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Differential effects of intensity and response preparation components of acoustic warning signals

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It is known that the increase of intensity on a warning signal (WS) usually decreases reaction times to targets and occasionally is accompanied by a startle reflex reaction that influences the speediness of response execution. In a simple detection task (Experiment 1), a detection task with catch trials (Experiment 2) and a Go-NoGo discrimination task (Experiment 3), we studied the relationship between response preparation and alerting mechanisms operating upon the presentation of warning signals. A WS was presented either synchronously with the target (simultaneous condition) or 1400 ms before it (delayed condition). In all three experiments, the intensity of the WS and the simultaneity between WS and target were orthogonally manipulated. Results confirmed shorter reaction times by increasing the WS intensity. In Experiment 1, all conditions presented a clear acoustic intensity effect. In Experiment 2 we observed shorter reaction times in higher intensity conditions but only when the WS and the target were presented simultaneously. In Experiment 3, the intensity effect was observed only when the WS preceded the target. In all experiments, trials where the WS triggered a startle reflex showed a systematic increase in reaction time, which was independent of response preparation and task demands. In general, our findings suggest that response preparation modulates the alerting mechanisms, as a function of task set, but not the startle reflex. The dissociation between intensity, response preparation and startle supports the interdependence between these mechanisms elicited by the presentation of warning signals.

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The alerting system is fundamental for humans to supply and sustain the processing of high priority signals (Posner, 2008; Posner & Petersen, 1990; Sturm & Willmes, 2001). The priority of these signals is established by an internal, general state of wakefulness (tonic alertness) or by a phasic alerting mechanism, usually triggered by an external and abrupt warning signal (WS). In particular, an acoustic WS presented before the imperative stimulus (target) provides information about the moment of its appearance, both modulating the preparatory state before the motor execution and informing about when and whether the target will be presented (Correa, Lupiáñez, Milliken, & Tudela, 2004; Correa, Lupiáñez, Madrid, & Tudela, 2006; Gabay & Henik, 2008; Hackley, 2009; Hackley, & Valle-Inclán, 2003; Mather & Sutherland, 2011). This means that a WS can leads to programing and preparation of the response in advance, thus optimizing response readiness and speeding up reaction times (RT) (Hackley et al., 2003).

On the other hand, it has been shown that the consequences of acoustic WS presentation vary depending on the accessory characteristics of the stimulus itself. In the case of acoustic WSs, one of the most relevant accessory dimensions is its intensity. When intensity is manipulated, reaction times are usually shorter for higher intensities (Kohfield, 1971; Angel, 1973). This phenomenon, that we call acoustic intensity effect, is usually observed in tasks where the intensity dimension is completely task-irrelevant (Jaskowsky, Rybarczyk, & Jaroszyk, 1994; Kohfield, 1971; Miller, Franz, & Ulrich, 1999; Ulrich, Rinkenauer, & Miller, 1998). These task-irrelevant characteristics can be considered as additional, or more precisely accessory, aspects of the stimulus itself. These characteristics, together with other non-accessory features (i.e., temporal predictiveness), are critical for response execution. It is nevertheless important to clarify whether these two aspects of WSs interact or affect performance independently from each other.

In a series of four experiments, Miller and collaborators examined the effect of the intensity of a pure tone used as WS in simple RT, Go/No-go and choice tasks (Miller, Franz, & Ulrich, 1999). Results showed that high acoustic WSs led to faster RT and stronger finger flexion force. This raised magnitude of the muscular activation was confirmed in later studies with simple and choice RT tasks (Włodarczyk, Jaśkowski, & Nowik, 2002); in choice RT and spatial incongruence stepping tasks (Watanabe, Koyama, Tanabe, & Nojiba, 2015); and in paradigms manipulating the luminance intensity of visual WS (Jaskoswki & Wlodarczyk, 2006). In their fourth series of experiments, Miller et al. (1999) manipulated the temporal interval between WS and target (-400, -50, 50 and 400 ms), the WS intensity and the predictability of target appearance, by not presenting the WS in half of the trials. Increased response force was reported again for the highest WS intensities, although the intensity-related RT shortening was small and not significant. Finally, the authors compared their results with those from studies where the WS always anticipated the target and concluded that the reduced intensity effect was due to the diminished predictability of the WS, which decreased the subject reliance on the warning stimulus as a temporal cue (Miller et al., 1999).

Miller´s study contributed significantly to our knowledge about the impact of temporal expectancy on the acoustic intensity effect. However, the way intensity and preparation were manipulated in their study presents two important limitations. The first one concerns the manipulation of target predictability. In Experiment four, the intensity of the WS was manipulated only in half of the trials, when the WS was presented before or after the target. The authors suggested that the reduced number of trials with WS was the cause of a diminished intensity effect (Miller et al., 1999). Nonetheless, this manipulation did not allow a direct comparison of the intensity effect between conditions with and without response preparation, as the target was presented in all trials and participants were always required to respond. In other words, it is not possible to clarify whether the decreased acoustic intensity effect was due to the specific intervention of response preparation mechanisms, or it was related to a more general coexisting manipulation of temporal expectation. Since the temporal expectation and response preparation following WS are considered separated, dissociable mechanisms (for a review, see Weinbach & Henik, 2012), this distinction is critical in order to understand their influence on the effects produced by the accessory characteristics of WSs.

The second limitation of Miller's study is related to the possibility that intense acoustic stimulations are particularly effective in triggering an automatic defensive response, commonly known in the literature as startle reflex (Davis, 1984; Valls-Solé, Kofler, Kumru, Castellote, & Sanegre, 2005). In mammals, startle responses are a characteristic sequence of muscular responses elicited by a sudden, intense stimulus. Following several studies reporting faster RT associated to overt automatic reflexes, some researchers proposed the idea that the startling motor activation is able to trigger response selection and programming. The startle reflex has been robustly obtained with different paradigms (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004; Carlsen, Chua, Inglis, Sanderson, & Franks, 2007; Carlsen, Maslovat, Lam, Chua, & Franks, 2011; but see Carlsen, Chua, Dakin, Sanderson, Inglis, & Franks, 2008), and different explanations have been proposed to explain it (Lipp & Hardwick, 2003; Lipp, Kaplan, & Purkis, 2006).

Trying to overcome these limitations, the aim of the present work was to investigate whether the intensity effect and response preparation induced by WS represent two independent aspects of alertness, and whether they are modulated by task sets requiring different levels of control. In particular, we intended to clarify the interaction between mechanisms of acoustic intensity and response preparation, while controlling for the presence of the startle reflex likely elicited by the more intense WSs. As explained above, the sound used as a WS may influence the response preparation in several ways: by anticipating the target (as it gives information about the temporal window of target appearance); by its intensity; or by triggering an automatic startle reflex. Despite the frequent use of acoustic WS and the manipulation of this sound dimension in experimental paradigms, the question of whether these behavioural changes rely on single or separate mechanisms is still under debate (Washington & Blumenthal, 2015; Carlsen et al., 2011; Lipp, Kaplan, & Purkis, 2006). In addition, the discrimination between the behavioural contribution of the intensity of the WS and that of the startle reflex is often missed. Nonetheless, understanding how these three mechanisms interact with each other is crucial to fully comprehend the nature of RT changes attributable to the WS manipulation.

With the purpose of exploring the relationship between the intensity effect and response preparation, we manipulated three levels of intensity of the acoustic WS, and expected shorter RT for the highest WS intensity. Moreover, in some conditions there was an interval between WS and target, which would allow participants to prepare in advance for responding to the target. We compared these conditions with those where the anticipatory preparation was not allowed, by presenting the WS simultaneously with the target. If preparatory and intensity effects are the behavioural reflection of independent mechanisms, response preparation should not interact with the intensity manipulation.

Also, we expected the startling response to be elicited by the WS, at least in the highest intensity condition (113 dB). It is worth noting that the startle reflex is a complex sequence of muscular response and there are several methods to record and quantify the electromyographic startling activity (Blumenthal, Filion, Hackley, Lipp, & van Boxtel, 2005; Carlsen et al., 2011). In this study we measured the startle responses by recording the orbicularis oculi (OOc) blink activity, through the identification of a significant increment from baseline of OOc activation. In trials where the OOc activity indicate a startle response manifestation, we expected to detect behavioural changes in response readiness. We did not expect to observe an interaction between the startle reflex and the level of response preparation, following the idea that startle reflexes are controlled by specific and more automatic mechanisms, and it has been previously reported independently of temporal uncertainty (Cressman, Carlsen, Chua, & Franks, 2006).

These hypotheses were tested in three task settings requiring different levels of control. In Experiment 1, we used a simple detection task without catch trials. In Experiment 2, a detection task with catch trials was used instead to investigate the effects of the "dispreparation" state induced by catch trials (Correa, Lupiáñez, Madrid, & Tudela, 2006). Finally, in a third experiment, participants performed a Go-NoGo discrimination task, where a response was required to the some targets but not to the NoGo target, in order to investigate the possible role of the inhibitory processes invoked by the presence of NoGo targets.

EXPERIMENT 1

METHOD

Participants. Thirteen students (mean age: 22.6 years; age range 19-31 years; 3 males) from the University of Granada participated voluntarily. All participants were right-handed, had normal or corrected-to-normal vision and none of them reported neurological disorders. The experiment followed the ethical guidelines for the Department of Experimental Psychology and was conducted in accordance with the ethical standards of the Declaration of Helsinki (1964).

Apparatus and Stimuli. Stimulus presentation, timing and behavioural data collection were controlled by a computer running E-prime 2 software (Schneider, Eschman & Zuccolotto, 2002). The target, either a white O or X letter, was used as detection stimulus and was centrally presented for 40 ms in a monitor (BenQ FP731), located at approximately 60 cm from the participant. Responses were recorded by means of a handle joystick button. The warning signal consisted of a 40 ms auditory burst of white noise (virtually instantaneous risetime) presented binaurally. The sound was amplified using a Logitec X-540 sound system and delivered via headphones (Philips SHP 2000), connected to a 220/240 V~ Fender 15 amplifier.

Physiological measurements. The eyeblink is the most persistent component of the startle reflex in humans and is usually measured by electromyography of the orbicularis oculi muscle (OOc) (Bradley &

Sabatinelli, 2003). The OOc EMG activity was therefore recorded using an EMG 100G module integrated in the Biopac MP150 system and stored for offline analyses with Acqknowledge 9.3.1 software (Biopac System Inc.). Miniature silver/silver chloride electrodes were placed at the inferior eyelid of the left eye (Blumenthal, Cuthbert, Filion, Hackley, Lipp & van Boxtel, 2005). Frequencies below 28 and above 500 Hz were filtered out. Sampling rates were set at 1000 Hz. The EMG raw signal was then rectified, integrated and finally analyzed using a graphic Matlab program complying with a physiological accepted protocol (Balaban, Losito, Simons & Graham, 1986). The startle responses were measured as the difference between onset and peak μVolts values. Startle always followed a startle probe and stayed within the 21-120 ms window after the probe. The first 20 ms of data were scanned in order to determine whether during the trial onset the eyelid was stable or in motion. Baseline activity (mean: 5.9 ± 1.6 μVolts) was estimated considering the last 10 ms before the WS presentation. Trials were excluded if any eye movement was detected during the baseline recording. Any trial with a noisy baseline, with eye blinks, without a clearly detectable peak, with peak amplitude lasting more than 10 ms, with a peak beyond the 80 ms and with onset-offset duration larger than 100 ms was excluded. If a blink that marked the peak latency was shortly followed by a second one, the trial was also excluded. Table 1 shows the mean amplitude and onset peaks for startle trials as a function of Simultaneity and WS intensity.

Table 1. *Peak amplitude in Volts and peak latency in millisecond (mean ± standard deviations) in startle trials for each Simultaneity and WS Intensity level.*

WS Intensity	53 dB	83 dB	113 dB
	Simultaneous conditions		
Experiment 1	.0692±.0248 94.71±18.32	.0766±.0326 88.26±11.59	.0848±.0369 85.94±8.69
Experiment 2	.0518±.0094 90.67±12.29	.0489±.0074 89.67±12.69	.0799±.0378 87.53±8.51
Experiment 3	.0560± .0467 109± 21.69	.0521±.0116 86.14±11.26	.0651±.0339 89.48±10.50
	Delayed conditions		
Experiment 1	.0628±.0216 103.1±30.74	.0756±.0373 84.55±8.81	.0852±.0359 85.46±7.49
Experiment 2	.0764±.0418 85.17±4.02	.0614±.0212 89.08±13.64	.0818±.0389 88.19±8.75
Experiment 3	.0521±.0056 76.25±15.84	.0538±.0136 83.36±7.23	.0608±.0257 87.30±8.82

Procedure and design. Participants were asked to detect as soon as possible the target letter by pressing the top button of the joystick with their right thumb. Both targets (a white O or X letter) required the same responses and where randomly presented to the participants to avoid perceptual habituation.

The experiment consisted in a practice block and fifteen experimental blocks, yielding a total of 768 trials. The warning signal lasted 40 ms and was disclosed either synchronously with the target (simultaneous condition) or 1400 ms before the target (delayed condition). We manipulated the intensity of the white noise in three different conditions: 53, 83 and 113 decibels (dB). The levels of WS intensity and Simultaneity were equally presented and crossed in each experimental block. The target was either the letter X (1/2 of trials) or O (1/2 of trials), and lasted for 200 milliseconds. In order to avoid participant's response synchronization, we presented two empty displays at the beginning and at the end of each trial with a variable duration depending on RT, for all trials to have a 5 seconds duration. The movement required to respond was a button press with the flexion of the right thumb.

In our manipulation the distinction between the effect of Startle and WS intensity in shortening RT is essential. Thus, with the aim of correctly identifying trials with startle response from those without startle response, once the task was concluded, the complete OOc EMG activity was tacked and analysed following the physiological protocol. We selected as valid startle responses any with EMG peak amplitude larger than 0.04 µVolts (within the 21-120 ms window following a startle probe, see Blumenthal et al., 2005). Trials with peak lower than 0.04 µVolts or without peak recorded were considered as no startle trials. Table 2 shows the proportion of startle and no startle trials per condition.

Table 2. *Percentage of startle trials for each level of Simultaneity and WS Intensity*

	Simultaneous conditions			**Delayed conditions**		
WS Intensity	**53 dB**	**83 dB**	**113 dB**	**53 dB**	**83 dB**	**113 dB**
Experiment 1	0.39%	6.46%	45.36%	0.45%	3.97%	43.38%
Experiment 2	1.04%	1.78%	47.19%	0.59%	2.96%	46.45%
Experiment 3	0.16%	1.75%	51.52%	0.32%	1.12%	45.14%

RESULTS

Error analysis. Mean RTs and error rates for each experimental condition are shown in Tables 3 and 4. Due to the low error rate, errors were not further analysed.

RT analysis. Incorrect trials (anticipations: 1.2%; missed responses: 2.1%) and trials with RTs faster than 200 ms or slower than 1000 ms (0.6% of incorrect trials) were excluded from the analysis. The RT filter in the time window 200-1000ms is a procedure widely used in the attentional literature (i.e. Boksem, Meijman, & Lorist, 2005; Correa et al., 2006; Lalor, Kelly, Pearlmutter, Reilly, & Foxe, 2007; Aasen, Håberg, Olsen, Brubakk, Evenseng, Sølsnesg, Skranesg, & Brunner, 2016) and has been reported to include almost all measurable responses for tasks like the one used in our experiments (Ledgeway & Hutchinson, 2008). Trials with EMG peak amplitude categorized as startle response (16.8%) were also excluded. A Simultaneity x WS intensity repeated measures ANOVA was performed on mean reaction times. One participant reported only startle trials in one or more levels of the WS manipulation and the data from this participant was therefore eliminated from analysis. A main effect of WS intensity was found, $F(2,22)= 21.32$, $p<.0001$, $\eta_p^2=.66$, such that RT decreased linearly as the WS intensity increased, $F(1,11)=24.15$, p=.0005. The main effect of Simultaneity (F<1) and the Simultaneity x WS intensity interaction were not significant, $F(2,22)=1.18$, p=.3249, $\eta_p^2=.09$.

Table 3. *Mean reaction times ± standard deviations (in milliseconds) and errors percentages ± standard deviations in "no startle" trials for Simultaneity and WS Intensity factors. In Experiment 1, percentages of errors include anticipations and missed trials. In Experiment 2, they include anticipations, missed responses in target trials and false alarm response in catch trials. In Experiment 3, they include anticipations, missed responses in "response" trials and false alarms in "no response" trials.*

	Simultaneous conditions			***Delayed conditions***		
WS Intensity	***53 dB***	***83 dB***	***113 dB***	***53 dB***	***83 dB***	***113 dB***
Experiment 1	*363±11*	*338±38*	*333±53*	*360±44*	*345±52*	*337±45*
	0±0	*0±0*	*7.7±2.7*	*5±.5*	*.9±1.4*	*5±9.2*
Experiment 2	*377±35*	*358±30*	*333±34*	*392±29*	*386±32*	*390±31*
	4.2±6.7	*2.5±3.3*	*8.8±12.8*	*2.8±3.8*	*2.8±3*	*1.5±2.9*
Experiment 3	*378±39*	*371±37*	*370±35*	*368±49*	*364±47*	*344±45*
	6.5±3.2	*6.1±2.7*	*5.3±3*	*4.1±2.7*	*4.5±3.9*	*3.3±3.5*

The next analysis focused on the startle response. Due to the small number of startle trials in the 53 and 83 dB conditions (see Table 2), only trials from the 113 dB condition were considered for the Simultaneity (simultaneous vs delayed) X Startle (no startle versus startle) repeated measures ANOVA (see Table 4). With this level of intensity, startle responses indeed occurred in both the simultaneous (45.4% of trials) and the delayed condition (43.4%). The ANOVA showed no significant effect of Simultaneity, F<1, and a marginal effect of Startle, $F(1,11)= 4.12$, $p=.0674$, $\eta_p^2=.27$. On startle trials participants responded slower (mean RT: 353 ms) than on no startle trials (mean RT: 335 ms). This tendency was similar in both the simultaneous and the delayed conditions, as can be observed in Figure 1 and is suggested by the lack of Simultaneity x Startle interaction, $F<1$.

Table 4. *Mean reaction times ± standard deviations (in milliseconds) and errors percentage ± standard deviations for Simultaneity and Startle factors (only 113 dB conditions included). In Experiment 1 percentages of errors include all anticipations and missed trials. In Experiment 2 they include anticipations, missed responses in target trials and false alarm responses in catch trials. In Experiment 3 they include anticipations, missed responses in Go trials and false alarms in No go trials.*

	Simultaneous conditions		***Delayed conditions***	
Presence of Startle	***No startle***	***Startle***	***No startle***	***Startle***
Experiment 1	*333±53*	*346±50*	*337±45*	*359±75*
	7.7±2.7	*0±0*	*5±9.2*	*1±1.3*
Experiment 2	*329±34*	*347±28*	*392±31*	*407±31*
	8±6.7	*.6±3.3*	*.7±2.9*	*.4±3.8*
Experiment 3	*370±35*	*391±34*	*344±45*	*373±37*
	5.4±3	*5.1±6*	*3.3±3.5*	*6.4±7*

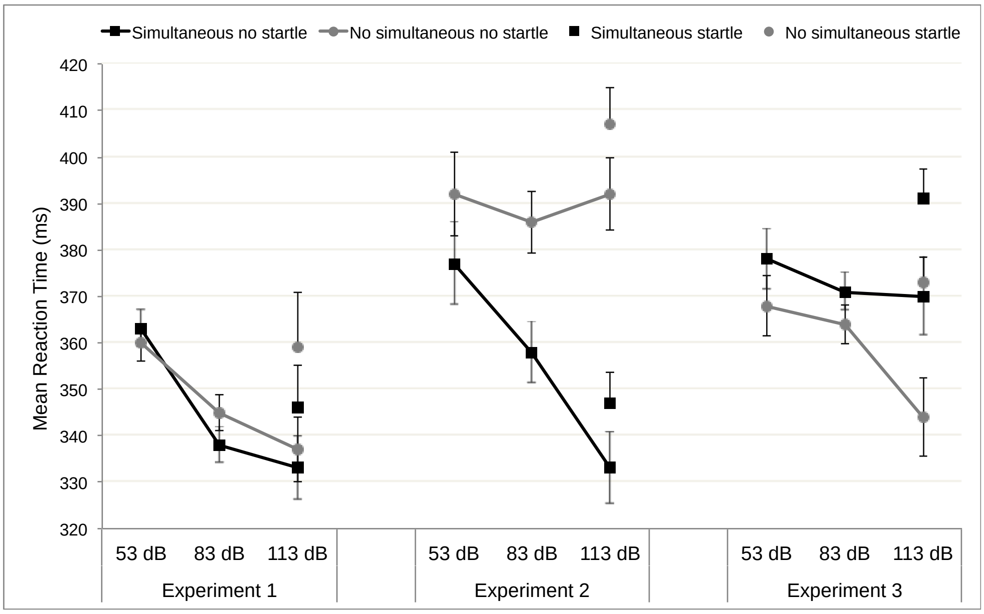

Figure 1. Relation between Simultaneity and WS Intensity across all experiments. Mean RT in Experiment 1(detection task), Experiment 2 (detection task with catch trials) and Experiment 3 (Go-NoGo discrimination task) as a function of WS intensities (53, 83 and 113 dB) for each Simultaneity and Startle level. Error bars represent standard error of the mean computed with Cousineau's (2005) method.

DISCUSSION

In line with our hypothesis, in Experiment 1 we found that responses to trials with high WS intensity were faster. Importantly, the effect was observed in the two Simultaneity conditions. Therefore, we could conclude that both response preparation and accessory characteristics of WS are mediated by independent mechanisms. However, no significant effect of Simultaneity was observed and therefore it seems that participants did not benefit from the temporal information supplied in the delayed condition, where the WS was presented before the target, to prepare their response in advance. One explanation for this lack of beneficial effect is that participants activated an automatic state of response at the beginning of each trial and were able to maintain that level of preparation for the whole trial. Since the target appeared in 100% of trials, in Experiment 1 the probability of making a mistake, besides missing or anticipating the response, was null. Thus, the level of response control applied was extremely low, which might explain the lack of interaction between Simultaneity and WS intensity.

Regarding the startle reflex, data from Experiment 1 showed a clear increase in RT for startle trials. This result contrasted with previous studies reporting RT shortening in conditions of startle reflex (i. e. Carlsen et al., 2004; 2007; 2011). It is important to note that in Experiment 1 participants were asked to respond by pressing a response button with the thumb´s flexion. This response is frequently required in behavioural studies concerning acoustic WSs in visual tasks, but is not usually the case for paradigms involving the startle reflex, where the more often responses are arm extension, wrist displacement and postural adjustments (Carlsen et al., 2004; 2007; 2008; Marinovic & Tresilian, 2016; for a review see Carlsen, 2011). Despite the fact that the type of motor response is usually neglected from the interpretation of startle effects on RT, it is possible that not only the task demand but also the type of response may have an influence on RT when a startle reflex occur, thus explaining why we observed a increase in RT instead of faster responses in this condition.

In any case, no matter the nature of the startle reflex on RT its mere presence allows the possibility to dissociate between the behavioural consequences of the intensity of the WS and the startle reflex. However, it is important to note that the effect was only marginally significant and therefore we cannot exclude the possibility that it might have been spuriously observed. Furthermore, no effect of simultaneity was observed, and therefore it was difficult to extract firm conclusions from this experiment.

EXPERIMENT 2

To test whether simultaneity (i.e., response preparation) and intensity of the WS did not interact because of a low or absent control setting that lead to absent response preparation, we designed a second experiment. In this new experiment, the Simultaneity and WS intensity manipulation remained unvaried from Experiment 1 but we added 1/3 of trials without target (catch trials), which required participants to hold the motor response on some trials. Catch trials intended to avoid the use of an automatic response strategy in order to reduce false alarms and anticipated responses and therefore required more response control. The level of control should be increased mainly after the WS presentation, i.e., in the delayed conditions, in order to avoid responding when no target was presented. Previous studies have also used catch trials to increase task control demands, leading to slower RT in conditions where response preparation is cancelled (Correa et al., 2006; Triviño et al., 2010).

Regarding the startle response, a replication seems necessary to investigate whether the increase in RT we reported for startle trials in Experiment 1 is confirmed in our procedure. Importantly, no matter whether a decrease or increase in RT is observed with startle, we expected the effect to be independent from both WS manipulations, in accordance with our hypotheses and results from Experiment 1.

METHOD

Participants. Eight students (mean age: 23.5 years; age range 18-30 years; 6 males) from the University of Granada took part voluntarily in Experiment 2. Participants were right-handed and had normal or corrected-to-normal vision.

Apparatus and Stimuli. Equipment and stimuli were the same as in Experiment 1.

Physiological measurement. Following the directions stated in Experiment 1, we measured the startle reflex elicitation as the μVolts difference onset-peak and we estimated the baseline (mean: 5.7±2 μVolts) considering 10 ms before WS. Trials with noisy baseline, eye blinks and protocol infringement (1.95 %) were eliminated.

Procedure and design. Stimuli duration and experimental procedure stayed unvaried. As in Experiment 1, three different levels of WS intensity and two levels of Simultaneity were orthogonally manipulated. The difference with the Experiment 1 consisted in the addition of 25% of catch trials, where no target was presented and participants were instructed to withhold responding until the end of the trial. In the remaining 75% of trials, as in Experiment 1, either the X or O letter was presented together with the WS (simultaneous conditions) or after a 1400 ms interval (delayed conditions).

ANALYSIS AND RESULTS

Means RTs and errors rates for "no startle" and "startle" trials in the 113 dB trials are presented in Table 3 and 4.

Error analysis. Errors were categorized as "anticipation" trials (0.9% of all trials), in which subjects responded before the target appearance; "false alarm" trials (1.3% of catch trials), in which subjects incorrectly responded to catch trials; and "missed" trials (3.6% of target trials), in which subjects did not respond to the target. The error analysis showed no significant main effect or interaction in false alarm and missed errors.

Error analysis. As in Experiment 1, we excluded from the RT analysis trials with incorrect response and those with responses faster than 200 ms or slower than 1000 ms (0.2% of correct target trials).

A Simultaneity x WS intensity repeated measures ANOVA was performed only considering trials classified as "no startle". The main effect of Simultaneity was now significant, $F(1,7)= 7.00$, $p= .0331$, $\eta_p^2=.50$, with faster responses for simultaneous than delayed conditions. The main effect of WS intensity was also significant, $F(2,14)= 4.79$, $p= .0260$, $\eta_p^2=.41$, with RT linearly decreasing as intensity raised, $F(1,7)= 7.01$, $p=.0330$. More importantly, the Simultaneity x WS intensity interaction was significant, $F(2,14)= 25.98$, $p<.0001$, $\eta_p^2=.79$: as can be observed in Figure 1, the intensity-related shortening of RT was observed only in the no simultaneous conditions, where the increased noise intensity was accompanied by a linear decrease of RT, $F(1, 7) = 23.14$, $p= .0019$. In contrast, no effect of WS intensity was observed for delayed conditions, $F<1$.

As in Experiment 1, the second ANOVA was performed only for 113 dB trials (see Table 2). One participant was eliminated due to the insufficient number of reported startle trials. The main effect of Simultaneity was significant, $F(1, 6)= 34.13$, $p=.0011$, $\eta_p^2=.85$. Although the main effect of Startle did not reach significance, $F(1, 6)= 2.18$, $p=.1899$, the tendency for slower RT in startle (377 ms) than in No startle trials (361 ms) was observed, as in Experiment 1. Again, the interaction was not significant, $F(1,6)=.19$, $p=.6776$, $\eta_p^2=.31$.

DISCUSSION

In line In line with our hypotheses, in no startle trials participants were faster as the intensity increased. Furthermore, a main effect of Simultaneity was found, with slower responses for delayed conditions. Furthermore importantly, the intensity effect was only observed for the simultaneous condition, with the no simultaneous condition showing no intensity effect. Previous studies have shown that catch trials modulate attentional orienting in time and in some cases inhibit the normal expression of temporal preparation (see Correa et al., 2004; Triviño, et al., 2010). In particular, catch trials generate target uncertainty and create a state of "dispreparation", which seems to prevent the orienting of attention at longer intervals. In other words, by including catch trials we induced in participants a state of increased response control, which helped to avoid responding before the target was presented. Note that this state of "dispreparation" was unnecessary in Experiment 1, where no catch trials were used and targets were presented in 100% of trials.

On the other hand, in Experiment 2 the pattern of results for the startle effect was similar to that reported in Experiment 1, with RT being slower in trials with a startle reflex response, although not significantly. Furthermore, this tendency to larger RT on startle trials was independent of the Simultaneity manipulation.

EXPERIMENT 3

The third experiment was designed to clarify the role of catch trials in response preparation, and thus further investigate the relationship between response preparation and the intensity and startle reflex effects. In Experiment 3 catch trials were replaced by NoGo targets, in which a number 8 was presented for which no response was required. As the discrimination of the NoGo target from Go targets (either the X or the O, as in Experiment 1 and 2) was necessary, we expected it to encourage participants to prepare in time. Indeed, a visual target-stimulus was always presented and therefore "dispreparation" would lead to either omission or commission errors (i.e., false alarms). Consequently, in Experiment 3 we expected RT to be faster in the delayed than in the simultaneous conditions. Note that to test our hypothesis regarding whether the intensity effect interact or not with other response preparation effects, observing the temporal preparation effect usually observed in the literature (i.e. faster RT when a WS anticipates the target appearance) was crucial.

In Experiment 2 the main effect of Startle was not significant and the interaction was unascertainable. Thus, before drawing any conclusion from this set of results, a further experimental confirmation is necessary. This was the second aim of Experiment 3, in which we expected to confirm the same tendency observed in Experiment 1 and 2.

METHOD

Participants. Eight students (mean age: 24.6 years; age range 19-33 years; 3 males) from the University of Granada took part in this study. Participants were right-handed, had normal or corrected-to-normal vision and no neurological disorders.

Apparatus and Stimuli. Equipment, stimuli and physiological measurement were the same as in Experiment 2. A display with a central white "8" number was used as NoGo target in substitution of catch trials. When the OOc activation value exceeding an amplitude threshold of 0.04 volts occurred, the trial was considered as startle response. A total of 2.28% of trials violated the reference protocol and were excluded from analyses (see Table 1).

Procedure and design. Participants were required to detect the presence of the X or O letters in the display using the handle grip button, taking care of not responding to NoGo targets, i.e., the "number 8", which was presented on 25% of trials. As in Experiment 1 and 2, conditions were equally balanced between the three levels of WS intensity and the two levels of Simultaneity.

RESULTS

Error analysis. Error rates on "no startle" trials for the three intensity conditions and on "startle" trials for the 113 dB condition are presented in Table 3 and 4. Errors were categorized as "anticipation" trials (0.1% of all trials) when participants responded before the target appearance, "false alarm" trials (12.5%) when subjects responded to a NoGo target, and "missed" trials (2.7%) when subjects failed to respond to Go targets. Only false alarm and missed errors were further analyzed. The analysis of missed response revealed no significant effects.

The Simultaneity x WS intensity ANOVA carried out on false alarms showed a main effect of Simultaneity, $F(1,7)=7.41$, $p=.0297$, $\eta_p^2=.51$, with fewer false alarms for the delayed condition. The effect of WS Intensity and the interaction were not significant (all p>.1). The analysis of Simultaneity x Startle (in 113 dB trials) showed no significant effects (all p>.1).

RT analysis. As in the previous experiments, incorrect trials and trials with responses faster than 200 ms or slower than 1000 ms (1.3% of correct response trials) were excluded from the RT analysis. Non target trials were also excluded from the analysis.

The Simultaneity x WS intensity ANOVA, where only "no startle" trials were considered, showed a significant main effect of WS intensity, $F(2,14)= 8.46$, $p= .0039$, $\eta_p^2=.55$; one more time, by increasing the WS intensity RT decreased linearly, $F(1,7)= 17.77$, $p= .0040$. Moreover, although the interaction was only marginally significant, $F(2, 14)= 3.42$, $p= .0616$, $\eta_p^2=.33$, the intensity effect was only observed in the delayed condition, $F(1,7)= 40.98$, $p=.0004$, but not in simultaneous conditions, $F(1,7)= 1.73$, p=.2299 (see Figure 1). Although RT was now faster in the delayed conditions (373 ms) than in the simultaneous one (359 ms), as predicted, the main effect of Simultaneity was not significant, $F(1,7)= 2.06$, $p=.1948$.

The second analysis (113 dB conditions only) with Simultaneity x Startle as factors showed a significant main effect of Simultaneity, F (1, 7)=9.70, $p=.0170$, $\eta_p^2=.58$, with faster RT for the delayed condition. Remarkably, the main effect of Startle was also significant, $F(1,7)=20.34$, $p=.0028$, $\eta_p^2=.74$; once more, participants were slower to respond when trials were categorized as "startle". This effect was observed in both Simultaneity conditions, as reflected by the lack of Simultaneity x Startle interaction, $F<1$. This result was perfectly in line with the outcomes of Experiment 1 and 2, where RTs increased in case of startle reflex release.

DISCUSSION

As previously observed in Experiments 1 and 2, when a startle reflex response was recorded, in Experiment 3 we reported a significant increase in RT. Importantly, the increase in RT induced by the startle reaction across experiments was independent of response preparation and task. This finding is in line with our original predictions and in sharp contrast with the intensity effect, also produced by the WS, which is quite robust (it was reported in the three experiments) but interacts in a complex way with preparation and the control set induced by task demands.

In line with our expectations, in Experiment 3 response preparation facilitated the behavioural expression of the intensity effect and we confirmed the dissociation between the effect of acoustic intensity and response preparation. Thus, importantly, the intensity effect was significant only in the delayed conditions. Across all three experiments, the task appears to be the key factor to interpret the presence of an interaction between preparatory and acoustic characteristic of the WS. Thus, we conducted an overall analysis including Experiment (1, 2 and 3) as a between-participants factor, in order to clarify the role of the task set on the expression of the WS intensity and WS-target simultaneity effects.

Overall RT analysis. A first analysis of variance was performed with Simultaneity and WS Intensity as within-participants factors and Experiment (1, 2 and 3 as levels) as between-participants factor, in trials classified as "no startle". The main effect of WS intensity resulted significant, $F(2,50)= 25.62$, $p< .0001$, $\eta_p^2=.51$; across the three experimental paradigms, faster responses were observed as sound intensity increased. The main effects of Experiment, $F(2,25)= 1.43$, $p=.2583$, and Simultaneity, $F(1,25)= 1.67$ $p=.2078$, were both not significant. Furthermore, the Simultaneity x WS Intensity interaction reached significance, $F(2, 50)= 4.13$, $p=.0220$, $\eta_p^2=.14$, although planned comparisons indicated that the intensity effect was significant for both the simultaneous condition, $F(1,25)=30.11$, $p<.0001$, and the delayed conditions, $F(1,25)=25.96$, $p<.0001$. The Simultaneity x Experiment interaction was also significant, $F(2,25)= 5.84$, $p=.0083$, $\eta_p^2=.32$, as well as the interaction between the three factors, $F(4, 50)=8.35$, $p<.0001$, $\eta_p^2=.40$. The significant interaction between the three factors statistically supports the idea that response preparation modulates the effect of WS intensity (i.e., faster RT for higher intensity) as a function of task set. Clearly, both the direction and magnitude of the Simultaneity x WS Intensity interaction seems to depend on task demands, as shown in the specific analyses of each experiment described above.

The second overall ANOVA was restricted to 113 dB trials to assess whether the startle reflex released by higher WS intensity affected the participant's readiness to respond, as a function of the task set. Like in the previous analysis, the Experiment was considered as a between-participants factor and Startle and Simultaneity as within-participants factors. We observed a significant main effect of Simultaneity, $F(1,24)= 5.69$, $p= .0253$, $\eta_p^2=.19$, but not of Experiment, $F(1,24)=1.39$, $p=.2674$. As in the previous analysis, the Simultaneity x Experiment interaction resulted statistically significant, $F(2,24)= 11.76$ $p= .0003$, $\eta_p^2=.49$. More importantly, in contrast to the effect of WS intensity measured on the no startle trials, the significant main effect of Startle, $F(1,24)= 14.01$, $p= .0010$, $\eta_p^2=.37$, was not modulated by any other factor (all $Fs<.1$).

These critical findings were confirmed with Bayesian analyses, which are especially relevant in case of non-significant effects with the null hypothesis testing approach. Importantly, the absence of statistical significance for an effect in the traditional null hypothesis testing approach is not evidence for the absence of such effect. However, Bayesian analyses help to assess whether our data either provide evidence favouring the alternative hypothesis (the larger the Bayes Factor -BF- the stronger the evidence), the null hypothesis (the lower the BF the stronger the evidence), or no evidence (BF between .33 and 3; see for references Jarosz & Wiley, 2014). Given the relatively high number for factors to be included in the Bayesian ANOVA to test the evidence against the three-way interaction, we report the Bayesian model averaging (see Wagenmakers et al., 2018). As shown in Table 5, the posterior probability of the models containing the Startle factor (i.e., P(incl|data)) is much higher than the a priori probabilities (i.e., P(inc)), thus leading to a high BF (27.820). Thus, taking the average across all candidate models, the data strongly support inclusion of the Startle model. In contrast, inclusion of the three way interaction model led to a reduction of the probability of this model after the inclusion (0.008), compared to the a priori probability (0.053), with a very low BF (.149), thus providing strong evidence against the three way interaction.

Table 5. *The Bayesian model averaging. The table includes the average models across all candidates containing that factor. Each BF sizes the increase in the odds of the models including each factor over the alternative models excluding it (see Wagenmakers et al., 2018).*

Effects	*P(incl)*	*P(incl|data)*	*BF $_{Inclusion}$*
Experiment	*0.737*	*1.000*	*2409.242*
Startle	*0.737*	*0.987*	*27.820*
Simultaneity	*0.737*	*1.000*	*3651.325*
Experiment * Startle	*0.316*	*0.152*	*0.389*
Experiment * Simultaneity	*0.316*	*1.000*	*8444.734*
Startle * Simultaneity	*0.316*	*0.230*	*0.646*
Experiment * Startle * Simultaneity	*0.053*	*0.008*	*0.149*

GENERAL DISCUSSION

In a series of three experiments we investigated the interaction between intensity effect of WSs, response preparation mechanisms and task setting, by controlling the presence of startle reflex. We presented an acoustic WS in three different intensities (53, 83 and 113 dB) and directly manipulated response preparation by using either no interval between WS and target or a 1400 ms interval between them. In Experiment 1 (simple detection task) the faster RT for the more intense WSs was observed in both the simultaneous and the no simultaneous conditions. However, in this experiment the target always appeared and the probability of incorrect responses was extremely low, thus leading to fast RT no matter whether WS and target were simultaneous or not. To test whether the lack of interaction between Simultaneity and WS Intensity was due to a low control setting, we ran Experiment 2, in which 25% of catch trials were added, and Experiment 3, in which 25% of the trials had a NoGo target to which participants had to withhold responding. In Experiment 2, the high intensity of WS led to shorter RT, but only in the simultaneous condition. As explained above, in this paradigm the use of catch trials possibly prevented the reorienting of attention and inhibited the normal preparatory process usually observed when the WS precedes the target (Correa et al., 2004; Triviño et al., 2010). Therefore, in Experiment 3 we used NoGo targets instead of catch trials, with the objective of eliminating the state of "dispreparation" induced by trials where the visual target was not presented. As expected, in Experiment 3 participants used the temporal information about the target presentation provided by WS and tried to avoid mistakes (e.g. false alarms and anticipations) by maintaining their response preparation active at the moment of target appearance. Consequently, we observed faster RT in the delayed condition, accompanied by a significant intensity effect. Finally, we ran an overall analysis to test the influence of task demand in the interaction between intensity and response preparation, concluding that the type of task modulates the interaction between simultaneity and intensity.

The first important finding from our experiments was the confirmation of the intensity and response preparation effects as two distinct aspects of alertness, together with the indication of a strong influence of task demand in their behavioural expression. In our first experiment (a simple detection task without catch trials) the target and the WS appeared simultaneously in 50% of the trials and it is likely that participants consequently started to get ready for the response at the beginning of each trial. As the target was presented in 100% of the trials, "dispreparation" would have been useless or counter-productive for participants. In fact, when the WS appeared alone (in the other 50% of the trials), they just had to maintain the preparation state until the target appeared, which always did. In fact, it has been shown that the level of motor hand readiness varies as a function of participant's expectancy (Burle et al. 2010). The maintenance of the high response preparation state seems to have been efficient in terms of hand readiness. A similar pattern is observed with no catch trials when a valid expectancy for the target to appear at a short interval is induced; responses are faster at the short interval and similarly fast at the longer interval (see, for example, Correa et al., 2006). This might thus explain why responses were faster overall in this experiment, as can be observed in Figure 1.

In contrast, catch trials generated target uncertainty and likely increased response control when the target did not appear together with the WS. Thus, in the delayed condition responses were slower; interestingly, the intensity effect was only observed for the simultaneous conditions. This seems to indicate that response control, as elicited by catch trials, induced a state of "dispreparation" for the delayed condition. As explained above, other studies have shown a modulation of temporal orienting attributable to catch trials (Correa et al. 2004; Triviño, et al., 2010). Furthermore, the "dispreparation" induced by catch trials made the intensity effect disappear. However, perhaps this is not the best way to test whether the intensity effect interacts or not with other response preparation effects in a paradigm where the preparation in time is discouraged. For this reason, in Experiment 3 we replaced the catch trials by NoGo targets and we finally confirmed the intensity effect and response preparation as two aspects of alertness, which nevertheless acted one more time in an interactive way. In this case, participants needed to control responding to the target-WS compound when they appear together in the simultaneous condition, in order not to commit false alarms on NoGo trials. This control over response preparation seems to have made disappear the intensity effect, now in the simultaneous condition.

In contrast to the intensity effect, which was clearly modulated by temporal orienting and the control induced by task set in an interactive way, another important result observed across the three experiments was the independence of the startle reflex response from any temporal information provided by the WS and the task manipulation. In all three experiments, when a startle reflex was recorded, RTs increased compared to when no startle was recorded, an effect that is opposite to the usual finding of RT speeding by the startle reflex (Carlsen et al., 2004; Carlsen et al., 2007). In any case, and importantly, the observed startle-related RT lengthening was not modulated by Experiment or the Simultaneity factor. The startle reflex produced an increase in RT that was robust across the three paradigms and independent from response preparation and task set manipulations, which fits with previous reports of Startle effects independently of temporal uncertainty (Cressman, Carlsen, Chua, & Franks, 2006).

Carlsen and collaborators studied the influence of startle in motor readiness and concluded that a startling stimulus represents the trigger for a faster release of previously prepared movements (Carlsen et al., 2011). Furthermore, a more recent study from his laboratory supported the physiological independence of startle reflex from the mechanism of response preparation (Drummond, Leguerrier, & Carlsen, 2016). Nonetheless, also in this study the direction of the startle RT effect was in line to the classic intensity effect (i.e., fastening RT) and opposite to our results. Some years before, Valls-Solé and colleagues proposed already the dichotomy between the startle response and the effects of a startling stimulus on reaction time (Valls-Solé, Kofler, Kumru, Castellote, & Sanegre, 2005). By analyzing the different response to prepulses, they suggested the existence of two separate phenomena for startle response and RT effects with high acoustic intensities. In particular, the fastening in RT might be the consequence of the progressive enhancement of excitability in the reticulospinal tract that takes place during movement preparation (Valls-Solé et al., 2005; Valls-Solé et al., 1999). In the same period, also Lipp and collaborators set the idea of independence between the RT facilitation and the startle reflex itself, claiming that they are dissociable (Lipp & Hardwick, 2003; Lipp, Kaplan, & Purkis, 2006). In general, as we found RT to be significantly slower in the startle than in the no startle condition, whereas more intense WSs led to faster RT, our outcomes sustain the dichotomy approach.

However, our results and those from previous studies reporting RT shortening in concomitance with startle reflex recording are in evident and sharp contrast. To analyse the possible reason behind this discrepancy it is crucial to understand how the startle reflex influences motor readiness, and the methodological differences between our manipulation and those from other studies. At least three methodological differences are worth considering. The first one is the locus of RT recording. In Carlsen´s studies (Carlsen et al., 2004; 2007; 2011) the response was recorded at premotor level. The premotor time is the interval between stimulus onset and the onset of EMG motor activity (Burle et al. 2010). Differently, in our series of experiments, the participant´s response was directly recorded from the key pressing, that is, the motor response. The motor response is considered as the sum of the premotor time and the motor time, i.e., the time interval between the onset of the first EMG burst and the start of the mechanical execution of the movement (Burle et al. 2010; Van der Molen, Bashore, Halliday, & Callaway, 1991). As consequence, RT in our experiments represent the sum of premotor and motor processes, which could be affected differently by the startle reflex. Thus, although unlikely, an effect startle on motor time larger and in the opposite direction to that on premotor time could explain ours and the previous results.

The second methodological difference is the identification of the startle effect through the OOc muscle. This method is diffusely widespread and accepted in the psychofisiological literature (Balaban et al., 1986; Blumenthal et al, 2005; Lang et al, 1990) and was thus chosen for the startle measures in the present study. However, in some cases the reference for the classification of startle reflex response are other muscles, as the esternocleidmastoid muscle activation (for a detailed explanation see Carlsen et al., 2011). This difference is extremely relevant, also considering the use of different criteria of electrophysiological measures (i. e., microvolt, signal filtering) for the classification of a muscular response as startle reflex.

Third, and perhaps more importantly, in Carlsen´s experiments the movement to perform was usually the arm extension or the angular displacement outwards the starting position of the right wrist (e. g. Carlsen et al. 2004; 2007). This means that the movement required for the response was the extension of limbs toward the external space. As a consequence, the movement in these studies is considerable as an external, outward, open movement. But in our studies participants held in the right palm a handle with a topper response button, and responded by pressing it with the thumb´s flexion. This is considered as a proximal, inward, movement. The type of movement required in an experimental task is a crucial aspect of the study because across evolution the natural selection developed complex behavioural sets, in order to dispose the organism to avoidance, escape and defence. It is possible that there are substantial differences between the startling stimuli exposition in case of flexion movements and extension movements. In fact, the startle reflex to a sudden noise is viewed as an aversive or defensive response and would be augmented in case of both ongoing aversive emotions and attentional allocation of foreground tasks (Lang, Bradley, & Cuthbert, 1990). We suggest that an aversive/defensive behaviour, as the startle reflex, is positively associated to movement patterns of avoidance (as the usual response used in previous experiments), and negatively associated with approaching patterns of movements (as the one used in our experiments). Future research should confirm the plausibility of this explanation of the contradictory results.

Before concluding, some methodological and theoretical limits of our experiments could be highlighted. First, our study included in the analysis a high number of observations per participant but a small number of them. This is an important issue, as a small sample size leads to low statistical power, which decreased the chance of discovering effects that are genuinely true, at the same time that increases the likelihood of falsely disclosing some non true effects. It is important to note, however, that the sample sizes used in our experiments are not unusual in this literature (see, for example, samples sizes of 8 participants in Carlsen et al, 2004, 10 in Carlsen et al, 2007, and 8 participants per group in Correa et al., 2006). Furthermore, the combined null hypothesis testing analysis of the data from the three experiments somehow solved the problem of sample size, leading to clear and robust intensity and startle effects, and a clear modulation of the former by simultaneity and tasks set (i.e., Experiment). On the other hand, Bayesian analysis was used to tackle the problem of the non-significant three-way interaction in the analysis of the startle reflex data, which provided strong evidence that the effect of startle on RT is independent from the modulation of simultaneity and task set.

Second, in our experimental design we manipulated two intervals between WS and target (0 and 1400 ms). However, in the analysis of attentional preparatory processes, it might be highly informative to have an intermediate short interval, that creates a context of temporal unpredictability. Within a combined paradigm with several WS-target intervals, a recent study examined the effect of alerting intensity on visual processing speed and threshold of conscious perception (Petersen, Petersen, Bundesen, Vangkilde, & Habekost, 2017). In this study, the pupil size was measured as a physiological marker of alertness and the increase in pupil size was associated to faster processing speed. Interestingly, the study reported a significant difference in pupil size between conditions without warning and those with a low intensity warning (40 dB), and between those with low and intensity warning (85 dB). Importantly, these differences were reported both for the early WS-target time window (300–800 ms after warning onset) and the late time window (800–2000 ms). Therefore, the processing speed seems to be affected by the intensity manipulation, both for short and long intervals after the WS. Thus, the next question would be whether pure alertness induced by intensity interacts with the process of response preparation in conditions of high temporal unpredictability, as a function of task set (as in our experiments), and whether this interactive modulation would also affect pupil dilation, not only RT. This debate is especially relevant also in relation to the startle reflex, as it has been shown that startling stimuli, in conditions of short temporal intervals, reduce the amount of muscle activation, delaying the preparation and execution of upcoming response (Maslovat, Drummond, Carter, & Carlsen, 2015).

To sum up, from the pattern of results obtained across our series of three experiments, we concluded that the task setting is critical to study the interaction between preparatory and perceptual aspects of alertness. In fact, task set clearly affected the behavioural expression of both response preparation and WS intensity. The phasic alertness, often defined as a purely automatic mechanism, has been demonstrated to be influenced by response preparation induced by temporal expectancies and temporally irrelevant characteristics of the warning signal as its intensity, which are both modulated in a complex way by task set. In contrast, response preparation and task set did not affect the startle response, for which RT increased in the opposite direction to the intensity effect and independently from the WS-target simultaneity and task set manipulations. In general, our findings supported the interaction between acoustic and response preparation mechanisms related to the presentation of a warning signal, as a function of task set, as well as the independence of the startle reflex response from any task-related manipulation.

Ethical Standards: the manuscript does not contain clinical studies or patient data.

RESUMEN

Efectos diferenciales de los componentes de intensidad y preparación de la respuesta para señales de alerta acústicas.

El aumento de la intensidad en una señal de alerta (warning signal, WS) generalmente disminuye los tiempos de reacción al estímulo objetivo y ocasionalmente está acompañada de un reflejo de sobresalto que influye en la rapidez de la respuesta. En tres experimentos, con tarea de detección simple (Experimento 1), de detección con ensayos sin estímulos objetivo (Experimento 2) y de discriminación “Go-NoGo” (Experimento 3), hemos estudiado la relación entre los mecanismos de preparación de la respuesta y de alerta que ocurren tras la presentación de señales de alerta. Se presentó una WS simultáneamente al target (condición simultánea) o con una antelación de 1400 ms (condición demorada). En los tres experimentos se manipuló ortogonalmente la intensidad de la WS y el intervalo entre WS y estímulo objetivo.

Los resultados confirmaron tiempos de reacción más cortos con el aumento de la intensidad de la WS. En el Experimento 1, todas las condiciones presentaron un claro efecto de intensidad acústica. En el Experimento 2 observamos tiempos de reacción más cortos en condiciones de mayor intensidad, pero solo cuando WS y estímulo objetivo se presentaron simultáneamente. En el Experimento 3, el efecto de intensidad se observó únicamente cuando la WS precedía al target. En todos los experimentos, los ensayos donde la WS provocaba un reflejo de sobresalto mostraron un aumento sistemático de los tiempos de reacción, que era independiente de la preparación de la respuesta y del tipo de tarea. En general, nuestros resultados sugieren que la preparación de la respuesta modula los mecanismos de alerta, en función de la tarea, pero no el reflejo de sobresalto. La disociación entre intensidad, preparación de la respuesta y sobresalto apoya la hipótesis de interdependencia entre estos mecanismos, provocados por la presentación de la señal de alerta.

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