotion circuitry, the intensity of the tasks appears to proportionally trigger a dysfunctional under-activation in the VLPFC of BD subjects relative to the HC. This gives the appearance, that the VLPFC "gives up" in response to severe or intense negative emotions. In response to reward anticipation, the NAc showed decreased activation in response to monetary reward in BD subjects relative to HC [84]. This is a pattern similar to that seen in MDD, suggesting the need for greater reward to obtain the same emotional impact as in HC. Thus, the pattern in BD differs from MDD in response to emotional stimuli based on pathophysiological differences, though leading to a similar behavioral response to the reward stimuli.

In explanation as to what could underlie clinical scenarios in BD, the physiological findings of the neuroimaging experiments complement the knowledge derived from animal studies. In this regard, it is possible that increased amygdala activity in BD projects a certain degree of intensity corresponding to the excitability. The decreased activity in the VLPFC and OFC regions may lead to disinhibition, and associated poor impulse control, and result in excessive pleasure seeking related to impairment in PFC-mediated decision-making. Based on animal studies [85] and BD human neuroimaging studies [86], connectivity between the amygdala and the NAc may be relevant in accentuating the "want" and the "like" in seeking rewards. Therefore, the intense reward-seeking behaviors (e.g., excessive shopping, drug use, food consumption, or sex) may be due to the interlinked dysfunction in the emotional and reward systems.

3.2.3. Substance Abuse Disorders

In addiction or substance abuse disorders, relative to HC, passive or implicit perception of craving-related stimuli leads to increased activation in the NAc [87]. This underlies the motivation bias associated with increased activation in the OFC, ACC, and amygdala, the regions that are linked to both reward and emotional circuitry [87]. These regions appear common to all reward seeking, regardless if the stimuli are or are not drugs [88,89]. While motivation toward seeking goals is dependent on the NAc in the ventral striatum, the progressive shift to habit formation appears dependent on the dorsal striatum [90]. This is in correspondence to the "liking" hypothesis in which with the initial observation of the reward is associated with NAc activation. In substance use disorders, relative to HC, decreased NAc activation occurs in this anticipatory observational phase, regardless of any subsequent loss or gain of a reward [91]. Increased DA release in the anterior ventral striatum, but not in the dorsal caudate, was shown to be positively correlated with the hedonic, or "liking", response to dextroamphetamine [92]. In actuality, the positive affective experience of hedonic "liking" is not readily disentangled from

"wanting" the drug [93]. Related to depression, seeking a hedonic response is a possible explanation of self-medicating through abuse of drugs. Similarly, stimulant use in a subpopulation of users may be primed due to seeking excessive rewards that is triggered by excessive dopamine.

3.2.4. Treatment implications through deep brain stimulation (DBS)

The DBS of the NAc was attempted for the treatment refractory obsessive-compulsive disorder where compulsion was considered to be similar to that of drug-seeking compulsivity, involuntary motor activity like Tourette syndrome, depression and drug and alcohol abuse [94]. All these attempts yielded no conclusive findings on outcome. Symptoms of depression were reduced by approximately 40% in this cohort [94,95].

3.2.5. Placebo effect in healthy individuals

When healthy adults were given a pain challenge, DA and opioid activity in the NAc were associated with subjectively perceived effectiveness of the placebo based on reductions in pain ratings [96]. Similar to reward expectation, this supports the NAc's involvement with anticipation of a positive response.

4. Summary and Conclusions

The foregoing discussion had the goal of providing an in-depth analysis of the NAc to allow scientists and educators to be aware of multiple aspects of its functionality. In relation to functional imaging, identifying the NAc requires careful analysis due to the multiple, small adjacent regions, such as parts of the caudate and putamen, that could be mistaken for the NAc or vice versa. With this in mind, the shape of the NAc means the best view is accomplished in the coronal section in interpreting the neuroimaging findings. Additionally, an understanding of the role of the NAc in a systems perspective of emotional and reward circuitry offers a broader perspective of its role in brain operations. The current paper has presented findings on the NAc from both human and non-human animal studies, with an examination of those findings as related to a clinical understanding. The existing scientific literature of both the basic and the clinical neuroscience paired with the acumen from clinical insights align a powerful triad toward translation to advance our understanding of the NAc's functional role, as has hopefully been illustrated in this manuscript. In summary, the clinically applicable derivatives of neuroscience, where the NAc plays a key role, are as follows:

1. The NAc plays a significant part in channeling DA, GABA and glutamate in modulating the reward and emotional systems.

2. Dissociable roles of the NAc core and the shell involve selecting the reward and evading distractions, respectively.

3. The NAc shows decreased activation to reward in individuals with MDD and BD, relative to that HC, and this can potentially explain the lack of pleasure with reward (akin to anhedonia) in MDD and the need for intense pursuit of reward in BD.

4. While the NAc shows increased activity in all substance use disorders, relative to HC, animal studies indicate joint increase in activity in the highly connected amygdala and V. pallidum. Anticipating and selecting reward with NAc involvement from human studies and the amygdala's excitability to accentuate the reward seeking in animal studies, can together inform the emotional overlay in addictive behavior.

5. It is also possible that inattention and impulse control associated with low DA or noradrenaline levels may lead to poor frustration tolerance, and potentially, seek reward as gratifying alternative. In this scenario, optimal treatment with psychostimulants could avoid being habituated to illicit drugs. It appears that adolescence is particularly a vulnerable time for the precipitation of any illness with accentuated glucocorticoid receptor sensitivity in the NAc. While there are no definitive answers, these unanswered questions pose research challenges for the future.

Conflict of Interest

All authors declare no conflicts of interest pertaining to this paper.

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