Harmonic amplitude summation for frequency-tagging analysis

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Abstract

In the approach of frequency tagging, stimuli that are presented periodically generate periodic responses of the brain. Following a transformation into the frequency domain, the brain’s response is evident often at the frequency of stimulation, \( F \), and its higher harmonics (\( 2F \), \( 3F \), etc.). This approach is increasingly used in neuroscience, as it affords objective measures to characterize brain function. However, whether these specific harmonic frequency responses should be combined for analysis, and if so, how, remains an outstanding issue. In most studies, higher harmonic responses have not been described or were described only individually; in other studies, harmonics have been combined with various approaches, e.g., averaging and root mean squared summation. A rationale for these approaches in the context of frequency-based analysis principles, and understanding of how they relate to the brain’s response amplitudes in the time domain, has been missing. Here, with these elements addressed, the summation of (baseline-corrected) harmonic amplitude is recommended.
1. Introduction

1.1 Frequency-tagging

It has long been known that a stimulus presented at a periodic rate elicits a response from an observer’s brain at exactly that rate. For example, a light flickering on and off at a periodic rate, 14 times a second, elicits a measurable response in the electroencephalogram (EEG) of a human observer 14 times a second (Adrian & Mathews, 1934). In the time domain, a response is evident as periodic changes in the brain’s response amplitude across time. Following Fourier transformation into a frequency domain representation (Fourier, 1822; Danielson & Lanczos, 1942), the response is evident as a high amplitude “peak” at exactly the fundamental stimulus presentation rate (frequency = F), and/or its higher harmonics, i.e., at frequencies that are integer multiples of F (2F, 3F, etc.; Regan, 1966; 1989).

The approach of presenting stimuli and analyzing neural responses at the frequency of stimulation is referred to by many names: “frequency tagging” (Tononi et al., 1998; Srinivasan et al., 1999) is the one that will be used here. Other names for this approach differ mainly on their point of reference: to the responses that appear consistently periodic to stimuli presented at high rates, i.e., “steady-state” responses, e.g., “steady state visual-evoked potentials” (SSVEPs; Regan, 1966; 1989; Di Russo et al., 2002; Heinrich, 2010; Norcia et al., 2015) and “auditory steady state potentials/responses” (ASSRs; Geisler, 1960; Watkin, 2008) or “travelling wave” responses (Engel, Glover & Wandell, 1997); to the stimulation mode itself (“fast periodic visual stimulation” (FPVS); Rossion, 2014; Rossion, Retter & Liu-Shuang, 2020); or the analysis occurring in the frequency domain (“Fourier analysis/synthesis”; Movshon, Thompson & Tolhurst, 1978; Bach & Meigen, 1999; Zhou, 2016; or simply “frequency(-domain) analysis”, e.g., as in McKeefry et al., 1996). Despite the varying terminologies, the principles of the approach are the same. In a similar vein, various types of stimulation modalities (visual, auditory, somatosensory, cross-modal) and recording methods (electroencephalogram, electroretinogram, functional magnetic resonance imaging, single cell recordings, etc.) may be applied with various participant groups (human adults, infants, non-human primates, rodents, insects, etc.), resulting in some practical differences, but the same fundamentals, of the approach.

In human cognitive neuroscience research, the frequency-tagging approach is associated with undeniable advantages. As noted early on, this approach is well-suited for specifically relating brain processes to external events (“This gives a method of tracing the visual messages...”)

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in the brain, for by means of the flicker rhythm they can be made easy to recognize,” Adrian, 1944, p. 361). More recently, its objectivity and sensitivity (i.e., high signal-to-noise ratio, SNR) have been highlighted, and the use of the paradigm is undoubtedly on the rise, having been extended from the study of basic sensory processes, and their modulation by spatial/selective attention, to the direct measurement of higher levels of cognition in recent years (Norcia et al., 2015, for review). However, frequency tagging is still fundamentally limited by outstanding conceptual and methodological ambiguities in dealing with responses occurring across harmonics.

1.2 Higher harmonics

Another way of describing frequency tagging is the following: given a periodic stimulus, responses of the brain periodic to that stimulus are investigated. In this formulation, it is evident that the brain’s responses may occur at the rate of stimulation, \( F \), but also at the other rates periodic to the stimulation: the higher harmonics (\( 2F \), \( 3F \), etc.). For example, a stimulus modulated 8 times a second, at 8 Hz, may generate responses that are evident as amplitude peaks in the frequency domain representation of the brain recording at 8 Hz (\( F \), the first harmonic corresponding to the fundamental frequency), but also at 16 Hz (\( 2F \), the second harmonic), and 24 Hz (\( 3F \), the third harmonic)\(^1\). Since only responses at higher harmonics are periodic to the fundamental frequency, it is uniquely at the higher harmonics, rather than a diffuse band, that higher frequency constituents of brain responses are present.

While responses of the brain are not always generated at the higher harmonics, they often do occur (Regan, 1966; Bach & Meigen, 1999; Heinrich, 2010; Vialatte, 2010; Rossion, 2014; Norcia et al., 2015; Zhou et al., 2016; Rossion, Retter & Liu-Shuang, 2020). Note that responses are not always generated at \( F \), either; for a classic example, in the case of alternating symmetrical stimulus inputs (e.g., pattern-reversing checkerboards), the brain responds only at \( 2F \) and higher even harmonics (Cobb, Morton & Ettlinger, 1967; reviewed in Norcia et al., 2015; for different examples: Movshon, Thompson & Tolhurst, 1978; Heinrich, 2010; Zhou, 2016). Further, note that throughout this manuscript, only harmonics that are specific to their fundamental frequency are addressed, which is always the case when a single stimulus presentation frequency is tagged.

\(^1\) A note on nomenclature: here, the “first” harmonic is the fundamental stimulation frequency. In another existent convention, the “first” harmonic is the double of the fundamental stimulation frequency.
(but for an extension to other cases, with more complex stimulation paradigms, see Section 4.1.2).

At present, while higher harmonic responses are an integral part of brain responses, they are not systematically addressed in frequency-tagging research. In many studies, higher harmonic responses are not even reported (e.g., Regan & Regan, 1988; Peterzell & Norcia, 1997; Muller, Teder & Hillyard, 1997; Tononi et al., 1998; Heinrich & Bach, 2001; Chen et al., 2003; Braddick et al., 2005; Müller et al., 2006; Wattam-Bell et al., 2010; Mouraux et al. 2011; Kus et al., 213; Coia et al., 2014; Paulk et al., 2015; Min et al., 2016; Bekhtereva et al., 2018; or are extirpated by narrow (band-pass, Gabor, etc.) filtering: e.g., Anderson & Muller, 2010; Miskociv & Keil, 2013; Davidson et al., 2020). Does it matter? Yes, at least when there is considerable amplitude at the higher harmonics, relative to $F$. In this case, higher harmonics do contribute significantly to the response measurement: for a dramatic illustration to this effect, see Fig. 1.

![Figure 1](image.png)

**Figure 1.** A demonstration of the importance of considering higher harmonics in frequency-tagged response analyses. A) Two synthetic periodic signals, each comprised of five harmonic frequencies. B) In the frequency domain, a consideration of only one harmonic (at the fundamental frequency; i.e., ignoring the four higher harmonics), describes signal 2 as larger than signal 1. This description is not in agreement with typical time domain response analyses, e.g., peak amplitudes, to compare these signals. Note that this figure will be revisited (expanded) in Section 5.

How often is there considerable amplitude at the higher harmonics? Can the cost of omitting higher harmonics in published studies be evaluated? Unfortunately, most studies do not report whether or not there were responses at higher harmonics, as mentioned above. Moreover, when higher harmonic responses were reported to be present, they were often not described (e.g., “Peaks were also present at the harmonics of the stimulus frequency but were not analyzed in this study”, Srinivasan et al, 1999, p.5438; “Note however that 2 Hz is a harmonic of 1 Hz and may actually be a relevant spectral region to consider (albeit outside the scope of this report)”,

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Kosem, Gramfort & van Wassenhove, 2014, Fig. S2; evident in figures but not discussed or included in analyses: Kaspar et al., 2001; Pastor et al., 2003; Honnegger et al., 2011; de Heering & Rossion, 2015; Eidelman-Rothman et al., 2019; Schettino et al., 2020), preventing a wide-scale review.

From some studies that have described the presence or absence of higher harmonics, there appear to be a couple, specific cases in which their amplitude was not considerable, and may be neglected with little cost. The most well-documented case is that in which high stimulus presentation rates are used (as will be demonstrated in Section 3.1; Van der Tweel & Verduyn Lunel, 1965; Regan, 1989; Luck, 2005; Vialatte et al., 2009; Capilla et al., 2011; Alonso-Prieto et al., 2013; Heinrich, Groten & Bach, 2015; Retter & Rossion, 2016; Retter, Webster & Jiang, 2019). However, the case of high stimulus presentation rates cannot be readily identified across studies using different recording methods to measure different brain processes in different populations. This is because “high” is dependent on the relationship of the stimulus presentation rate to the duration of the brain responses being measured (Keysers & Perrett, 2002; Retter et al., 2020; see also Heinrich, 2010). A second case is that in which low-amplitude first harmonic responses were reported, such as responses elicited with subtle stimuli (e.g., with no higher harmonic amplitude above noise: Brazier, 1964; Retter & Rossion, 2017; Park, 2018; Lochy, Schiltz & Rossion, 2020; with very low higher harmonic amplitudes: Ales et al., 2012; Moungou, Thonnard & Mouraux, 2016, McFadden et al., 2014). However, this case also does not allow general inferences: low first harmonic amplitudes may still coincide with large higher harmonic amplitudes (see Section 4; also, e.g., Vialatte et al., 2009; Capilla et al., 2011; Alonso-Prieto et al., 2013; Gaume, Vialatte & Dreyfus, 2014), such that the amplitude of the first harmonic itself is not diagnostic.

On the other hand, considerable amplitude at higher harmonics has been reported in a wide array of studies. For example, higher harmonics often exceed the fundamental in studies on the brain’s responses for an extensive range of processes (with $F$ usually below 8 Hz, recorded with EEG): from luminance (patterns: Davilda, Srebo & Ghaleb, 1998; Vialatte et al., 2009; Capilla et al., 2011; Gaume, Vialatte & Dreyfus, 2014), to color and motion (Tyler & Kaitz, 1977; McKeefrey et al., 1999), to face perception (Alonso-Prieto et al., 2013). Higher harmonics may also be present, each with a lower amplitude than the fundamental, but with their amplitude distributed across a large range of harmonic frequencies (e.g., Vialatte et al., 2009; Capilla et al.,
Considerable higher harmonics have also been demonstrated with low-temporal resolution techniques, including fMRI, given appropriately slow stimulus presentation frequencies (e.g., with $F$ well below 0.1 Hz; motor activity: Bandettini et al., 1993; luminance patterns: Engel, Glover & Wandell, 1997).

In some cases, the conclusions of studies considering and not considering higher harmonics can be compared: e.g., without considering higher harmonics, maximal visual responses were reported to stimuli modulated at about 10-15 Hz with EEG (e.g., Regan, 1966; Pastor et al., 2003; Ding, Sperling & Srinivasan, 2006). However, when higher harmonics were considered, the lowest stimulation frequency tested (3 Hz) yielded the maximal visual EEG responses, being over three times higher than the responses to 12 Hz stimulation with natural images (Retter et al., 2020: Fig. S3). Overall, while it is thus impossible to ascertain what the impact of unreported, or uncharacterized, higher harmonic responses in most studies may have been, it is likely that it was often considerable.

1.3 Should higher harmonic responses be combined, and if so, how?

At present, harmonics are surrounded by many questions: why do they occur? What do they represent? Which, or how many, harmonics should be considered? Should they be taken into account for response identification and measurement, and if so, how? Indeed, the lack of understanding and standard practice regarding higher harmonics has limited the ease (i.e., objectivity) of frequency-tagged response identification and measurement. This is particularly significant because objectivity is given as a primary advantage of the frequency tagging technique, contributing to its increasing application in (cognitive neuroscience) research and clinical applications (e.g., see Norcia et al., 2015; Rossion, Retter & Liu-Shuang, 2020).

In previous studies that reported harmonic responses, most often these responses have been described individually, e.g., at $F$, $2F$, $3F$, etc., and have not been taken into account for response measurement (e.g., Tyler & Kaitz, 1977; Bandettini et al., 1993; Srinivasan et al., 1999; Herrmann, 2001; Vialatte et al., 2009; Capilla et al., 2011; Rossion & Boremanse, 2011; Ales et al., 2012; Alonso-Prieto et al., 2013; Painter et al., 2014; Moungou, Thonnard & Mouraux, 2016; Cunningham, Baker & Pierce, 2017). While considering harmonic responses separately is considerably better than not at all, individual harmonic responses do not represent independent or
temporally separated aspects of a time-domain response (e.g., Tang & Norcia, 1995; for dependent harmonic amplitude examples: Retter & Rossion, 2016; Zhou et al., 2016; for qualitatively similar neighboring harmonic examples: Rossion, 2014; Jacques, Retter & Rossion, 2016; Rossion, Retter & Liu-Shuang, 2020; Zemon & Gordon, 2018; see Section 5).

In practice, considering higher harmonic responses improves response detection, measurement and classification (e.g., Davilda, Srebo & Ghaleb, 1998; Retter & Rossion, 2016; Zemon & Gordon, 2018; for brain-computer interfaces: Muller-Putz et al., 2005; Chen et al., 2015; Cetin, Ozekes & Varol, 2020). Combining harmonics is particularly useful for comparing response amplitudes across experimental conditions. Otherwise, if one input produces a response with inconsistently larger harmonic amplitudes than another input, how could these responses be evaluated overall? Or, how could the relative change (e.g., percent increase) of one response relative to another be calculated overall? In few previous studies, harmonic responses have been combined with various approaches, such as root mean squared summation (e.g., Hou et al., 2003; Appelbaum et al., 2006; 2010), a (weighted) sum of powers (e.g., Wang et al., 2008; Zhang et al., 2011), or averaging (e.g., Liu-Shuang et al., 2014; Lochy, Van Belle & Rossion, 2015). However, these approaches have not been justified, and have not been related to approaches analyzing the brain’s response amplitudes in the time domain (or physiologically: see Heinrich, 2010).

In the following, a validated methodology for combining (baseline-corrected) harmonic amplitudes through simple summation will be provided. This approach derives from a theoretical bases of how signals over time are represented through mathematical transformations into the frequency domain (Section 2), extended to experimental responses in practice (Section 3). This approach was indicated empirically by Retter and Rossion (2016), and it has since been applied in a number of studies, however, primarily by those authors or associated research groups (e.g., Xu et al., 2017; Beck, Rossion & Samson, 2018; De Keyser et al., 2018; Leleu et al., 2018; Chemin et al., 2018; Guillaume et al., 2018; Gwinn et al., 2018; Gwinn & Jiang, 2019; Dwyer, Xu & Tanaka, 2019; Damon et al., 2020; Fisher et al., 2020). To be of further use to the scientific community, the approach requires deeper methodological evaluation and, especially, evaluation in a theoretical context, which is the goal throughout the present manuscript. From this, some practical guidelines are offered (Section 4) and implications are drawn for the interpretation of harmonic responses more generally (Section 5).
2. Frequency-domain representations

2.1 Sine waves

When a signal is transformed into the frequency domain (by means of a Fourier transform), it becomes represented through a combination of sine waves, which are the fundamental units of the frequency domain. While there are many texts on the mathematics of frequency transformations and representations (e.g., Press, Falnery & Teukolsky, 1993; Smith, 1997; Strang, 2007; Patel, 2012; Forinash & Christian, 2016; Gonzalez & Woods, 2018), a basic understanding of sine waves and their combination is a sufficient foundation for the interpretation of multi-harmonic responses of the brain (Regan, 1989).

Briefly, sine waves are trigonometric functions that describe periodic signals in terms of frequency, amplitude, and phase (Fig. 2). The frequency of a sine wave describes the number of cycles (of 360°; equivalent to $2\pi$ radians) per unit of time or space (time is typically given in units of cycles/sec = sec$^{-1}$ = Hertz = Hz). Note that the cycles of sine waves are periodic, and could repeat their pattern infinitely, like endlessly tracing a circle (the sine wave, as in Fig. 2B, derives from the y-axis values of a unit circle, as shown in Fig. 2A). The amplitude of a sine wave spanning from -1 to 1 in y-axis units (e.g., in EEG recordings, the unit is typically microvolts = µV), as in Fig. 2B, has an amplitude of 1. The phase of a sine wave is a measure of its starting angle (indicated by theta in Fig. 2A), with an arbitrary beginning at zero (in units of degrees (deg; °) or radians (rad)), as is shown in Fig. 2B. Changes in frequency, amplitude, and phase, that help demonstrate these properties, are illustrated in Fig. 2C. For those whom it helps to see it mathematically, the expression of a sine wave, as a function of $x$, is: $y(x) = a \sin(2\pi fx + \phi)$, where $a =$ amplitude (scaling on the y-axis); $f =$ frequency (by cycles); and $\phi =$ phase (x-axis shifts).
2.2. A lot of sine waves

A frequency-domain representation of a signal is essentially a lot of sine waves. That is, when a signal is transformed into the frequency domain, the resultant x-axis describes the frequency of its constituent sine waves. The other descriptors of sine waves, amplitude and phase, are described in the transformed, complex-valued y-axis at each frequency, which is typically plotted as separate amplitude and/or phase frequency spectra.\(^2\) The resolution (x-axis sampling) of the frequency-domain spectrum is the inverse of the signal recording length, and the range spans from 0 to half of the signal sampling rate (note that these properties have practical implications for frequency tagging experimental design, e.g., as addressed in Bach & Meigen, 1999). The combination, through summation, of these sine waves described in the frequency domain reconstructs the original signal in the time domain. Here, the focus will be on periodic signals over time, but note that frequency-domain analyses can be applied in many settings, e.g., signals over space or over two dimensions.

\(^2\) Note that in frequency-domain transformations, technically a complex-valued combination of sine waves and cosine waves is used to represent the signal. In many discrete, fast Fourier transforms, the sine component carries only the amplitude information and the cosine component carries only the phase information. Here, “sine waves” are referred to as complex entities themselves, combining both amplitude and phase information. Additionally, note that phase spectra are rarely plotted in the same format as amplitude spectra, because: 1) their values are circular, e.g., with equivalent distance from 0-359° and 0-1°; see an alternative plotting example in Fig. 6D; and 2) the phase at non-frequency-tagged signal frequencies is random, i.e., full-range noise.
In the simplest case, a periodic signal that is a perfect sine wave is represented in the frequency domain by a single frequency, representing a single sine wave (Fig. 3A). Another way to understand this is to observe that in this case the frequency, amplitude, and phase of a single sine wave in the frequency domain are sufficient to reconstruct the original signal in the time domain. In most cases, signals are more complex (i.e., non-sinusoidal), but this does not pose a problem: a combination of sine waves at different frequencies can sum to model any signal. In a classic example, a periodic squarewave signal is shown to be represented with a sum of sine waves specific to its periodicity (Fig. 3B). Non-periodic signals, e.g., event-related potentials (ERPs) to temporally jittered stimuli can also be represented in the frequency domain, but since they are not specific to limited frequencies, their interpretation does not correspond to that of frequency-tagged signals (Fig. 3C). Although frequency-based analyses of non-periodic signals may be applied (e.g., see Regan, 1989; Basar & Shurmann, 1994; Herrmann et al., 2014; see also Chemin et al., 2018), these are outside the present focus on frequency tagging.

It may be observed that a simple sine wave ranging from -1 to 1 in the time domain has an amplitude of 1 in the frequency domain, but that the relationship between the time-domain and frequency-domain amplitudes for multi-harmonic signals is more complex (compare Figs. 3A and 3B). However, there is a direct relationship between these dimensions: because energy is conserved from the time to frequency domain, the sum of the squared amplitudes of the time-domain signal equals the sum of the squared root mean amplitudes of the frequency-domain signal (Parseval’s relation; Parseval des Chênes, 1806; e.g., see Smith, 1997)\(^3\). For example, the sum of the squared amplitudes per cycle of the time-domain signal in Fig. 3A is equal to 0.5, and the sum of the squared root mean amplitudes of its discrete frequency-domain signal is equal to 0.5. Multi-harmonic signals also preserve this relationship, although their time-domain amplitude range does not directly relate to their frequency-domain amplitude (being affected by phase; see Section 4.1.1).

\(^3\) In the time domain, energy is equal to power over time, which is equal to the sum of the squared amplitudes. In the frequency domain, energy is equal to the sum of the squared root mean amplitudes (root mean amplitude = amplitude/\(\sqrt{2}\)).
Figure 3. Lots of sine waves build frequency-domain representations of signals. **Upper row:** time-domain signals. **Lower row:** these signals transformed into the frequency domain. **A)** A periodic sine wave is represented with a single frequency in the frequency domain. **B)** A periodic squarewave (thick, black line) is represented with a combination of many, specific harmonic frequencies (lines colored correspondingly across top and bottom panels). Literally, the sum of these (and higher, not illustrated) colored lines’ amplitude at each time point reconstructs the original signal. **C)** A non-periodic, event-related potential (ERP) signal is represented with a combination of many nonspecific frequencies. Note several properties of the frequency-domain signal: 1) the 0 frequency bin reflects the mean amplitude (DC offset) of the signal; 2) the x-axis resolution is the inverse of the signal recording duration; 3) although the frequency domain is plotted only until 10 Hz here, its range spans further (up to half of the signal sampling rate); and 4) although only the phase of tagged frequencies are indicated on the lower row here, each frequency in the spectrum has a corresponding phase value.

3. Frequency-tagged responses in the frequency domain

3.1 One harmonic; a lot of harmonics

Frequency tagging is an approach in which stimuli are presented *periodically* in order to generate periodic responses of the brain, that can thus be identified in the frequency domain at specific frequencies harmonic to the stimulation, i.e., the fundamental and the higher harmonic frequencies. In the following, examples will be taken only for harmonics that are specific to a
single tagged frequency: again, for determining specific harmonics in the context of multiple
tagged frequencies, please see Section 4.1.2.

According to the principles of frequency analyses, a simple, sinusoidal brain response
would be represented only at the fundamental frequency $F$, while more complex brain responses
would be represented with a combination of $F$ and its higher harmonics, $2F$, $3F$, etc. Generally,
this is evidenced with experimental brain responses. In the event that the brain responses are
nearly sinusoidal, the response is dominated by amplitude at $F$ (e.g., at a high stimulus
presentation rate: Fig. 4A); in the event that the responses of the brain are complex, a
combination of sine waves at different frequencies (i.e., the higher harmonics) can sum to model
any signal (Fig. 4C). In many studies, complex, non-sinusoidal responses of the brain evoked
over time are represented in the frequency-domain not only at $F$, but with considerable amplitude
at its higher harmonics (as addressed in the Introduction, e.g., Brazier, 1964; Van der Tweel &
Verduyn Lunel, 1965; Regan, 1966; 1989; Donker, 1975; Bach & Meigen, 1999; Vialatte et al.,
2009; Heinrich, 2010; Vialatte, 2010; Alonso-Prieto et al., 2013; Norcia et al., 2015; Retter &
Rossion, 2016; Zhou et al., 2016; Rossion, Retter & Liu-Shuang, 2020).

Figure 4. The higher the stimulus presentation rate ($F$), generally the lower the amplitude
of higher harmonic responses, $2F$, $3F$, etc., relative to the fundamental, $F$. Upper row: the example
brain responses, recorded with EEG (channel POO6 displayed here), were elicited from periodic
visual stimulation of natural object images at various presentation frequencies (thick black lines;
data from Retter et al., 2020). Harmonic sine waves from the frequency-domain analysis, as
represented below, are superimposed in color, to illustrate their relationship with the original signal. **Lower row:** Frequency-domain representations of these signals. The amplitude of harmonic responses above 5% of that of the fundamental are plotted in color, corresponding with the upper row. A) 12 Hz stimulation elicits nearly sinusoidal brain responses. B) Intermediate, 6 Hz stimulation. C) 3 Hz stimulation elicits more complex brain responses in the time domain, represented with frequency-domain amplitude more distributed across higher harmonics.

Indeed, higher harmonic responses may be accounted for in relation to the complex (i.e., non-sinusoidal) responses of the brain, in accordance with the principles of frequency-domain analysis of periodic signals (as in Regan, 1989; Heinrich, 2010; Zhou et al., 2016; Norcia et al., 2015; Rossion, Retter & Liu-Shuang, 2020). This account explains that higher harmonics are present when complex brain responses are present, but does not implicate a specific source of complex brain responses (see Heinrich, 2010). However, it is important to note that complex brain responses are not a product of frequency tagging, and may equivalently occur with non-periodic (event-related) stimulus-presentation modes⁴. The harmonics do not represent new information, specific to the frequency-domain: they are merely highlighted in an alternative, frequency-domain representation of the original time-domain signal (certain variations may be represented more or less clearly in each domain).

### 3.2 Imperfect signals: accounting for baseline noise

In theoretical examples of signal transformation into the frequency domain (as in Section 2), the signal is pure signal. In frequency-tagging, as in all brain recordings, the signal (here, i.e., the responses of the brain at the tagged frequencies), also carries “noise”, a term that refers to both non-event-related brain activity and artifacts (e.g., Regan, 1989; Luck, 2005)⁵. Note that there are many methods for correcting for noise in frequency-tagging research, although a discussion of these is more general than the scope of this manuscript (see instead, e.g., Meigen & Bach, 2004; Appendix 2 of Norcia et al., 2015). In the examples given here, a simple correction for

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⁴ Although largely beyond the scope here, note that it is extensively debated whether frequency-tagged EEG responses reflect the (linear) super-position of event-related potentials (ERPs), or whether they reflect an interaction with endogenous oscillations in the brain (see Donker, 1975; Herrmann, 2001; Makeig et al., 2002; Heinrich, 2010; Capilla et al., 2011; Gruss et al., 2012; Keitel, Quigley & Ruhnau, 2014; Heinrich, Groten & Bach, 2015; Norcia et al., 2015; Retter & Rossion, 2016; Zoefel, Oever & Sack, 2018).

⁵ Note that, further, the signal and noise may interact non-linearly (supra-additively), but this modest contribution is negligible when the signal is much greater than noise (Strasburger, 1987; Norcia et al., 1989; Peli, McCormack & Sokol, 1988; see Bach & Meigen, 1999).
noise will be applied that subtracts a local baseline from the amplitude of the frequencies of interest (e.g., Retter & Rossion, 2016). The baseline is defined as the mean amplitude of a range of neighboring frequency bins (for theoretical justification, see Regan, 1989; Norcia et al., 2015; e.g., Peterzell & Norcia, 1997; Boremanse, Norcia & Rossion, 2013; with power rather than amplitude: Srinivasan et al., 1999; Vialatte et al., 2009; Mouraux et al. 2011). This method is used to provide a measure of signal amplitude in the frequency domain that is relatable (i.e., both in the same unit) to amplitude in the time domain, while compensating for local variations of noise inherent to human brain recordings across the frequency spectrum.

4. Combining harmonic responses

4.1 Combining harmonic response amplitude

The combination of sine waves is simple: sine waves sum linearly to reconstruct a signal. However, with the goal of identifying and measuring overall response amplitude in the frequency domain, since sine waves carry both amplitude and phase information, their sum is not intuitive to interpret in terms of amplitude only (or phase only). (Note that there are alternative approaches for combining harmonics that incorporate both amplitude and phase, however these approaches make use of phase as an indicator of reliability (coherence), typically across short stimulation durations (e.g., Jervis et al., 1983; Delorme & Makeig, 2004).) Further, while the amplitude of a single sine wave in the frequency domain directly relates to its time-domain peak amplitude, i.e., half its positive to negative peak range (e.g., Fig. 3A; similarly, see Fig. 4A), the frequency-domain amplitude of complex time domain signals (summed sine waves) is not as easily visualized from the time domain. Perhaps for these reasons, various approaches have been taken for the combination of frequency-tagged multi-harmonic brain response amplitude in the frequency domain (e.g., as mentioned previously, averaging or root mean squared summing, e.g., respectively, Liu-Shuang et al., 2014; Hou et al., 2003).

The summation of harmonic amplitudes is recommended here for identifying and measuring the overall brain response (based on Retter & Rossion, 2016; see also Heinrich, 2009). In the study of Retter & Rossion (2016), this approach was validated empirically, by qualitative comparison of time and frequency domain responses, in the situation where several equivalent time-domain EEG responses were produced by several slow target stimulus presentation frequencies. There, it was observed that despite different distributions of harmonic
amplitudes stemming from the different fundamental target stimulus presentation frequencies (1.1 to 2.5 Hz), the summation of baseline-subtracted harmonic amplitude across a common frequency range led to equivalent overall amplitudes, that related to approximately equivalent response amplitude peaks in the time domain by visual inspection (see Fig. 5A&B, row 1, here, for examples of reprocessing of that data in combination with the underlying harmonic distributions). Moreover, a faster stimulus presentation rate (4.2 Hz), which produced visually lower amplitude deflections in the time domain (Fig. 5C, row 1), also produced a lower summed-harmonic response amplitude.

Here, these data are revisited quantitatively, with typical time-domain interpretations of response amplitudes: peak-to-peak amplitude of the largest deflections, and the area under the curve of the response deflections (Fig. 5, row 4). Note that an exact comparison of specific deflections, as is more commonly done in relating brain responses, is possible for the conditions at 1.1 and 1.4 Hz, but that a different response pattern is observed for the condition at 4.2 Hz, preventing such a direct comparison.

The summed-harmonic response amplitude is shown to be congruent with these measures (Fig. 5D). Critically, other approaches for harmonic combination would not have led to these conclusions when comparing conditions. In the frequency domain here, a large fundamental harmonic amplitude relates to a smaller number of harmonic responses (with an amplitude above 0.1 µV). Therefore, for example, averaging the harmonic responses would have generated lower amplitude responses the slower the stimulus presentation frequency (1.1 Hz < 1.4 Hz < 4.2 Hz; Fig 5D). For another example, the root-mean-squared harmonic amplitude would have generated the highest amplitude for the highest stimulus presentation rate (4.2 Hz; Fig 5D). For a last example, using uncorrected amplitudes would have produced a larger response at 1.1 Hz than 1.4 Hz, since “noise” would have been included at more, and lower-frequency (noisier), harmonics at 1.1 Hz.
Figure 5. The combination of harmonic amplitude. **Row 1:** example brain responses, recorded with EEG (channel PO10 displayed here), were elicited from periodic visual stimulation of natural face (vs. object) images at various frequencies (from Retter & Rossion, 2016). **Row 2:** Frequency-domain representations of these responses. The amplitude of harmonic responses above 0.1 µV are plotted in color, and these harmonic sine waves are superimposed in the corresponding color in Row 1. **Row 3:** The colored harmonic responses above are summed (shown at bin 0), following
a baseline subtraction of “noise”, defined as the average amplitude of the two adjacent frequency bins. **Row 4:** Similar response amplitudes are demonstrated in the time domain in Panels A and B, consistent with Row 3. Time outside of one cycle duration is shadowed in gray, and the response amplitude range is emphasized between the red and blue lines. **A)** 1.1 Hz target (face) stimulation elicits complex brain responses. **B)** 1.4 Hz target stimulation elicits a similar response to 1.1 Hz stimulation in the time domain and the amplitude of summed, baseline-subtracted harmonics in the frequency domain, despite a different distribution of harmonic frequency amplitudes. **C)** 4.2 Hz target stimulation elicits more simple and lower amplitude neural responses in both the time and frequency domains. **D)** Quantification in the time domain (amplitude range) is compared with different methods of harmonic assessment in the frequency domain, with baseline-subtracted amplitudes. The sum of harmonics provides a better correspondence with the time domain, across conditions, than the fundamental harmonic \( F \) only, average of harmonics, or root-mean-square (RMS) of harmonics.

Thus, summing baseline-subtracted harmonic amplitudes is advantageous for a correspondence with interpretations of time-domain brain responses. This approach has been used to quantify and compare overall response amplitude in a number of studies following Retter & Rossion (2016) as mentioned previously (e.g., including time-domain correspondences: De Keyser et al., 2018; Leleu et al., 2018; frequency-domain analyses only: Xu et al., 2017; Beck, Rossion & Samson, 2018; Chemin et al., 2018; Guillame et al., 2018; Gwinn et al., 2018; Gwinn & Jiang, 2019; Dwyer, Xu & Tanaka, 2019; Damon et al., 2020)\(^6\).

If measures other than amplitude are desired, e.g., signal-to-noise ratio, z-scores, or another statistic, the harmonic amplitudes can be extracted with an inclusion of a baseline frequency range (i.e., as a “chunk” of X Hz, centered around each frequency-of-interest), and then summed prior to these baseline-relative computations (Retter & Rossion, 2016; see also appendix 2 of Norcia et al., 2015; Box 2 of Rossion, Retter & Liu-Shuang, 2020). In this way, a single statistical measure can be applied to the combined harmonic amplitude relative to its combined baseline amplitude, i.e., “noise”. Note that different approaches for combining harmonics may serve different ends, in that they describe different aspects of the signal, e.g., the root-mean-squared amplitude relates to the equivalent power of a flat (non-sinusoidal) signal, however, these aspects must be justified in relation to their physiological meaning.

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4.1.1 What about phase?

As addressed in Section 2.2, there is a direct relationship between signal amplitude in the time and frequency domains. As a reminder, this relationship is given by Parseval’s relation, which states that energy is conserved across the time domain (where energy equals the sum of the squared amplitudes) and frequency domain (where energy equals the sum of the squared root mean amplitudes). In light of this, the amplitude across the harmonics relates to the overall amplitude of the signal in the time domain, regardless of phase.

However, in order to fully relate signals across the time and frequency domains, both the amplitude and phase of the representative frequency domain sine waves need to be taken into account. Without phase information, the fluctuation of amplitude across time, e.g., affecting local amplitude peaks, cannot be determined. Therefore, there is a cost towards relating time- and frequency-domain signals when excluding phase information. However, this cost is reasonably minor: as relative phase changes across harmonics, it is possible that the latency of signal peaks varies, but that their amplitude does not (Fig. 6A&B). When relative phase does affect peak amplitudes, this influence is limited (e.g., compare Fig. 6B&C). Moreover, despite relative phase changes, the area under the curve of the time-domain signal may remain approximately constant (Fig. 6A-C; see also Heinrich, 2010).

Moreover, in frequency tagging, it is worth remembering that the phase is not arbitrary: the phase of each relevant harmonic is determined relative to the time domain signal. In other words, the aligning positive and negative peaks of the sine waves across harmonic frequencies correspond to the time of the positive and negative peaks of the signal in the time domain. For example, this leads to phase differences across harmonics that are similar to describe time-domain signals with similar temporal dynamics, despite the use of different stimulation frequencies (Fig. 6D). Thus, the influence of phase on combined harmonics is largely invariant of the stimulus presentation frequency, given consistent temporal dynamics of the response (as hinted at empirically, e.g., Appelbaum, 2006; Retter & Rossion, 2016). Finally, it is worth noting a couple helpful restrictions in the context of frequency-tagging for combining harmonic responses: only one sine wave is represented at each frequency bin, and non-harmonic

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7 In any case, it remains unclear at present how phase could be combined meaningfully across harmonics, beyond time-domain latency, to relate to functional, physiological processes.
frequencies are not considered, such that the response is fully periodic at the cycle duration of the fundamental frequency, $F$.\(^8\)

Figure 6. The influence of harmonic phase on the combination of harmonic amplitude. Synthetic time-domain signals (in thick black lines; Row 1) are the sum of two harmonic sine waves: a 1 Hz sine wave with varying phase (A: 0°; B: 180°; C: 45°; plotted in red) and a 2 Hz sine wave with constant phase (0°; plotted in orange). Each sine wave has an amplitude of 1. Row 2: Frequency-domain representations of these signals show the consistent 1 Hz amplitude at 1 and 2 Hz across the Panels. In the time domain, the area under the curve (absolute value) also remains approximately consistent, although the amplitude peaks (positive and negative) and range may be influenced by the relative harmonic phase. D) 1.1 Hz and 1.4 Hz EEG responses (data from Fig. 8).

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\(^8\) In frequency-tagging, there cannot be multiple sine waves of the same frequency that interfere with each other, e.g., sine waves with a 180° phase difference that produce complete interference (zero amplitude sum). Further, when a complete $F$ cycle is considered, the amplitude is representative of the complete signal (which is not the case for multiple (non-harmonic) frequencies that may sum differentially across short time segments of evaluation). Note that these conditions are not always met in other contexts in which frequency-based analyses are performed.
5). Left panel: Time-domain responses, highlighting the phase of the first four harmonics. Middle panel: Polar plots, in which each of these four harmonic frequency-domain responses is represented with a vector: angle = phase; length = amplitude. Right panel: Despite the different fundamental frequencies, the difference across sequential harmonics’ phase is similar.

4.1.2 Which harmonics to consider?

Before combining harmonics, a decision of which harmonics to consider is required. To this extent, harmonics-of-interest (similarly to a region-of-interest) must be defined. The first criterion for determining harmonics-of-interest is whether a higher harmonic is specific to its fundamental frequency. As mentioned previously, in frequency-tagging paradigms using a single stimulation frequency, the higher harmonics are always specific to the fundamental frequency. However, in paradigms deploying multiple stimulation frequencies, unspecific harmonics may occur, which are often excluded from the analyses (for a paradigm-focused review: Norcia et al., 2015). For example, two stimuli may be simultaneously presented at different spatial locations, one at 8 Hz ($F_1$) and the other at 6 Hz ($F_2$). If a response occurred at 24 Hz, it would not be specific to either stimulus, being the 3rd harmonic of 8 Hz ($3F_1$), and the 4th harmonic of 6 Hz ($4F_2$), and would likely be excluded from all analyses (for further examples: Table 1).

A second consideration for determining harmonics-of-interest aims to exclude extreme harmonic frequencies (e.g., the 30th harmonic of 8 Hz, at 240 Hz) at which no signal is expected or found. A limited selection of harmonics has been made based on various types of criteria: 1) amplitude, power, signal-to-noise ratio, or significance thresholds (e.g. Donker, 1975; Hou, Pettet & Norcia, 2008; Rossion et al., 2015); 2) frequency range (e.g., Jacques, Retter & Rossion, 2016; Leleu et al., 2018; Zemon & Gordon, 2018); 3) harmonic series number (e.g., Donker, 1975; Appelbaum et al., 2006; Wittevrongel et al., 2018); 4) in relation to other stimulation frequencies (e.g., Heinrich et al., 2009); and 5) correlation with the time-domain response (e.g., Badettini et al., 1993; Engel, Glover & Wandell, 1997).

The use of a limiting frequency range is recommended here, either as determined a priori, or from an assessment of the highest harmonic meeting a threshold (in terms of amplitude, signal-to-noise ratio, or significance). This is recommended because the upper frequency limit of harmonic responses, although affected by the overall strength of the signal, generally relates to the highest frequency that is strongly represented in the signal (see Section
The upper frequency limit of harmonic responses is thus often conserved across fundamental stimulation frequencies (see Fig. 2 of Retter & Rossion, 2016).

Note that the highest harmonic-of-interest can be determined either at the group level across conditions, or as presented by any participant for any condition, but that typically a common range of frequencies-of-interest should be used across participants and conditions (e.g., Jacques, Retter & Rossion, 2016). In this approach, there may be harmonic frequencies included for consideration at which there is no signal (e.g., in some participants, conditions, or regions-of-interest); however, including a small number of such frequencies is likely less detrimental (given that an appropriate baseline noise correction is applied, so that approximately zero amplitude values are added) than missing some frequencies containing a weak signal. Similarly, although responses are typically expected to occur consecutively across harmonic frequencies, in the event a small number of within-range harmonic frequencies do not contain signal (above threshold), including them is typically tolerable (e.g., Liu-Shuang, Norcia & Rossion, 2014; Rossion, Retter & Liu-Shuang, 2020).

Finally, in some cases harmonic responses appear to be qualitatively different from one another. This may occasionally be related to physiological sources: for example, different harmonic response patterns are generated from the recordings of frequency-tagged responses from single- vs. double-opponent cortical cells (Movshon, Thompson & Tolhurst, 1978). However, more often, physiological sources may only be tentatively inferred, e.g., when different EEG scalp topographies are observed at different harmonic frequency ranges (e.g., Rossion, 2014; see Section 5.2). In this case, is it appropriate to select subranges of qualitatively homogeneous harmonics to consider and/or combine? Perhaps, although it should be remembered that harmonic responses are not independent of one another (e.g., Tang & Norcia, 1995; Retter & Rossion, 2016; Zhou et al., 2016), and therefore should also be described individually and/or summed all together (Section 5.3). It is not advised to select or subgroup harmonics in accordance to only their number (e.g., only the 1st vs. 2nd harmonic: Pastor et al., 2007; the 1st vs. 2nd harmonic, rather than odd vs. even harmonics: Kim et al., 2007; 2011), unless this is explicitly derived from the stimulation paradigm (see Table 1; see also Section 5.3).
Table 1. Identifying specific harmonics for consideration in response analysis, according to different frequency-tagging stimulation paradigms. In the paradigm example sequence illustrations: A = one stimulus or stimulus type; B = another stimulus or stimulus type. *Special cases: In the case that A and B stimuli in a symmetry/asymmetry paradigm lead to symmetrical brain responses, e.g., if representing pattern-reversals, only even harmonics are observed (Cobb, Morton & Ettlinger, 1967; Norcia et al., 2015); in a combined symmetry/asymmetry and oddball design (Braddick et al., 2005), the odd harmonic analysis is unaffected. In the case that multiple frequencies lead to intermodulation, i.e., additive and subtractive interaction frequencies and their harmonics, the analysis of the intermodulation harmonics should exclude the overlapping harmonics of F1 and F2 (e.g., Zemon & Ratliff, 1984; Hou et al., 2003; Applebaum et al., 2009; Boremanse, Norcia & Rossion, 2013; Gordon et al., 2019). In the case that a sweep design is applied to a symmetry/asymmetry paradigm, this does not affect the harmonic analysis (see Norcia et al., 2015).

5. Interpreting harmonics

5.1 Why are there higher harmonics?
5.1.1 Non-sinusoidal brain responses

Higher harmonic responses represent complex neural responses in the time domain. At a fundamental level, these harmonic responses are like any other frequency-domain representations: they are sine waves described by frequency, amplitude, and phase (Fig. 2). While only one sine wave is required to describe a sinusoidal signal in the time domain, a combination of (a lot of) sine waves is required to describe complex signals in the time domain (Fig. 3). Frequency-tagged brain responses are periodic in the time domain, and thus only sinewaves periodic to their fundamental frequency, i.e., the harmonics, are mathematically available to describe them. Simple brain responses require few harmonics, while complex responses require more harmonics (Fig. 4). For example, lower stimulation frequency responses often have more harmonics, since there are relatively more harmonic frequencies available within a relevant frequency range ceiling (Fig. 5).

5.1.2 Limitations of a non-linearity account

Higher harmonic responses have often been interpreted as being caused by non-linearities in the stimulus presentation and/or brain responses (e.g., Van der Tweel & Verduyn Lunel, 1964; 1965; Spekreijse, 1969; Regan, 1966; 1989; Norcia et al., 2015; Gordon et al., 2019; see also Shapley, 2009). This relates to early attempts to present stimuli perfectly sinusoidally (e.g., a light being modulated sinusoidally in luminance; since van der Tweel, 1958). If the brain’s response to a sinusoidal stimulus was linear at the level of recording, it too would be perfectly sinusoidal in following this stimulus, and would be represented in the frequency domain by a response only at the fundamental frequency, i.e., without higher harmonics (Section 2). Contrary to expectation, higher harmonic brain responses were produced in most cases and were attributed to non-linearities in the brain’s responses themselves (e.g., non-linear action potential firing, neural population response dynamics, etc.; e.g., Movshon, Thompson & Tolhurst, 1978; Skottun et al., 1991; Shapley, 2009). More recent studies suggested that the amount of non-linearity, or complex temporal frequency content, in stimulus presentation may not correspond with the amount of non-linearity in the brain’s response at the population level: there was no difference in the amplitude of higher harmonics in response to (imperfect) sinusoidal vs. squarewave (i.e., abrupt on/off) complex stimulus presentation (Fawcett et al., 2004; Retter et al, 2016: Dzhelyova et al., 2017). Moreover, while the inherent non-linearity of the brain’s responses could account
for higher harmonics, in practice the amplitude of the higher harmonics is not always above noise level (as addressed in Section 1.2), or is very low, suggesting only a modest contribution of this factor (Section 3). However, one source of complexity in the brain’s responses is likely these non-linearities.

5.2 What do higher harmonic responses represent?

Higher harmonic responses represent the relevant frequency characteristics of the response in the time domain (e.g., Galambos, Makeig & Talmachoff, 1981; Gaume, Vialatte & Dreyfus, 2014; Heinrich, 2010; Zhou, 2016; Zemon & Gordon, 2018; Rossion, Retter & Liu-Shuang, 2020; and Fig. 2 of Retter & Rossion, 2016, showing how frequency characterizes harmonic EEG responses, in terms of amplitude and scalp topography, across conditions with different fundamental frequencies). That is, dynamics of the time domain response best represented at different frequency ranges will produce more amplitude in those frequency ranges in the frequency domain (Fig. 7; compare with Fig. 3C, and other such frequency representations (over time), e.g., Makeig et al., 2002; and see again Heinrich, 2010). Note that the amplitude distribution of harmonics across frequencies is, however, affected by the fundamental stimulation frequency and the overall amplitude of the signal. Moreover, individual harmonics do not represent independent or temporally separated aspects of a time-domain response (e.g., Tang & Norcia, 1995).

Figure 7. A time-domain signal can be fit with segments of sine waves of different frequencies. While this is not analogous to a frequency transformation, it hints at the range of frequencies that may be optimal for representing this signal.

Nevertheless, harmonics may thus provide insight into the functional dynamics of the neural processes that occur at their respective frequencies. In some cases, harmonic responses may be (gradually) influenced (quantitatively and/or qualitatively) by the frequency at which
they fall (e.g., Retter & Rossion, 2016; Rossion, Retter & Liu-Shuang, 2020). In other cases, harmonic responses may appear to group into somewhat distinct frequency ranges, e.g., visually-evoked EEG responses above about 10 Hz having a more medial-occipital (low-level) scalp topography (Rossion, 2014; Jacques, Retter & Rossion, 2016; see also Zemon & Gordon, 2018). Additionally, individual participants may vary in their distribution of harmonic response amplitudes (Heinrich & Bach, 2001; Dzhelyova et al., 2019). Note that since harmonic responses are characterized by their frequency, they are generally not well characterized by their sequential number, irrespective of frequency, unless differentially tagged in the stimulation paradigm (Section 4.1.2).

5.3 Should higher harmonic responses be combined, and if so, how? (Reprise)

Limiting the analysis of frequency-tagged responses to a non-predominant fundamental harmonic is not recommended: taking harmonic responses into account leads to substantially improved response quantification and classification (Fig. 8; Davilda, Srebo & Ghaleb, 1998; Muller-Putz et al., 2005; Chen et al., 2015; Retter & Rossion, 2016; Zemon & Gordon, 2018; Cetin, Ozekes & Varol, 2020). The combination of harmonic amplitudes through summation is justified through the principles of frequency-based analyses, and it leads to a combined response measurement that relates to typical time-domain amplitude measurements. It is useful for comparing brain response amplitudes overall, especially those with different temporal dynamics or following different stimulus presentation rates. Note that it is does not preclude, but rather is complementary, to the description of harmonic responses individually.
Figure 8. Combining higher harmonics in frequency-domain analyses, expanded from Fig. 1. A) Row 1: Two periodic signals (thick lines), with their five constituent harmonic frequencies (in thin lines, with colors corresponding to the frequencies below). Row 2: Frequency-domain representations of these signals. B) In the frequency domain, a consideration of only the fundamental harmonic describes signal 2 as (two times) larger than signal 1. A summation of the five harmonic amplitudes more appropriately describes signal 1 as (50%) larger than signal 2.

6. Conclusion

Stimuli that are presented periodically generate periodic responses of the brain that are often complex, i.e., non-sinusoidal. To capture and describe these complex brain responses overall, (baseline-corrected) frequency-tagged harmonic response amplitude can be combined through simple summation.

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8. Competing interests

None.

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