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The N400 as an Indicator of Semantic Memory for Music

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Table of Content

Abstract (German)

Eine einzigartige und faszinierende Charakteristik von Musik ist ihre Einprägsamkeit. Sogar Patient:innen die an Alzheimer leiden sind immer noch in der Lage auf Musik zu reagieren und zu erlernen, wodurch Rückschlüsse auf positive Effekte von musikbasierten Interventionen gezogen werden können. Ein ereigniskorreliertes Potenzial, welches als Marker für ein semantisches Musikgedächtnis in Erwägung gezogen wird, ist das N400, das sich als Reaktion auf Verletzungen musikalischer Erwartungshaltungen artikuliert. Wir präsentieren ein zeiteffizientes, 20-minütiges Paradigma zum Hervorrufen von musikbasierten N400s. Wir haben elektroenzephalographische (EEG) Daten als Reaktion auf 40 Standardnoten und 40 Verletzungsnoten gemessen, die in 17 bekannten Melodien und 17 darauf basierenden Neukompositionen enthalten waren. Die allgemeine musikalische Erfahrenheit wurde mithilfe des GOLD MSI erhoben. EEG Daten von 39 Versuchspersonen (Alter: M = 31.7 Jahre, SD = 15.5) wurden mit ANOVAs über die Faktoren Familiarität (bekannt/neu), Ereignisart (Standardnote/Verletzungsnote), und Hemisphäre (links/rechts) ausgewertet und wiesen signifikante Interaktionen zwischen Familiarität und Ereignisart auf, $F(1,38) = 4.452$, $p = .041$. Zusammenfassend fanden wir signifikante N400s in bekannten Melodien nach Verletzungsnoten. Wir fanden außerdem kleinere N400s in Neukompositionen, was darauf schließen lässt, dass auch wenige Wiederholungen innerhalb eines Liedes ausreichend für Erinnerung im Kurzzeitgedächtnis sind. Wir fanden keine Unterschiede in Hemisphären. Korrelationen zwischen GOLD MSI Skalen und Differenzwellenamplituden traten nur bei bekannten Melodien auf. Unsere Ergebnisse lassen darauf Rückschlüsse ziehen, dass 20 Minuten ausreichend für N400 Reaktionen sind und zeigen den Nutzen unseres verkürzten Paradigmas auf.

Schlagwörter: N400, ERP, EEG, semantisches Gedächtnis, Musik, Alzheimer

Abstract (English)

A unique and intriguing characteristic of music is its memorability. Even patients suffering from Alzheimer's disease (AD) are still capable of learning and reacting to musical inputs, suggesting positive effects of music based intervention. One event-related potential (ERP) which has been discussed as a candidate marker for semantic memory of music is the N400, occurring in response to violations of musical expectancy. Here we introduce a time-efficient, 20-minute paradigm to elicit musical N400s. Electroencephalographic (EEG) data were measured in response to 40 standard and 40 in-key violation notes contained in 17 familiar and 17 novel melodies composed in the familiar melodies' style. General musical sophistication was measured using the GOLD MSI inventory. Repeated-measure ANOVAs were conducted on the N400 data of 39 participants (Age: M = 31.7 years, SD = 15.5) with the factors familiarity (familiar/novel), event type (standard/violation), and hemisphere (left/right) revealing significant interactions of familiarity and event type, $F(1,38) = 4.452$, $p = .041$. In summary, significant N400s followed in-key violations in familiar melodies. Smaller N400s were observed in novel melodies suggesting that repetition of musical phrases within a song is sufficient for their retention in short-term memory. No hemispheric differences were observed. Correlations between GOLD MSI subscales and difference wave amplitudes were only observed in familiar songs. These results suggest that 20 minutes are sufficient for establishing N400s, demonstrating the utility of this shortened paradigm for assessing semantic memory for music.

Keywords: N400, ERP, EEG, semantic memory, music, Alzheimer's disease

Introduction

One unique characteristic of music is its memorability even in the context of dementia. What constitutes memory of music? Which aspects of music are remembered? Researchers have suggested that music is processed in a modular fashion. Following this logic, music is also remembered in a modular way, such that different aspects of music are processed by different parts of the human brain (Park & Friston, 2013; Peretz & Coltheart, 2003). In recent years, an ever growing body of research on the processing of music in the brain has been conducted, in part via research on the relationship between the processing of music - as a time-varying auditory signal - and speech - as perhaps the more common time-varying auditory signal. This research has produced evidence showing that both language and music seem to follow specific sets of rules and are interpreted in a hierarchical, modular fashion (Koelsch et al., 2000, 2002; Kunert et al., 2016; Kunert & Slevc, 2015; LaCroix et al., 2015; Maess et al., 2001; Patel et al., 1998; Slevc et al., 2009).

For example, music engages neural networks responsible for action, emotion and learning in conjunction with the auditory system. This allows the listener to actively form a model for predictive coding, developing hierarchical structures for melody, harmony, tonality and rhythm, thus establishing a tonal centre or a recurring rhythm pattern (Vuust et al., 2022). Language can be seen as a computational system with a neural foundation being grounded in the collaboration of the frontotemporal network, the posterior temporal cortex and white fibre connections. When forming and interpreting sentences, syntactic and semantic interactions take place, such that single units like words (syntax) are recursively merged together in a hierarchical structure to convey a semantic meaning; something that is presumably a unique characteristic of human language (Friederici et al., 2017).

Multiple studies have investigated the shared neural resources for the processing of linguistic and musical components in songs (Besson et al., 1998; Gordon et al., 2010; Patel, 2003), pointing towards a strong connection between music and language. However, other findings state that music perception remains largely intact despite damaged language domains - such as seen in patients with aphasia (Chen et al., 2023; Chiappetta et al., 2022; Faroqi-Shah et al., 2020). These accounts indicate that damage to one modality doesn't necessarily result in a weaker performance of the other modality, adding to the complicated connection of music and language. Especially in light of theories suggesting that musical training can positively influence language processing (Patel, 2014; Patel & Morgan, 2017), this calls for further research on the common and/or different neural underpinnings of music and language (Peretz & Zatorre, 2005; Slevc & Okada, 2015). In this thesis, using EEG methods, we want to test whether an ERP commonly shown for semantic interpretation of language, that is, the interpretation of language based on semantic memory, is also found when we semantically interpret music, that is, interpret the melodies based on musical semantic memory.

To gain insight into the workings of the brain, the measurement of ongoing electroencephalographic (EEG) data via investigation of event-related potentials (ERPs) represents a standard technique of assessing cognitive processes (Luck, 2012). As a non-invasive, direct way to measure electrical currents and neuron activity in the brain with a high level of temporal resolution (Biasiucci et al., 2019; Blackwood & Muir, 1990; Mulert, 2013), the method is suitable for measuring a broad range of cognitive and affective processes within almost any group of participants (Luck, 2012). ERPs are assessed by recording EEG data during the presentation of a number of experimental stimuli and calculating the average of the EEG data immediately following presentation of the stimuli in the time domain, with naming conventions being based on either the direction or time of the ERP's occurrence after stimulus onset (Herrmann et al., 2014). They can furthermore be divided into exogenous/sensory or endogenous/cognitive components. The former are triggered by physical parameters of a stimulus appearing roughly within the first 100 ms after stimulus onset. The latter occur at later times due to evaluation-based and task-dependent cognitive processes. They can additionally also be classified as preparation/execution-based motor response components (Luck, 2012; Sur & Sinha, 2009).

One ERP which has been discussed as a candidate marker for semantic memory of music, and the main focus of this thesis, is the N400. It was first found in the context of language and has been associated with semantic incongruity. Early studies found that the presence of the N400 is usually indicated by a large amplitude peak around 400 ms after a stimulus onset (Kutas & Hillyard, 1980) and has a negative-going nature relative to a 100 ms pre-stimulus baseline at a specific reference location (Kutas & Federmeier, 2011). In language, an example stimulus which might elicit an N400 component could be the sentence “The pizza was too hot to cry”, where a person would usually expect the word “eat” instead of “cry” (Besson & Schön, 2001). This grammatically intact, but semantically incorrect sentence results in an N400 emerging due to a contextually inappropriate combination of words (Osterhout & Holcomb, 1992). Smaller N400s can also be found when the expected word would result in a lesser violation of the sentence’s content, such as “drink” instead of “eat” (Kutas & Hillyard, 1984). Similar findings have also been found in pairs of words and sounds (van Petten & Rheinfelder, 1995) or pairs of pictures and objects which are either more or less related to each other (McPherson & Holcomb, 1999). This might be due to the fact that the N400 reflects representations in the long-term memory and integration of context into long-term knowledge (Kutas & Federmeier, 2000).

Researchers have since also investigated the EEG responses to violations of long-term knowledge of musical rules. Early studies found a so-called early right-anterior negativity (ERAN) peaking at around 200 ms when investigating the effects of violations on musical syntax by examining expectancy violating chords in chord progressions (Koelsch et al., 2000; Maess et al., 2001; Patel et al., 1998). Further studies investigated the role of selective attention when listening to chord progressions (Loui et al., 2005), and whether this ERAN is a result of violating listener's long-term knowledge of the rules of harmony and tuning of chord progressions known in western music (Leino et al., 2007).

Further, researchers have investigated the EEG responses to violations of expectations based on long-term knowledge of specific music. Miranda and Ullman (2007) tasked participants with listening to more than 240 familiar and unknown melodies covering a broad spectrum of musical genres. These stimuli could contain in-key or out-of-key violations: Out-of-key violations can be assumed to violate expectations based on long-term knowledge of harmonic progression and can additionally violate expectations based on long-term knowledge of the specific melody if the melody is known. In-key violations were assumed to only violate expectations based on long-term knowledge of a specific melody, that is, semantic musical memory, if the melody is known; there is no expectation violation on long-term knowledge of harmonic progression, i.e. musical syntax. Their results suggested that both in-key and out-of-key violations elicited a N400 reaction around 220-380 ms for their familiar melody violations following the right-anterior negativity (RAN), whereas in-key violations for novel melodies did not (Miranda & Ullman, 2007). Musical education of the test subject also affected their ERP components, as those with a more musical background displayed a P3a in addition to the N400, suggesting additional attentional resources were allocated to musical stimuli (Miranda & Ullman, 2007; Tervaniemi et al., 2005).

A later study conducted by Calma-Roddin and Drury (2020) adopted a new interference approach incorporating language and music in a paradigm to test the processing streams simultaneously. Here, violations could be introduced either in sentences, in concurrently presented melodies, or in both streams at the same time (double-violation).

They reaffirmed Miranda and Ullman's findings for the presence of the N400 in familiar melodies, as well as further findings concerning delayed N400 and RAN reactions: Delays of linguistic N400 reactions occurred when attending to familiar melodies. They also found delays of up to 200 ms in familiar melodies between RAN and musical N400s, when processing of concurrent language occurred. Their findings also implied that within double-violations of familiar melodies, the linguistic N400 remained relatively unperturbed in the presence of a music violation. In contrast, the musical N400 was suppressed, leading to a weaker sub-additive N400 response instead of a summation of amplitudes from both domains. These results were interpreted as a potential competition of processing resources for both modalities simultaneously. Furthermore, double-violations in unfamiliar melodies and violations in familiar melodies without violations in the concurrently presented sentences showed delayed RAN reactions. Attentional focus might have been shifted towards the more important interpretation of language when being exposed to unfamiliar melodies, explaining the delayed RAN reactions in the first case. Within familiar melodies – although their predictability could have caused the attention shift towards language – the opposite effect occurred, delaying the language N400, most likely due to attentional focus being shifted towards the familiar melodies. These findings indicate the important role of attention for cross-domain research, as well as the role of long-term memory representation for both RAN and N400 reactions (Calma-Roddin & Drury, 2020).

Summarizing scientific findings regarding the N400, topography and overlap of areas are sometimes hard to distinguish based on the types of stimuli presented, which might be due to the distributed networks involved in storing and processing of semantic memory (Kutas & Federmeier, 2011). For example, linguistic stimuli with semantic or syntactic violations seem to generally result in reactions with a stronger left and a weaker, but still substantial, right lateralization (Friederici, 2011; van Petten & Luka, 2006), while music based stimuli, as seen with the E(R)AN, can either be right or bilaterally lateralized mainly in frontal areas (Koelsch et al., 2002; Maess et al., 2001; Patel et al., 1998). However, the music-based N400 elicited by Miranda and Ullman's stimuli were recorded at central and posterior electrodes and were being evenly distributed over both hemispheres, giving their findings a novelty factor for music-based ERP studies (Miranda & Ullman, 2007).

The fact that an N400 can be elicited for isolated musical stimuli also provides an avenue for investigations into the workings of long-term memory more generally. In the context of ongoing research, adapting a music based test for semantic memory of music to be usable with special populations is particularly relevant for diseases in which dementia presents as a symptom (Lichtensztejn et al., 2024). Despite the aforementioned influence of attention, a music-based N400 paradigm may serve to test the presence of musical semantic memory without requiring participants to explicitly comment on this memory.

A special form of dementia is Alzheimer's disease (AD), a complex neurodegenerative disorder with varying cognitive impairments based on age, genetics, education and environment. The root causes of AD are β-amyloid-containing plaques and tau-containing neurofibrillary tangles accumulation in the brain (Knopman et al., 2021; McGirr et al., 2020). This leads to the disruption of the function of previously healthy neurons, as these obstructions lead to connection loss with other neurons, eventually resulting in neuronal death and consequent significant shrinking of the affected brain tissue (NIH, 2023). AD, currently affecting around 55 million people worldwide, accounts for over 60% of all types of dementia, mainly impacting short term memory and learning processes (Alzheimer's Association, 2025; WHO, 2023). As AD entails a rather significant socioeconomic burden (WHO, 2023), depending on an impaired individual's stage of dementia (Vermunt et al., 2019), different avenues for disease prevention have been explored over the years to account for the diversity of AD's representation. These avenues include monitoring cardiovascular risk factors, nutritional counselling, physical exercise, cognitive training and social activity, as well as identification of at-risk individuals via the usage of biomarkers or genetic risk profiles (Crous-Bou et al., 2017).

Although no definitive cure has been found for the severe deficits imposed by AD on memory domains, positive effects can be found with music-based intervention (Fusar-Poli et al., 2018). For instance, patients are still capable of learning new melodies and verbal information, as well as react on an emotional level to these types of inputs (Matziorinis & Koelsch, 2022; Samson et al., 2009). Those only suffering from mild to moderate AD seem to preserve engagement and enjoy seeking out music, evoking music-based memories in largely spared long term memory domains (Cuddy et al., 2015). Considering that these patients require more repetitions of music-based stimuli in order to retain musical memory compared to healthy adults (Coppalle et al., 2019), it is even more remarkable that patients with stronger disease onsets and no prior music training are still capable of learning new songs via individual music intervention (Lichtensztejn, 2022). Being embedded in the memory for music project (M4M) (Lichtensztejn et al., 2024), this paper is specifically aimed at first exposing healthy young adults to a similar but adapted paradigm as suggested in the Miranda and Ullman 2007 study to test for N400 effects. Given the long duration of their paradigm, one goal of this master’s thesis is to introduce a paradigm with a length that is not prohibitive to usage with AD patients.

Methods

In this experiment, participants listened to familiar and novel melodies while their brain activity was recorded via EEG. In this section, we describe the participants, the stimuli, and the procedure of the experiment.

Participants

A total of 40 healthy adults (Age: *M* = 32.3 years, *SD* = 15.8; 14 men, 26 women) were tested. None of the subjects attested to having any developmental, neurological or psychiatric disorders and had no hearing or vision impairments. Most participants spent their youth in Austria, and can thus be assumed to possess familiarity with Austrian children and folk songs. Of the 6 participants who were born outside of Austria, only 2 spent their youth in Germany and Italy (South Tyrol), respectively. Both participants affirmed their knowledge of Austrian children and folk songs when prompted. Musical sophistication was measured via the German version of the GOLD MSI questionnaire and data was compared to the results of a German demographic's sample (Schaal et al., 2014). Average subscale values fell between the 40th and 60th percentile, with the overall general music sophistication (*M* = 67.6, *SD* = 21.3) ranking the sample between the 45th and 50th percentile compared to the demographic's population data.

Table 1

Average Gold MSI Scores

	General Sophistication	Active Engagement	Perceptual Abilities	Musical Training	Singing Abilities	Emotions
M (SD)	67.6 (21.3)	35.7 (9.8)	44.5 (8.3)	21.2 (11.0)	26.5 (9.9)	31.6 (5.6)
percentile	45 to 50	55 to 60	40 to 45	45 to 50	45 to 50	45 to 50

Note. Mean, standard deviation and percentile values are compared to the German GOLD MSI; higher mean values indicate a higher ranking within the specific (sub)scale

All participants gave informed consent. Research methods were approved by the Ethics Committee of the University of Vienna in March 2024. Participation was possible from the start of April to the end of May in 2024. Participants were recruited either via posters or word-of-mouth. Participants were given 15€ compensation for their participation.

Stimuli

Familiar Stimuli

The familiar stimuli were identified using an online questionnaire. A total of 41 participants (Age: *M* = 33.0 years, *SD* = 12.0, 11 men, 30 women) were recruited from mailing lists and word-of-mouth to evaluate the familiarity of a selection of 30 songs. In order to participate, participants had to have grown up in Austria. None of these participants participated in the EEG study. To enhance the probability of familiarity of songs, the selection focused on children and folk songs known in Austria, where the main study took place. Songs lasted between 8 to 29 seconds (*M* = 15.9 s, *SD* = 5.3 s) and were presented in a randomized order as a piano-only melody that participants could listen to indefinitely. Participants were tasked to assign recognisability values on a scale from 1 (lowest) to 10 (highest). Participants also indicated with yes or no, whether they perceived the songs to be singable, whether they knew the lyrics, and whether they had listened to the song in childhood.

Once a participant answered the questions for each song, they were not able to go back and change their answers. Only completely filled out forms were used for the analysis of song familiarity. The questionnaire was made available on a version of SoSciSurvey hosted on the servers of the University of Vienna for the duration of one month (29.10.23 - 26.11.23). The 17 most recognisable songs (*M* = 9.2, *SD* = .6) were used in the EEG study. For the EEG study, these were all transcribed to the key of C-major on piano as a MIDI file in Musescore (Version 4) (MuseScore, 2025). All songs were also set to 100 beats per minute (BPM) ranging between 13 and 39 seconds of playtime (*M* = 24.9 s, *SD* = 7.6 s).

Table 2

List of familiar songs used in the online questionnaire

Name	Familiarity	Singability %	Lyrics %	Childhood %	Study Usage
O Tannenbaum	10	92.7	97.6	100	Yes
Bruder Jakob	9.95	95.1	100	100	Yes
Alle meine Entchen	9.88	97.6	100	97.6	Yes
Happy Birthday	9.85	92.7	95.1	95.1	Yes
Morgen kommt der Weihnachtsmann	9.63	80.5	87.8	97.6	Yes
Hänschen Klein	9.61	73.2	95.1	95.1	Yes
Fuchs du hast die Gans gestohlen	9.56	80.5	87.8	92.7	Yes
Kling Glöckchen Kling	9.51	75.6	92.7	95.1	Yes
Guten Abend gute Nacht	9.32	65.9	85.4	90.2	Yes
Alle Jahre Wieder	9.29	75.6	92.7	95.1	Yes
O du lieber Augustin	9.07	63.4	90.2	92.7	Yes
Alle Vögel sind schon da	8.8	65.9	85.4	90.2	Yes
Hänsel und Gretel	8.76	53.7	78	87.8	Yes
O du Fröhliche	8.56	58.5	78	85.4	Yes
Leise rieselt der Schnee	8.39	70.7	80.5	82.9	Yes
Stille Nacht heilige Nacht	8.22	65.9	75.6	78	Yes
Ihr Kinderlein kommet	8.2	58.5	78	75.6	Yes
Schneeflöckchen Weißröckchen	8.15	61	70.7	75.6	No
Kommt ein Vogel geflogen	7.73	51.2	68.3	75.6	No
A A A der Winter der ist da	7.68	39	73.2	73.2	No
Fröhliche Weihnacht	7.59	48.8	68.3	73.2	No
Schlaf Kindlein schlaf	7.07	36.6	56.1	61	No
Hopp hopp hopp Pferdchen lauf Galopp	6.78	26.8	56.1	68.3	No
Backe backe Kuchen	6.56	41.5	56.1	56.1	No
Still still still	6.44	36.6	48.8	58.5	No

Weißt du wie viel Sternlein stehen	6.44	31.7	56.1	68.3	No
Der Kuckuck und der Esel	6.1	39	51.2	68.3	No
Hoppe hoppe Reiter	6.1	19.5	39	53.7	No
Das Wandern ist des Müllers Lust	4.56	22	31.7	34.1	No
Der Mond ist aufgegangen	3.9	29.3	22	29.3	No

Note. Familiarity is based on a 1-10 scale; singability, lyrics and childhood are percentage values; higher values indicate higher rankings; the final selection of songs is indicated in the right column

Novel Stimuli

The novel stimuli consisted of newly composed songs based on the final selection of familiar stimuli from the online questionnaire's analysis. We analysed the structure of the familiar songs and composed 17 completely novel songs using data regarding pitch step distribution, intervals, transition frequency, number of notes per song and rhythm contained in the familiar stimuli. Pitch step distribution was analysed in half steps going from C to B. Intervals ranged from Unison to Eight and were analysed as either absolute or relative intervals, with absolute intervals referring to the interval step itself (i.e. a Third, Fourth, etc.), and relative intervals referring to musical contour of up and down movements of an interval (i.e. a Third up or a Fourth down). Transition frequency was analysed for each note and each possible half step (i.e. how many C to C, C to B, D to D, D to B, etc. movements were in each individual song). Notes per song accounted for the total amount of notes contained in each familiar song. Rhythm accounted for the longest and shortest note durations (ranging from sixteenth through whole note durations) in each song. Based on the analysed transition frequency data, random melody fragments were generated and then manually adapted to reflect the compositional style of the familiar songs. Each familiar song was assigned with a novel counterpart containing similar song length, phrase repetitions and time signature. Newly composed songs were also transcribed to the key of C-major on piano and were set at a tempo of 100 BPM for the closest resemblance of structure to their familiar counterparts; these novel songs were also generated as a MIDI file in MuseScore (Version 4) (MuseScore, 2025). The final songs individually ranged between 13 and 39 seconds of playtime (M = 26.6 s, SD = 6.5 s).

Song Violation Notes

To elicit N400s in both familiar and novel songs, both stimulus types were checked for possible violation note locations. A total of 80 target notes were selected in the final 34 musical pieces for each condition. To allow time for music recognition, target notes could not be placed within the first four bars of a melody. Target notes were first marked in the musical score and needed to minimally have a duration of 600ms. Longer notes were always preferred over short notes in order to allow longer epoch generation for easier N400 analysis. Half of these target notes were left unchanged (standard note), the remaining 40 target notes were replaced with in-key violations (violation note). Violations could either be a violation of musical contour, interval or both types of violations combined. Because musical contour is more easily retained in short term memory, contour violations were minimised in favour of interval violations, and if unavoidable, balanced in terms of contour violation direction for the final selection (Bella et al., 2003; Schmuckler, 2010; Schmuckler & Moranis, 2023). An additional level of unpredictability was achieved by adding violations either in the middle, the end of the phrase, or at the end of the song. To shorten the paradigm of Miranda and Ullman, each song could contain multiple violations and standard notes, with violations always being in-key violations.

Setup and Procedure

The study setup consisted of two PCs: a desktop PC for stimulus presentation via PsychoPy and a laptop for EEG data collection using a g.tec HIamp and the corresponding software g.Recorder. Stimuli were played over loudspeakers. PsychoPy stimuli triggers were matched with the refresh rate of the PC's monitor. Participants were fitted with a cap containing 64 electrodes arranged in the 10-20 international system. While being fitted, participants filled out a form of consent for data usage as well as the music familiarity questionnaire – the German version of the GOLD MSI. Participants were seated ~70 cm away from the PC screen. Before the start of the recording session, they were instructed to remain as relaxed and still as possible while tasked with looking at a fixation cross in the middle of the PC screen. Participants were informed about the general testing modalities, but not about the mix of familiar and novel songs, or that each song would contain violations. After being instructed all 34 stimuli were played in succession in a randomised order with an applause indicator at the end of each stimulus. Once participants heard the applause, participants were instructed to press the spacebar on a computer keyboard to continue with the next stimulus. This method was chosen to ensure participants remained awake and engaged throughout the entire duration of the study. The study lasted around 20 minutes and was conducted in a silent and darkened room to prevent distractions.

Analysis

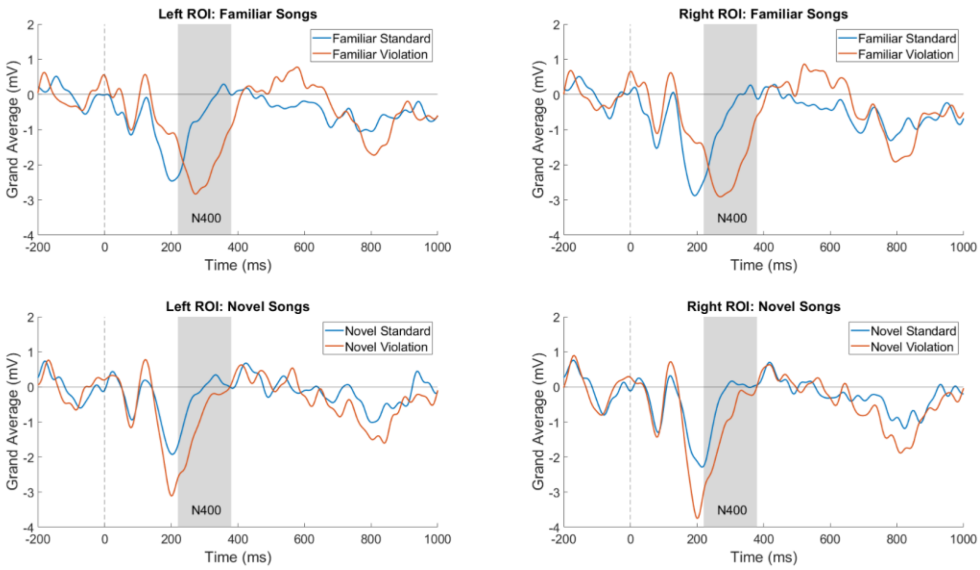
EEG Pre-processing

Data was pre-processed in MATLAB on a PC computer using the EEGLAB library (Delorme & Makeig, 2004). The reference electrode was re-assigned to the CZ electrode. Electrodes P9 and P10 were removed for all data sets, as they consistently had high impedance during testing. Data was resampled to 256 Hz and bandpass filtered, 1-30 Hz. Additional channels were removed if impedance exceeded 50 kOhm. Epochs were first created as time periods of -0.5 seconds before target note onset to 1.5 seconds after the target note onset. Trials with large, paroxysmal artifacts were manually removed for each participants' data sets before running ICA. Due to noisy and unsalvageable data one participant was entirely removed before ICA. ICLabel was used to flag components with a probability of more than 90% of representing eye, muscle, or channel noise (Pion-Tonachini et al., 2019). Additional components were removed if manual checks afterwards indicated that further data cleaning was necessary. Further Epochs were removed to clean the data from remaining artifacts. On average, 38 familiar standard note epochs (M = 38.2, SD = 1.7), 37 novel standard note epochs (M = 37.4, SD = 2.9), 38 familiar violation note epochs (M = 38, SD = 2.2), and 37 novel violation note epochs (M = 37.9, SD = 2.4) remained. After epoch removal, all of the previously removed electrodes were interpolated. Epochs were further shortened by trimming down the time period to -0,2 seconds before and 1,0 second after the target note onset.

After baselining, the average amplitude was computed for the N400's predefined time window and region of interest (ROI) for each participant. The time window for the N400 was defined with 220-380 ms and was based on Miranda and Ullman's results for the posterior negativity (Miranda & Ullman, 2007). Posterior right and left lateralized ROIs were defined and modelled as closely as possible to reflect the ROIs used by Miranda and Ullman. For analysis of the right hemisphere a 5-electrode ROI consisting of P2, P4, P6, PO4 and PO8 was used. For analysis of the left hemisphere a 5-electrode ROI consisting of P5, P3, P1, PO7 and PO3 was used. Separate averages for each ROI were computed for standard and violation notes in familiar and novel melodies, resulting in four averages per participant. Figure 1 shows the grand averages. Additionally, we calculated difference wave amplitudes in the N400's time window (violation - standard), averaged over both hemispheres, separately for the familiar and novel melodies, for each participant.

Figure 1

Grand average amplitudes



Note. Grand average amplitudes of 39 participants split into left ROI and right ROI for familiar melodies (top row) and novel melodies (bottom row); the shaded area labelled "N400" indicates the time window of 220-380 ms used by Miranda and Ullman (2007)

Statistical Analysis

Repeated measures ANOVA was performed for the posterior ROIs using JASP (Version 0.19.1.0) (JASP Team, 2025). The ANOVA included three factors: familiarity (2 levels: familiar vs. novel), event type (2 levels: standard vs. violation) and hemisphere (2 levels: left vs. right). Post hoc tests were conducted with Bonferroni correction applied for familiarity vs. event type. Correlation analysis using Pearson's r was performed to check possible correlations between GOLD MSI subscales and the difference wave amplitudes of familiar and novel notes averaged per hemisphere. The threshold for significance used in all analyses was $p \leq 0.05$. P-value and confidence intervals were adjusted for comparing a family of six estimates in the post-hoc test using a Bonferroni adjusted $\alpha = .008$.

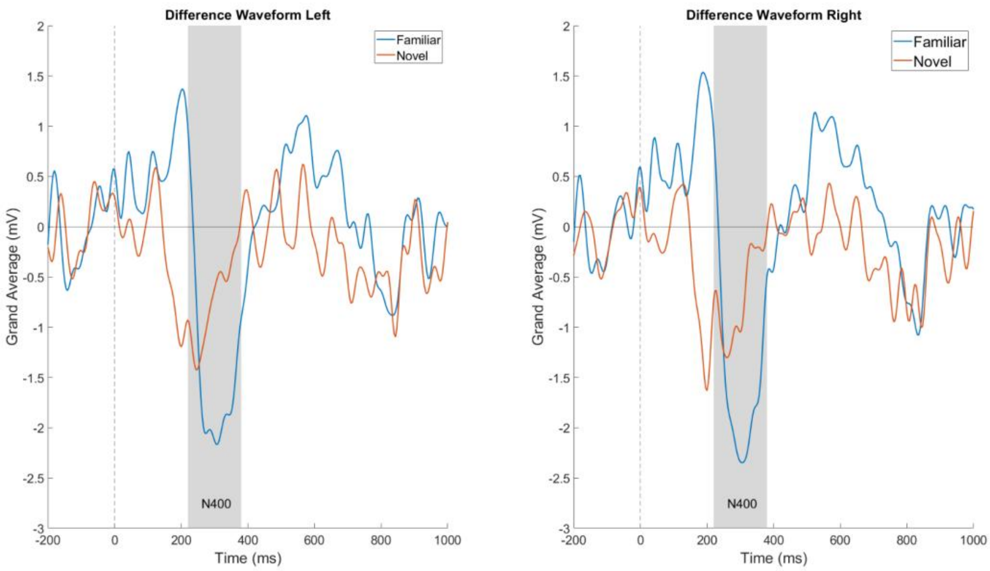
Results

ANOVA and post-hoc tests

The grand average data of 39 participants (Age: *M* = 31.7 years, *SD* = 15.5) shows a possible N400 effect for violation notes matching Miranda and Ullman's time window of 220-380 ms in familiar songs. The N400 was also present in novel stimuli, although being smaller in amplitude compared to the N400 of the familiar songs. No hemispheric differences were observed.

Figure 2

Difference wave amplitudes



Note. Difference wave amplitudes (calculated as violation - standard waveforms) are split per hemisphere; the shaded area labelled "N400" indicates the time window of 220-380 ms used by Miranda and Ullman (2007)

Accordingly, repeated-measures ANOVA on the N400 with the factors familiarity (familiar song vs. novel song), event type (standard note vs. violation note) and hemisphere (left ROI vs. right ROI) revealed significant effects on each factor (familiarity: $F(1,38) = 11.189$, $p = .002$; event type: $F(1,38) = 30.972$, $p = <.001$; hemisphere: $F(1,38) = 5.565$, $p = .024$). Significant interactions were found only for within-subject factors of familiarity and event type, $F(1,38) = 4.452$, $p = .041$, such that significant N400s followed violations in familiar and novel melodies, albeit larger effects were found in familiar melodies.

Further post hoc t-test comparisons using Bonferroni correction for familiarity and event type confirmed statistical significance between familiar standard and familiar violation notes $p = < .001$, novel standard and familiar violation notes $p = < .001$, novel standard and novel violation notes $p = .001$, as well as familiar violation and novel violation notes $p = .016$. The pairs of familiar standard and novel standard $p = .481$, as well as familiar standard and novel violation $p = .626$, showed no statistical significance after post hoc comparisons.

The ANOVA results for familiarity and hemisphere, $F(1,38) = 1.632$, $p = .209$, as well as event type and hemisphere, $F(1,38) = 0.003$, $p = .955$, showed no significant interactions, suggesting that the effect of familiarity on brain activity did not vary based on hemisphere. No significant interaction was found for all three factors – familiarity, event type and hemisphere, $F(1,38) = 0.192$, $p = .664$.

Gold MSI Correlations

Follow up analyses were conducted on the difference wave amplitudes for violation versus standard notes, averaged over the two hemispheres (given the absence of interaction effects involving hemisphere), for familiar and novel songs separately. The familiar difference wave amplitude averaged over both hemispheres correlated significantly with participants' values for the GOLD MSI’s general musical sophistication as well as with subscale values of perceptual abilities, musical training, and singing abilities. There were no significant correlations between the novel difference wave amplitude averaged over both hemispheres with any of the GOLD MSI subscale values (see Table 3).

Table 3

Correlations between difference wave amplitudes and the GOLD MSI inventory

	Familiar difference wave average	Novel difference wave average
Active Engagement	0.216	-0.067
Perceptual Abilities	0.591***	0.196
Musical Training	0.535***	-0.03
Emotions	0.195	0.126
Singing Abilities	0.492**	0.091
General Sophistication	0.502**	0.02

Note. Significance is indicated as follows: *p < .05, ** p < .01, *** p < 0.001

Summary and discussion

Our main factors revealed significant effects of each main factor (familiarity, event type and hemisphere). We also found a significant interaction of familiarity and event type, such that N400 amplitudes were largest in familiar songs with violations. This indicates that it is possible to measure an EEG marker that is sensitive to semantic memory of music using a 20 minute long paradigm. Unexpectedly we also found smaller N400s in our novel songs, albeit with smaller amplitudes compared to the N400s found in the familiar songs with violations. These results hint at the possibility of short-term memory learning effects based on repetition of phrases we used in our newly composed stimuli. Although hemispheres initially showed a significant interaction, no hemispheric differences were observed upon further inspection. Correlations between GOLD MSI factors and difference wave amplitudes of familiar songs were observed, suggesting that musical sophistication subscales might play a major role when perceiving violations in familiar melodies. We discuss our results in detail in the next sections.

Novel melodies and learning effects

Familiar violation N400s were significantly larger than novel violation N400s. Our results are consistent with previous findings (Calma-Roddin & Drury, 2020; Miranda & Ullman, 2007), as our stimuli succeeded in eliciting the N400 in familiar songs. In contrast to their findings, our paradigm resulted in additional N400s in novel melodies. Learning effects in short term memory paradigms generally seem to be strongly intertwined with repetition (Kahana & Wagner, 2024). Our familiar stimuli using children and folk's songs are mainly aimed at a younger audience and can be regarded as having relatively simple and repetitive structure. Compared to more complex music aimed at adults, their simplistic nature makes them still engaging enough, optimizing the relationship between predictability and uncertainty for a rewarding musical experience (Gold et al., 2019; Labendzki et al., 2025). Similarly simplistic and engaging approaches can also be seen when investigating speech and performance aimed at infants, making them prime candidates for teaching the younger generation due to their nature (Eaves Jr. et al., 2016; Español & Shifres, 2015). While we did not specifically aim for our stimuli to incorporate strong learning effects, novel musical pieces closely resembling their familiar counterparts might have led to this effect regardless. This also suggests that even a few repetitions of musical phrases within a song might be sufficient for their retention in short-term memory, making the N400 a strong contender as a marker for semantic memory of music.

Topography Findings

As previously discussed, the N400s topography is typically recorded in posterior electrodes, though the ERP is sometimes also recorded in central region electrodes, depending on the involved memory domains and stimuli presented (Kutas & Federmeier, 2011; Lau et al., 2008; van Petten & Luka, 2006). Regarding studies with a music focused design, the ERP was mainly recorded in posterior regions with an even hemispheric distribution (Calma-Roddin & Drury, 2020; Miranda & Ullman, 2007). To these results we add our own findings of the N400 being present in both familiar and novel violation conditions in posterior ROIs. No hemispheric differences in posterior ROIs were observed when comparing the difference wave amplitudes per hemisphere, suggesting an even spread of the music based N400.

Musicality and the N400

In terms of our sample's musicality, three of the five GOLD MSI's subscale scores – namely perceptual abilities, musical training and singing abilities – showed significant correlations with our stimuli. Considering the fact that our sample in total ranked slightly below the 50th percentile in terms of musical sophistication (Schaal et al., 2014), our paradigm seems to be suitable for eliciting the N400 in the average population. However, these correlations between difference wave amplitudes and musical sophistication were only observed in familiar songs, leading to more pronounced measurable brain potentials. There is a possibility that we gain semantic knowledge about musical rules independent of musical education, interest or emotional attachment, based on implicit memory acquisition and intuition (Bigand & Poulin-Charronnat, 2006; Koelsch et al., 2000; Rohrmeier & Rebuschat, 2012). However, our results suggest that access to this semantic knowledge might be easiest for people with high musical training and/or musical abilities, as the purposefully placed mistakes within a familiar song could have been easier to detect for participants with more extensive music education, leading to their stronger conceptual activation (Lörch et al., 2023; Yee et al., 2018).

Future Directions

Our results could prove to be useful for further usage with special populations. As AD patients suffer mainly from loss in short term-memory domains, further analysis will be conducted in a follow-up study, using a singing-based, individually administered intervention (Lichtensztejn et al., 2024). Judging based on previous results, AD patients still retain musical memory and are capable of learning new songs (Lichtensztejn, 2022; Matziorinis & Koelsch, 2022; Samson et al., 2009). Based on our results, which show that the average musicality level does not appear to be a defining factor when eliciting the N400, there seems to be a strong case for the feasibility of our approach with the general population. Due to the paradigms' short runtime, our approach also provides the added benefit of not putting too much of a time burden on patients when conducting future research.

The importance of lyrics might also need further investigation. Our novel stimuli, as well the ones used in the two studies we modeled our paradigm after (Calma-Roddin & Drury, 2020; Miranda & Ullman, 2007), are based on familiar songs associated with lyrics. Arguments for both the independence of melody (Besson et al., 1998), their tight link with lyrics (Peretz et al., 2004), or a middle ground for varying degrees of integration between both can be made (Sammler et al., 2010). As pointed out by Chien and Chan (2015), studies investigating song perception are split on whether processing of melody and lyrics occurs independently versus interactively. Furthermore, independent processing often deviates from a normal everyday listening experience due to the way lyrics are presented or due to studies having their participants sometimes intentionally attend to the meaning of lyrics (Chien & Chan, 2015). To fully understand the musical N400, designing a study either using familiar stimuli without any associated lyrics whatsoever, or a long-term study teaching participants new songs without lyrics - to the point of being fully familiarized with them - might be needed.

Following up on the importance of lyricism, learning effects for novel songs could be investigated by assessing whether positive or negative associations arise in the presence or absence of lyrics. On one side there have been accounts showing that learning materials through lyrics is a viable way of memorizing (Dickson & Grant, 2003; McCullough & Pepper, 2023), resulting in a deeper semantic understanding and association when lyrics are accompanied by images (Napadow & Harmat, 2024). On the other hand, music containing lyrics compared to purely instrumental music seem to either lead to neutral results (Kämpfe et al., 2011) or hinder cognitive learning if the music is presented in a background context (Souza & Barbosa, 2023), which might hint to the role of attention and divided focus. Within the context of our study design, comparing two samples – both groups being exposed to newly composed songs, with one of them listening to songs with lyrics and the second one to songs without lyrics – might be required to assess the viability of potential learning effects in newly taught songs.

Conclusion

In this study we investigated the semantic memory of music using a 20-minute paradigm and posed the question whether a paradigm that is shorter than the ones used in previous studies would suffice in eliciting the N400. Not only was the N400 present in familiar stimuli but also in novel stimuli, although being smaller in amplitude. Our results revealed significant effects of each main factor (familiarity, event type and hemisphere) with significant interactions between familiarity and event type. We observed no hemispheric differences upon further investigation of difference wave amplitudes. Furthermore, correlations between GOLD MSI factors and difference wave amplitudes of familiar songs were observed. Our results suggest that 20 minutes are sufficient for establishing N400s, demonstrating the utility of this shortened paradigm for assessing semantic memory for music. This makes our approach feasible for shortening study designs and provides usability for special populations such as patients suffering from AD. Potential follow-up studies investigating the role of lyrics and learning effects in novel songs could pose to be fruitful endeavours.

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