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The gap-startle paradigm to assess auditory temporal processing: Bridging animal and human research

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Abstract

The gap-prepulse inhibition of the acoustic startle (GPIAS) paradigm is the primary test used in animal research to identify gap detection thresholds and impairment. When a silent gap is presented shortly before a loud startling stimulus, the startle reflex is inhibited and the extent of inhibition is assumed to reflect detection. Here, we applied the same paradigm in humans. One hundred and fifty-seven normal-hearing participants were tested using one of five gap durations (5, 25, 50, 100, 200 ms) in one of the following two paradigms—gap-embedded in or gap-following—the continuous background noise. The duration-inhibition relationship was observable for both conditions but followed different patterns. In the gap-embedded paradigm, GPIAS increased significantly with gap duration up to 50 ms and then more slowly up to 200 ms (trend only). In contrast, in the gap-following paradigm, significant inhibition—different from 0—was observable only at gap durations from 50 to 200 ms. The finding that different patterns are found depending on gap position within the background noise is compatible with distinct mechanisms underlying each of the two paradigms.

Descriptors: Startle, Temporal processing, Prepulse inhibition, Gap-startle, Hearing, Gap-prepulse inhibition of the acoustic startle reflex (GPIAS), Gap-embedded, Gap-following

Temporal processing is a major property of the mammalian auditory system thought to be critical in speech perception and sound localization. In animals, one of the most often-used techniques to assess temporal acuity is the acoustic gap-startle paradigm. The acoustic startle reflex is a primitive reflex that consists of contraction of the major muscles of the body following a loud and unexpected sound (Koch, 1999). This reflex is reduced when preceded by a silent gap embedded in a soft background noise or tone, a technique also known as gap-prepulse inhibition of the acoustic startle (GPIAS). The investigation of auditory temporal resolution capacity by this technique involves short silent gaps of various durations as prestimuli, with the assumption that the amount of inhibition produced by the gap reflects detection, or temporal processing. A consistent finding is that the percentage of inhibition of the startle reflex increases as the gap duration increases (Allen, Schmuck, Ison, & Walton, 2008; Barsz, Ison, Snell, & Walton, 2002; Bowen, Lin, Merrit, & Ison, 2003; Cranney, Cohen, & Hoffman, 1985; Dean, Sheets, Crofton, & Reiter, 1990; Harbin & Berg, 1983; Ison, Allen, Rivoli, & Moore, 2005; Ison & Bowen, 2000; Ison, O'Connor, Bowen, & Bocinea, 1991; Ison & Pinckney, 1983).

Human studies using the gap-startle paradigm are scant (Cranney, Hoffman, & Cohen, 1984; Fournier & Hébert, 2013; Harbin & Berg, 1983; Ison & Pinckney, 1983; Lane, Ornitz, & Guthrie, 1991). Only two studies have reported increased reflex inhibition with increasing gap duration (Harbin & Berg, 1983; Ison & Pinckney, 1983). Using the gap-startle paradigm with a shock to the forehead to elicit the startle reflex, Ison and Pinckney (1983) estimated a threshold of 5 ms. Using a psychophysical gap detection task in the same subjects, the estimated threshold was 5.4 ms. This finding led authors to suggest that gap-startle detection thresholds and psychophysical gap detection share neural mechanisms.

Harbin and Berg (1983) used the gap-startle paradigm for gap durations of 10 to 120 ms to compare young to older adults. The startle reflex was elicited by an air puff stimulation. Increased inhibition with increasing gap durations from 10 to 80 ms was found for young adults but with a sudden decrease at 120 ms. No interpretation was provided for this surprising finding: If inhibition provided by the gap-startle paradigm reflects perceptual detection, then why would a 120-ms gap provide less inhibition than an 80-ms gap? Also, does inhibition decrease or increase for values greater than 120 ms?

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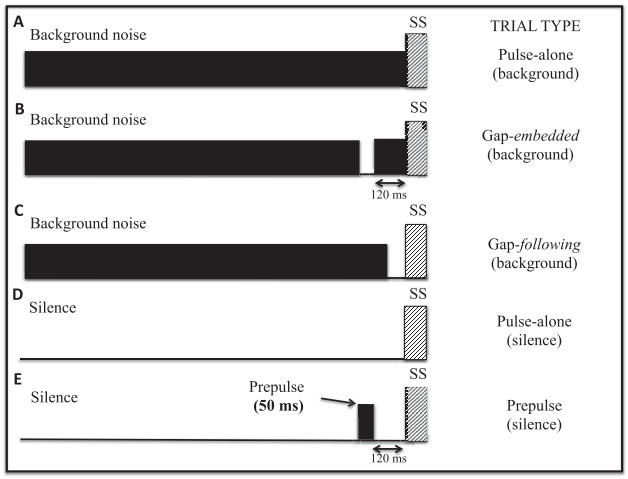

Figure 1. A schematic view of pulse-alone in background noise (A), gap-embedded (B), gap-following (C), pulse-alone in silence (D), and prepulse in silence (E) trials (SS = startle sound). Pulse-alone trials consisted of a startle sound in either a silent or continuous noise background. Gap-embedded trials consisted of a continuous noise background and a gap presented 120 ms before the SS. The gap-following condition consisted of a continuous background noise and a gap of silence presented just before the startle sound. Prepulse trials consisted of a silent background with a 50-s prepulse presented 120 ms before the SS.

The present study aims to determine the duration-inhibition relationship of very short (5 ms) up to long-gap durations (200 ms) using the gap-startle inhibition paradigm with auditory stimulation only. In addition, since gaps embedded in a noise background (hereafter gap-embedded) and gaps following a noise background (hereafter gap-following) yield different patterns of results in animal studies (Hickox & Liberman, 2014; Ison et al., 1991; Threlkeld, Penley, Rosen, & Fitch, 2008), both types of gaps were used with the assumption that they would not produce the same inhibition patterns. More specifically, differences in inhibition patterns should be observable for gap durations < 50 ms, since gap-embedded of longer durations have been suggested to be processed by the brainstem, similarly to gap-following (Threlkeld et al., 2008).

Another aspect that was examined here is the frequency specificity of the inhibition using high- and low-frequency prestimuli (background and prepulse). One animal study (Hoffman & Searle, 1967) and one human study (Cranney et al., 1984) found no effect of background frequency on acoustic startle inhibition (broadband and narrow-band noises in Hoffman & Searle, 1967; 1 or 2.5 kHz pure tones in Cranney et al., 1984). Yet, two human studies found greater inhibition for white noise prepulse compared to a tone (Blumenthal & Berg, 1986; Wynn, Dawson, & Schnell, 2000). Also, we (Fournier & Hébert, 2013) reported more inhibition of the GPIAS using a low-frequency (centered around 500 Hz) compared to a high-frequency narrow-band noise (centered around 4 kHz). The effect of low- and high-frequency background noises will be reexamined here.

Method

Participants

One hundred and seventy-six participants (mostly students at Université de Montréal) were recruited through word of mouth and paper ads. Inclusion criteria included having hearing thresholds ≤ 30 dB HL at any frequency between 250 Hz and 4 kHz in either ear as assessed by a standard clinical procedure. Exclusion criteria were uncontrolled medical conditions (e.g., hypertension, diabetes), middle and/or outer ear pathology, and heavy smokers (>10 cigarettes/day; Kumari, Cotter, Checkley, & Gray, 1997). Participants who were nonresponsive to the acoustic startle ($N = 17$, see below) and participants with noisy electromyography (EMG; $N = 2$) were excluded. The final sample totaled 157 participants who were assigned to one of the following 10 groups based on gap durations (5, 25, 50, 100, or 200 ms) and gap types (gap either embedded or following; see Figure 1). Sociodemographic characteristics of all groups are presented in Table 1. The study was approved by the local ethics committee of Université de Montréal and was conducted with the understanding and written consent of each participant.

Materials

Startle stimuli and task. A schematic view of startle with background noise (pulse-alone), gap-embedded or gap-following, startle in silence (pulse-alone), and prepulse trials are shown in Figure 1. Startle trials consisted of startle noises (50-ms broadband noise bursts set at 105 dBA SPL with near-instantaneous rise and fall time < 1 ms) preceded by either a low- or high-frequency continuous background noise set at 65 dBA SPL. The low-frequency background noise was centered at 500 Hz (200–1200 Hz) and high-frequency background noise at 4 kHz (3.5–4.5 kHz). Gap-embedded trials were similar to startle trials, except that a silent gap of 5, 25, 50, 100, or 200 ms was inserted between two segments of background noise with a constant interstimulus interval (ISI) of 120 ms before the startle noise, producing stimulus onset asynchrony (SOA) of 125, 145, 170, 220, and 320 ms, respectively. Gap-following trials were similar except that the different silent gaps were following the end of the background noise, producing different ISIs of 5, 25, 50, 100, 200 ms, equivalent to SOA. Prepulse trials were either low- or high-frequency 50-ms noise bursts set at 65 dBA SPL presented in silence, followed by a 120-ms (ISI: 120 ms) interval of silence and a startle noise. The ISI of 120 ms was selected to maximize inhibition (Braff et al., 1978). The intertrial interval (ITI) time was randomly set at a value between 15 and 23 s in each block. Both background noise and silence were present for the entire ITI duration of the gap and prepulse conditions, respectively. Finally, startle trials in silence consisted of a silent background (no background noise) with a startle noise as described above. All stimuli were created using Max/MSP software program (Cycling 74, San Francisco, CA). All stimuli were calibrated before each testing session with an SE SoundPro DL 1/3 octave level meter (Quest Technologies, USA) using an EC-9A artificial ear coupler (Quest Electronics, Oconomowoc, WI) with appropriate rates, that is, impulse for startle noises/prepulse and slow rate for background noise, using the A-weighting frequency curve.

EMG measures. Eyeblink activity was measured using two 4-mm Ag/AgCl shielded recording electrodes positioned 1.5 cm apart on the orbicularis oculi muscle under the left eye and a ground electrode on the forehead, according to guidelines (Blumenthal et al., 2005). Signal acquisition was made using an IMac running the Acqknowledge 4.1 software connected to a Biopac MP150 system (Biopac Systems, Inc., Santa Barbara, CA) using the following settings: $\times$1,000 amplification, 90–500 Hz band-pass filter, root mean square (RMS) transformation, A/D conversion at 1 kHz. The stimulus presentation system was coupled to a Fireface sound card (RME, Haimhausen, Germany) hosted by a PC computer. Startle

Table 1. *Sociodemographic Characteristics (SD) of Participants in Each Gap Duration Group for Each Gap Type*

Gap duration (ms)	5	25	50	100	200	*p* value
Gap-embedded in the background						
Number of participants	12	12	32	15	13	
Gender						
Male	7	8	19	12	5	*n.s.*
Female	5	4	13	3	8	
Age in years (*SD*)	22.1 (3.3)	23.7 (3.6)	23.2 (2.9)	24.7 (2.0)	24.3 (2.5)	*n.s.*
Education in years (*SD*)	15.6 (3.1)	16.5 (2.7)	16.8 (2.9)	16.8 (1.4)	17.4 (3.3)	*n.s.*
Gap-following the background						
Number of participants	12	12	14	22	13	
Gender						
Male	4	4	6	7	4	*n.s.*
Female	8	8	8	15	9	
Age in years (*SD*)	21.8 (2.6)	21.2 (3.0)	23.6 (2.7)	24.4 (2.9)	23.3 (3.3)	*n.s.*
Education in years (*SD*)	16.1 (2.0)	16.1 (2.5)	16.2 (2.2)	18.0 (2.1)	16.9 (2.4)	*n.s.*

noise presentation was synchronized with eyeblink activity recording via a square-wave trigger signal to precisely determine the window of responses for magnitudes and latencies of the eyeblink (see Data Processing below).

Procedure

Participants were instructed to sit quietly in a soundproof booth, refrain from moving, and listen to the sounds presented binaurally via closed dynamic headphones DT 770 Pro/250, while watching a white cross projected on a dark screen. The test session began with a 2-min acclimatization period consisting of a high-frequency background noise of 65 dBA SPL that ended with four pulse-alone stimuli for habituation before the beginning of Block 1. The task consisted of three blocks. In the first block, five high- and five low-frequency background startle trials were randomly mixed with five low- and five high-frequency gap trials. Block 2 started with a 1-min acclimatization period of silence followed by two startle noises, and then by 10 high- and 10 low-frequency prepulse trials, randomly mixed with 10 startle trials in silence. The third block was identical to the first one except that the acclimatization period was low-frequency background noise followed by two startle noises. Short breaks between blocks allowed the experimenter to monitor participants' drowsiness or lack of attention. There were 70 stimuli, lasting for a total duration of about 25 min.

Data Processing

All trials were visually inspected for excessive noise in the EMG signal and for any spontaneous blink occurring immediately before the startle stimuli. These occurrences were very few (2.7%) and were rejected from further analysis. The baseline was assessed for each participant by selecting the highest RMS amplitude value occurring between −20 ms to startle noise onset, averaged across startle-alone trials only. The peak-to-peak amplitude of each startle response occurring between 20 and 120 ms from pulse onset was extracted from the transformed RMS data. Data for each trial type were averaged for each background noise for each participant. Any peak-to-peak amplitude value of any trial (i.e., prepulse, gap, startle) that was smaller than two standard deviations above the average baseline was considered a nonresponse, which were assigned a magnitude of zero. In addition, participants displaying more than 25 nonresponses out of a total of 70 stimuli were considered nonresponders and were excluded from the study ($N = 17$). Percentage of inhibition was calculated for each condition (gaps or prepulse) using the following formula: % inhibition = [(pulse-alone) − (gap/prepulse)]/(pulse-alone) × 100. Startle facilitation was assessed by comparing the magnitude of the mean response for pulse-alone trials in the three different conditions (silence, low-, and high-frequency background). Peak latency was obtained from the same time window but calculated from the raw EMG waveform following guidelines (Blumenthal et al., 2005). Latency facilitation was calculated for each condition (gaps or prepulse) using the following formula: latency facilitation = (pulse-alone latency) − (gap or prepulse) latency. Data for each trial type were averaged for each background noise (high, low, silence) for each participant. For percentage of inhibition, data above two standard deviations from the group mean were replaced by the average value of the appropriate group for each trial type, gap duration, and background noise (total of 4.9%).

Statistical Analyses

The effects of gap duration and gap type on percentage of inhibition were assessed by a 5 × 2 × (2) mixed analysis of variance (ANOVA) with gap duration (5, 25, 50, 100, 200 ms) and gap type (gap-embedded or gap-following) as between-subjects factors and frequency (high vs. low) as within-subject factor. Similar ANOVAs were run on latency facilitation, magnitude, and latency of the startle-alone, and percentage of inhibition and latency facilitation of the prepulse. Significant interactions were followed up by appropriate ANOVAs, *t* tests, or Sheffé's post hoc comparisons. Bonferroni correction for multiple comparisons was used for *t* tests when appropriate in order to keep the alpha level to .05 throughout all analyses. Therefore, the reported *p* values are corrected values. Greenhouse-Geisser degrees of freedom were used to evaluate significance and are reported when sphericity assumptions were violated.

Results

Effects of Gap Duration and Gap Type on Percent (%) Inhibition

Figure 2 displays % inhibition for all gap durations across high and low frequencies. The expected two-way interaction between gap duration (5, 25, 50, 100, 200) and gap type (embedded or following the noise) was significant, $F(4,147) = 5.1$, $p = .001$, $\eta^2 = .12$. There was also a significant interaction of Frequency × Gap Type, $F(1,147) = 8.8$, $p = .004$, $\eta^2 = .06$, and Frequency × Gap

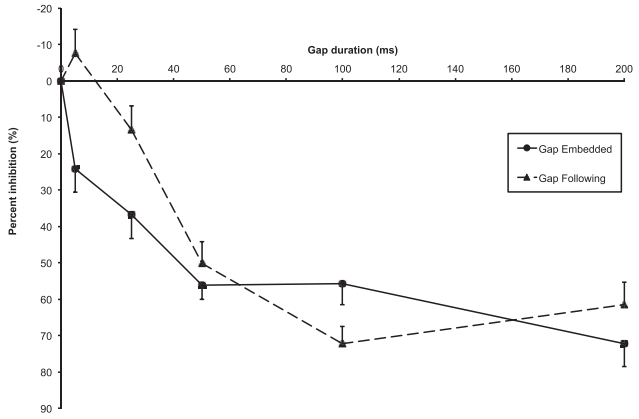

Figure 2. Percentage of inhibition (*SEM*) for each gap duration group (5, 25, 50, 100, 200 ms) and gap type (embedded or following).

Duration, $F(4,147) = 2.8$, $p = .027$, $\eta^2 = .07$. For gap-embedded, overall % inhibition was greater for low (54%) than for high (44%) frequency, $F(1,79) = 10.8$, $p = .002$, $\eta^2 = .12$. There was also a main effect of gap duration $F(4,79) = 9.5$, $p < .001$, $\eta^2 = .33$. For the gap-following, there was only a main effect of gap duration, $F(4,68) = 30.5$, $p < .001$, $\eta^2 = .64$. No effect involving frequency was found here, $p = .29$.

For the gap-embedded condition, 5-ms gaps produced significantly less inhibition (24.2%) than 50 (56.1%), 100 (55.8%), and 200 (72%) ms, but not less than 25 ms (36.8%), and the latter differed significantly only from 200-ms gaps (see Table 2). The lower limit of the 99% confidence interval (CI) for each gap duration was calculated to assess differences from 0% inhibition, that is, no inhibition (see Table 3). All gap durations produced significant measurable inhibition that was different from 0%.

For the gap-following condition, both 5 (−7.7%) and 25 (13.4%) ms gaps produced significantly less inhibition than 50 (50.2%), 100 (72.2%), and 200 (61.6%) ms. Percent inhibition did not differed significantly between 5 and 25 ms (see Table 2). However, the lower limit of the 99% CI did include 0% for 5- and 25-ms gap durations, meaning that 5- and 25-ms gaps did not produce significant inhibition (see Table 3).

In summary, in the gap-embedded condition all gap durations produced significant and increasing inhibition whereas in the gap-following only gaps of ≥ 50 ms produced significant and similar inhibition.

Table 2. *Post hoc Comparisons (p Values) of % Inhibition Between Each Gap Duration for Each Gap Type*

	5 ms	25 ms	50 ms	100 ms	200 ms
Gap-embedded					
5 ms	–	–	–	–	–
25 ms	0.73	–	–	–	–
50 ms	**.002**	.16	–	–	–
100 ms	**.011**	.29	1	–	–
200 ms	**< .001**	**.004**	.28	.42	–
Gap-following					
5 ms	–	–	–	–	–
25 ms	0.3	–	–	–	–
50 ms	**< .001**	**.005**	–	–	–
100 ms	**< .001**	**< .001**	0.11	–	–
200 ms	**< .001**	**< .001**	0.78	0.78	–

Note. The *p* values in bold represent cases in which groups significantly differ from one another.

Effects of Gap Duration and Gap Type on Latency Facilitation

Figure 3 displays latency facilitation for all gap durations and gap types. The expected two-way interaction of Gap Duration (5, 25, 50, 100, 200) × Gap Type (embedded or following the noise) was significant, $F(4,147) = 5.68$, $p < .001$, $\eta^2 = .13$. Only main effects of gap duration were found in the gap-embedded, $F(4,79) = 5.36$, $p = .001$, $\eta^2 = .21$, and gap-following, $F(4,68) = 3.83$, $p = .007$, $\eta^2 = .18$, conditions. Sheffé's post hoc comparisons showing group differences between gap durations are presented in Table 4.

For the gap-embedded condition, 50-ms gaps significantly produced less latency facilitation than 100 and 200 ms. The lower limit of the 99% CI for each gap duration confirmed that only 100- and 200-ms gap durations produced significant measurable latency facilitation different from 0 ms.

A different scenario was found for the gap-following condition, with the 50-ms gap producing significantly more latency facilitation than 5 ms and 25 ms (the latter was marginally significant). Consistent with amplitudes, the lower limit of the 99% CI for each gap duration confirmed that only 50-, 100-, and 200-ms gap durations produced significant latency facilitation.

Magnitude and Latency of the Startle Sound: Control Condition

A 5 × 2 × (2) mixed ANOVA was performed on startle stimuli to ensure that all groups responded in a similar way. For magnitude, there was only a significant main effect of frequency, $F(1,147) = 8.53$, $p = .004$, $\eta^2 = .06$, with greater startle reactivity in high-frequency (213 uV) than low-frequency (199 uV) background. When comparing the startle magnitude in high- or low-frequency background to startle magnitude in silence with paired sample *t* tests, startle in silence was significantly lower (140 uV) than high-frequency, $t(74) = 6.4$, $p < .001$, and low-frequency background, $t(74) = 5.3$, $p < .001$. No main effect of gap duration ($F < 1$), gap type ($F < 1$), or interaction between these two factors ($F < 1$), was significant. For latency, there were no main effects or interactions. The mean latency was 61 ms.

Percent of Inhibition and Latency Facilitation of the Prepulse: Control Condition

A 5 × 2 × (2) mixed ANOVA was performed to ensure that all groups had similar sensorimotor gating abilities. For % inhibition, there was a significant main effect of frequency, $F(1,147) = 5.3$, $p = .023$, $\eta^2 = .04$, with more inhibition for the lower frequency (78.8%) compared to high-frequency (75.4%) prepulse. There was also a main effect of gap duration, $F(4,147) = 2.7$, $p = .033$, $\eta^2 = .07$. There was only one significant difference of the order of 14% between 50 ms and 100 ms ($p = .009$ by Sheffé's comparisons). For latency facilitation, there were no main effects or interactions.

Gap Versus Prepulse

Comparisons between % inhibition of the prepulse versus the gap condition for each group (paired sample *t* tests) revealed that prepulse produced more inhibition than any gap duration (5, 25, 50, 100, 200) and gap type (embedded or following), with the exception of 200 ms in the gap-embedded condition, with which it did not differ significantly (see Table 5).

Table 3. *Lower Limits of the 99% CIs (% Inhibition and Latency Facilitation) for Each Gap Duration Group for Each Gap Type*

	Gap-embedded		Gap-following	
Gap duration	% of inhibition	Latency facilitation	% of inhibition	Latency facilitation
5 ms	**7.3**	−7	−24.6	−7
25 ms	**20.0**	−4	−3.5	−4
50 ms	**45.7**	−5	**34.5**	**6**
100 ms	**40.7**	**1**	**59.7**	**1**
200 ms	**56.1**	**1**	**45.3**	**1**

Note. Values in bold represent cases in which the lower limit of the 99% CI is greater than 0, suggesting the presence of a reliable inhibition and/or latency effect.

Discussion

Herein, we report the important finding that patterns of startle reflex % inhibition and latency facilitation in relation to silent gaps of various durations differed substantially depending on gap position within the background noise (i.e., embedded vs. following), suggestive of distinct mechanisms underlying each of the two paradigms.

For the gap embedded within background noise paradigm, inhibition of the startle reflex increased with gap durations of up to 50 ms. These results are consistent with previous animal (Cranney et al., 1985; Ison, 1982; Ison et al., 1991; Threlkeld et al., 2008) and human studies (Harbin & Berg, 1983; Ison & Pinckney, 1983) employing various types of background (tone or noise) and startle stimuli (tone, white noise, noise burst, air puff, shock to the forehead; see online supporting information Table S1) suggesting that such factors—modality and type of stimulus—exert only minor influences on the pattern of inhibition and, conceptually, that a commonality in GPIAS exists across species. The startle reflex inhibition-gap duration relationship from very short to protracted duration was such that statistically significant increments occurred up to 50 ms, whereas there was only a trend for further inhibition between 50 ms, 100 ms, and 200 ms. Similar patterns have been reported using a wide range of gap durations with rapid maximum inhibition occurring at approximately 50 ms (80 ms, Cranney et al., 1985; 40 ms, Harbin & Berg, 1983; 30 ms, Threlkeld et al., 2008).

In contrast, the gap-following paradigm did not produce any significant inhibition at 5- and 25-ms gap durations, lying within the 99% CI of no inhibition. However, inhibition values from 50- to 200-ms gap durations were of similar magnitudes as to the ones determined using the gap-embedded paradigm, with 50% inhibition at 50 ms, 72% at 100 ms, and 62% at 200 ms. These findings, reported herein for the first time in humans, are consistent with previous animals studies (Bowen et al., 2003; Ison & Allen, 2003, 2012; Ison et al., 1991). One possible line of interpretation for the discrepancy between the two paradigms at 50-ms gap duration (or lower) might be related to the fact that, in the gap-following paradigm, only a single cue (i.e., gap onset) is available compared to double cues in the gap-embedded paradigm (i.e., gap onset and offset), thereby making—in the latter—the gap more perceptible and more efficient as an inhibitor of the startle. Moreover, increasing the duration between a gap's offset and onset increases the inhibition in a way similar to a phenomenon observed in prepulse studies: increasing either the separation between two clicks, or increasing the duration of a single prepulse, increases the inhibition up to values of approximately 50 ms, a phenomenon called "temporal summation" (Blumenthal, 1995). Conversely, gap offsets at 125 and 145 ms SOA could be seen as interfering with the effectiveness of the gap onset at 120 ms: the offset of the gap could exert a negative influence on the inhibition driven by the onset, as an onset cue produces more inhibition than a gap-embedded (offset-onset) of a few milliseconds at similar onset ISI. Accordingly, auditory cortex deactivation studies have shown that GPIAS was diminished for gap durations ≤ 50 ms in gap-embedded but not in gap-following noise, suggesting the involvement of cortical neural substrates in the former (Ison et al., 1991; Threlkeld et al., 2008) and the possibility of brainstem involvement in the latter. Cortical involvement is supported by experimental data showing that auditory cortical neurons respond to the gap offset with a characteristic burst of spikes (termed *the gap termination response*) presumed to be a neural correlate of brief gap detection (Eggermont, 1999; Recanzone, Engle, & Juarez-Salinas, 2011). Moreover, a recent

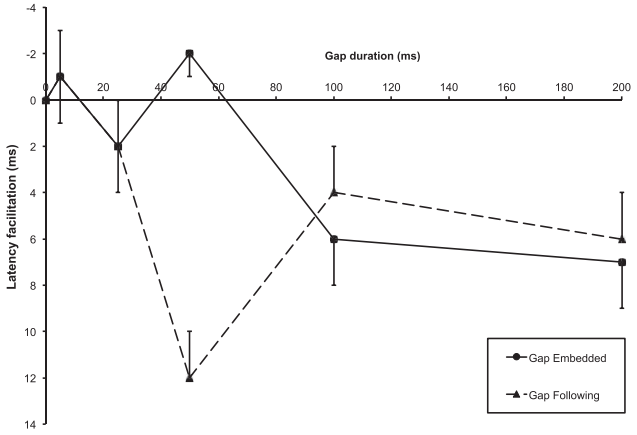

Figure 3. Latency facilitation (*SEM*) for each gap duration group (5, 25, 50, 100, 200 ms) and gap type (embedded or following).

Table 4. *Post hoc Comparisons (p Values) of Latency Facilitation Between Each Gap Duration Group for Each Gap Type*

	5 ms	25 ms	50 ms	100 ms	200 ms
Gap-embedded					
5 ms	–	–	–	–	–
25 ms	.91	–	–	–	–
50 ms	.99	.7	–	–	–
100 ms	.16	.66	**.02**	–	–
200 ms	.13	.57	**.016**	1.0	–
Gap-following					
5 ms	–	–	–	–	–
25 ms	.94	–	–	–	–
50 ms	**.015**	.13	–	–	–
100 ms	.61	.98	.2	–	–
200 ms	.35	.83	.67	.97	–

Note. The *p* values in bold represent cases in which groups significantly differ from one another.

Table 5. *Individual Differences Between % Inhibition by the Prepulse (Control) and Gap Conditions Averaged Across Each Gap Duration and Gap Type*

	Mean difference (%) prepulse-gap	Paired sample *t* test	*p* value
Gap-embedded			
5 ms	56.5 (33.3)	$t(11) = 5.9$	<.001**
25 ms	33.1 (32.3)	$t(11) = 3.5$	.005*
50 ms	11.5 (24.8)	$t(31) = 2.6$	.014*
100 ms	28.3 (22.6)	$t(14) = 4.9$	<.001**
200 ms	6.4 (15.5)	$t(12) = 1.5$	.16
Gap-following			
5 ms	72.1 (26.5)	$t(11) = 9.4$	<.001**
25 ms	70.9 (29.5)	$t(11) = 8.3$	<.001**
50 ms	24.0 (20.4)	$t(13) = 4.4$	.001*
100 ms	12.1 (16.4)	$t(21) = 3.5$	.002*
200 ms	21.5 (17.9)	$t(12) = 4.3$	.001**

*$p < .01$. **$p < .001$.

study has shown that gap detection (as measured by GPIAS) appears to be processed by interneurons that allow ongoing comparisons between pre- and postgap spiking activity (Weible et al., 2014). Interestingly, this ongoing comparison held for gap duration ≤ 25 ms, but not for 50 ms. Possibly then, gaps ≤ 25 ms embedded in a background noise could be processed as a whole rather than consisting of distinct features such as offsets and onsets. If this were not the case, then cortical deactivation would not have any effect on gap startle inhibition since both offsets and onsets would be processed and inhibition would occur even without active cortical areas. Therefore, temporal summation is present in the gap-embedded for values ≤ 50 ms. For greater values, separation between the onset and offset is too large to consider the gap as a whole, and the latter is then processed by its distinct features.

Finally, another explanation for the discrepancies between the inhibition of the gap-embedded and gap-following condition at 5 ms and 25 ms might be the lead time or SOA difference between the two conditions. Indeed, it is well known that the optimal lead interval range producing maximal inhibition is 60–240 ms for auditory prepulses (Braff et al., 1978; Graham & Murray, 1977). By reducing the gap duration to 25 and 5 ms in the gap-following condition, we are consequently reducing the lead times to values lower than the optimal interval range and thus jeopardizing inhibition. For the gap-embedded condition, the SOA is reduced with the reduction of the gap but never less than 125 ms, since the ISI is fixed at 120 ms in this condition. A follow-up study should focus on the effect of lead times on GPIAS using fixed gap durations at different SOA times.

It is noteworthy that latency facilitation occurred only under conditions associated with significant % inhibition in the gap-following paradigm, again supporting the notion that the two paradigms rely on distinct neural networks, at least at short gap duration values. Thus, latency facilitation would occur only under conditions in which inhibition is driven by brainstem mechanisms (gap-following: 50, 100, and 200 ms; gap-embedded: 100 and 200 ms). However, this proposition was not subjected to direct testing herein, and further research will be needed to clarify the origin of latency facilitation.

Our results demonstrate greater inhibition of the startle reflex when low frequency prepulses are used or when gaps are embedded within a low-frequency background noise. These results are in line with our previous findings (Fournier & Hébert, 2013) but contrast with some previous human (Cranney et al., 1984) and animal (Cranney et al., 1985; Hoffman & Searle, 1967) studies that have found no effect of frequency. However, these studies have used pure tones rather than narrow-band noise, the latter being more effective than pure tones to generate inhibition in a prepulse paradigm (Blumenthal & Berg, 1986; Wynn et al., 2000).

Although the noise centered around 4 kHz spanned less critical bands than the one centered around 500 Hz (two vs. 16, see Moore, 2003), and thus the latter might have sounded louder than the former, it is unlikely that loudness is involved in this frequency difference. Indeed, extant data are that louder background noise would be less effective in inhibiting the startle by the prepulse since it is the difference between the background noise and the prepulse level, rather than the absolute level, that is the critical factor (e.g., Blumenthal, Noto, Fox, & Franklin, 2006). In addition, if sound level were a critical factor here, all three types of stimulus would produce similar differences, which is not the case. Furthermore, a recent study using a similar GPIAS paradigm but applied to auditory evoked potentials also demonstrated greater inhibition of components N1, N2, P2 (particularly P2) when using a 8 kHz pure tone compared to a 600 Hz one as background noise (Ku et al., 2015). These findings cannot be explained in terms of critical bands (or loudness) since pure tones presented at similar dB sensation level, and thus loudness levels, were used. One possible explanation to reconcile the discrepancy among studies is the size of the difference in Hertz used between the low- and the high-frequency stimuli. Indeed, Cranney and colleagues (1984) used very close frequencies only 1500 Hz away from each other (1000 and 2500 Hz) compared to the present study (3500 Hz of difference between 500 and 4000 Hz) and the Ku and colleagues' (2015) study (7400 Hz of difference between 600 and 8000 Hz). The small difference in Hz might have thus been insufficient to generate a difference of inhibition between high and low frequencies.

Implications for Hearing Disorders

Inhibition of the acoustic startle reflex by a variety of prestimuli such as prepulses and gaps has been widely used in animal research to assess physiological changes within the central auditory system, notably temporal acuity related to age-related hearing loss (Barsz et al., 2002; Ison, Agrawal, Pak, & Vaughn, 1998; Swetter, Fitch, & Markus, 2010), and modifications of auditory functions with deletion of specific genes (e.g., K channels; Allen et al., 2008; Ison & Allen, 2012) or pharmacological treatment (Ison & Bowen, 2000; Leitner & Girten, 1997). Gap-prepulse inhibition of the acoustic startle (GPIAS) has become the gold standard to assess behavioral gap detection in animals. This method has been proposed as an objective measure of tinnitus in animal models (Turner et al., 2006) under the rationale that tinnitus might "fill in" the gap and thus prevent inhibition of the startle. Moreover, the lack of inhibition would be specific to the tinnitus frequency; that is, a high-frequency tinnitus would produce more GPIAS deficit (i.e., less inhibition) when the gap is embedded in a high-frequency noise background than in a low-frequency background. Since it is based on a reflex rather than costly and time-consuming conditioning, this method has been enthusiastically adopted by many as a behavioral measure of tinnitus in several animal species (for a review, see Galazyuk & Hébert, 2015). However, the interpretation of the tinnitus filling in the silent gap has been recently challenged in human (Boyen, Baskent, & van Dijk, 2015; Campolo, Lobarinas, & Salvi, 2013; Fournier & Hébert, 2013) as well as in animal studies (Hickox & Liberman, 2014). Since tinnitus is usually in the high-frequency range ($\sim$10 kHz and above in animals when

assessed by operant conditioning paradigms), one reason for being cautious about interpreting a decrease in inhibition as an objective marker of tinnitus is that, as shown herein, high-frequency produce less inhibition than low-frequency background noises (also found in Fournier & Hébert, 2013). Therefore, identifying the source of a decrease in inhibition between high- and low-frequency backgrounds might be difficult when criteria for a deficit are not clearly defined. Moreover, although in one study GPIAS deficits were identified in human tinnitus participants at both low- and high-frequency background noises without precisely controlling resemblance with tinnitus frequencies (Fournier & Hébert, 2013), some studies have found normal psychophysical gap detection abilities in tinnitus subjects (Boyen et al., 2015; Campolo et al., 2013), questioning the link between GPIAS and behavioral detection abilities. In psychophysical studies conducted in normal adults without hearing disorders—similar to the participants in the present study—threshold values of ∼4.19 ms were identified, and the vast majority of young adults were able to detect 5-ms gaps (Hoover, Pasquesi, & Souza, 2015; Samelli & Schochat, 2008). Herein, we showed that inhibition produced by a 5-ms gap-embedded (but not gap-following) stimulus is ∼24% and constitutes a robust measure, with a lower limit of the conservative 99% CI higher than null inhibition. This is consistent with the notion that the shortest gap was indeed detected. However, tinnitus data suggest that GPIAS and psychophysical detection might not be as straightforwardly linked as previously proposed, and that additional attentional processes might be involved in the latter (Li, Du, Li, Wu, & Wu, 2009).

Conclusion

It is concluded that, in any study using the GPIAS method, there is a necessity to consider the type of gap paradigm (gap-embedded vs. gap-following) and the duration selected to establish the inhibition (or lack thereof) as fundamentally different outcomes might arise.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1: Patterns of GPIAS for gap-embedded background sound across species.