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Orientação da atenção seletiva visual por pistas auditivas em ratos

São Paulo
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Ph.D. Thesis presented at the University of São Paulo, Institute of Biosciences, Brazil, to obtain the title of Doctor of Sciences. Concentration area: General Physiology.

Advisor: Gilberto Fernando Xavier

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Prof(a). Dr(a).:_____.

Institution:_____.

Decision:_____.

Prof(a). Dr(a).:_____.

Institution:_____.

Decision:_____.

Prof(a). Dr(a).:_____.

Institution:_____.

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Dedication

To my nuclear family–
Conceição, Deraldo and Simão –
for inspiring me to follow this path
and for doing their best to give me
all the support I needed to succeed.
To my spiritual brother – Natan – for
taking and throwing many
punches for and with me.
To my spouse – Zeni – for
helping me become a better human
being.
To my master – Jesus – for
teaching me how to live.
To God, for allowing me to be
co-creator of myself.

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A minha família nuclear – Conceição, Deraldo e Simão – por me inspirarem a seguir este caminho e por fazerem o melhor que puderam para me dar todo o suporte que eu precisava para vencer.

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A Deus, por me permitir ser cocriador de mim mesmo.

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“I promise that, for the rest of my existence, I will express my gratitude for all individuals that somehow contributed to me during this work through my efforts of becoming a better human being and making the world a better place”.

Although that sentence is true, it did not seem enough as an acknowledgement.

After giving it some thought, and reading other acknowledgements of other people, I realized that there is something unique about thanking people by name. Our names act as a unique identifier for our identities, such that saying or writing a person’s name makes direct reference to all that person represents in the real world. So by thanking someone by name, I would not only be acknowledging that the effort the person made to help me finish my work was crucial to me, but saying that that person was essential for me to be where I am; and that feels powerful.

Besides that, although this work is not a part of me, it is a product of who I am. It is what it is because I became who I needed to be to complete it, and I would not be who I am today without the help of many people.

These realizations made me want to also evoke the names of those that, by being who they are and being where they were, allowed me to be a person I needed to be to finish this work.

So here we go.

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Thank you, Natan, for being my brother, not by blood, but by heart and always being there for me. Your love makes me feel truly at home, whenever and however I am.

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Thank you, “Comunidade Espírita Arvorecer”, for teaching me to pursuit my true calling.

Please note that if you feel sad I did not cite your name here, it is because you should be here. I am sure that my memory has failed me more than once and I hope you can forgive me for that.

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Inicialmente, tudo o que eu ia escrever aqui era:

“Prometo que, pelo resto da minha existência, vou expressar minha gratidão a todas as pessoas que de alguma forma contribuíram para mim durante este trabalho através do meu esforço para me tornar um ser humano melhor e fazer do mundo um lugar melhor”.

Embora essa frase seja verdadeira, não pareceu um agradecimento suficientemente bom.

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Essas constatações me fizeram querer também evocar nominalmente as pessoas que, sendo quem são e estando onde estavam, me permitiram ser a pessoa que precisava ser para concluir este trabalho.

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Se você que lê esse agradecimento se sente triste por eu não citar seu nome aqui, é porque você deveria estar aqui. Tenho certeza de que minha memória, que já é muito falha, falhou mais uma vez e espero que você possa me perdoar por isso.

Epigraph

To live is to learn; and to learn is to try to live better."

(Nyerere, J. K. (1973). Adult education year. *Freedom and development*. p. 138)

Abstract

Cruz, M. T. (2022). Orienting of visual selective attention following auditory cues, in rats (Ph.D. Thesis). Institute of Biosciences, University of São Paulo, São Paulo.

Orienting of **selective attention** is a widely studied phenomenon in the Neurosciences. Studies in both humans and non-human animals, employing a wide range of methods – from behavioral tasks, to optogenetic manipulations –, have advanced the knowledge in the area. Thanks to these advances two main types of orienting of attention have been describe, **endogenous orienting**, which depend on the internal expectations of the animal about its environment, and **exogenous orienting**, which depends on how salient environmental stimuli are. Although important advances have been made, the understanding of both the phenomenology and physiological underpinnings of selective attention is far from complete. Animal models in biological studies have historically allowed great leaps forward for scientific knowledge. The present work adds to the field of attention by further investigating the use of in rats in the study of attention. Specifically, by using a behavioral task – a Posner-like covert orienting of attention task using auditory cues and visual targets – we investigated select attention shifts between two different sensory modalities, i.e., vision and hearing. In Experiment 1 experimentally demonstrated, for the first time, that exogenous orienting of visual attention by auditory stimuli is possible in rats. Experiment 2 investigated how exogenous and endogenous orienting are affected by whether visual and auditory stimuli are presented at the same or at different locations, i.e., by their degree of spatial superposition. The results show that the intermodal attentional shift does not seem to depend on presentation of the auditory cue and visual target exactly in the same location. By bringing to light these previously unknown aspects of the orienting of attention in rats, this study contributes to the use of this animal model in future investigations of the physiological underpinnings of attention.

Palavras-chave: Posner task, Covert orienting of attention task, Operant conditioning, Mental chronometry, Spatial orienting.

Resumo

Cruz, M. T. (2022). Orienting of visual selective attention following auditory cues, in rats (Ph.D. Thesis). Institute of Biosciences, University of São Paulo, São Paulo.

A orientação da atenção seletiva é um fenômeno amplamente estudado nas Neurociências. Estudos em humanos e animais não humanos, empregando uma ampla gama de métodos – desde tarefas comportamentais, até manipulações optogenéticas –, têm ampliado o conhecimento na área. Graças a esses avanços, dois tipos principais de orientação da atenção foram descritos, a orientação endógena, que depende das expectativas internas do animal sobre seu ambiente, e a orientação exógena, que depende de quão salientes são os estímulos ambientais. Embora avanços importantes tenham sido feitos, a compreensão tanto da fenomenologia quanto dos fundamentos fisiológicos da atenção seletiva está longe de estar completa. Modelos animais em estudos biológicos têm, historicamente, permitido grandes avanços no conhecimento científico. O presente trabalho contribui para o campo da atenção, investigando o uso de em ratos no estudo da atenção. Especificamente, usando uma tarefa comportamental – uma tarefa de orientação encoberta da atenção do tipo Posner, usando pistas auditivas e alvos visuais – investigamos deslocamentos da atenção seletiva entre duas modalidades sensoriais diferentes, i.e., visão e audição. O Experimento 1 demonstrou experimentalmente, pela primeira vez, que a orientação exógena da atenção visual por estímulos auditivos é possível em ratos. O Experimento 2 investigou como a orientação exógena e endógena é afetada por estímulos visuais e auditivos serem apresentados no mesmo local ou em locais diferentes, ou seja, pelo seu grau de superposição espacial. Os resultados mostram que o deslocamento da atenção intermodal parece não depender da apresentação da pista auditiva e do alvo visual exatamente no mesmo local. Ao trazer à luz esses aspectos até então desconhecidos da orientação da atenção em ratos, este estudo contribui para o uso deste modelo animal em futuras investigações das bases fisiológicas da atenção.

Palavras-chave: tarefa de Posner, tarefa de orientação encoberta da atenção, condicionamento operante, cronometria mental, orientação espacial.

List of Figures

List of Acronyms

ANOVA	Analyses of Variance
GLMM	General Linear Mixed Models
IOR	Inhibition Of Return
IQR	interquartile range
ITI	Inter-Trial Interval
LED	Light-Emitting Diode
MT	Movement Time
NP	Non-Predictive
OCC	Operating Conditioning Chamber
P	Predictive
RT	Reaction Times
SEM	Standard Error of the Mean
SOA	Stimulus-onset asynchrony

Summary

1 Introduction

At every moment of our everyday lives, we are continuously facing countless sensory stimuli. As only a portion of them is relevant, the ability to focus only on the most relevant stimuli at each time is crucial to behave optimally. This capacity must also be flexible because a stimulus that is important in a given context might not be in another. For example, traffic lights are extremely important when one is driving a car, but not important when one is having a conversation in a nearby coffee shop. This flexible and selective ability of either filtering out or enhancing certain stimuli depending on the context is called selective attention, or simply attention.

William James, the father of Northern American psychology, was one of the first to scientifically investigate this phenomenon. In his “The Principles of Psychology” he summarizes the nature of attention in a quote that has already became a classic in the field:

> Everyone knows what attention is. It is the taking possession by the mind in clear and vivid form, of one out of what seem several simultaneously possible objects or trains of thought....It implies withdrawal from some things in order to deal effectively with others. (James, 1890)

This quote illustrates an important aspect of attention: the fact that it can be voluntarily oriented (“taking possession by the mind in clear and vivid form”) towards specific “objects or trains of thought”. This is called **endogenous orienting** and is how we direct attention when reading a book or while looking for someone wearing a red hat in a crowd. Attention, however, can also be “caught” automatically by salient features, like strong colors, movements, flashing lights. This is what happens when someone suddenly barges in through a door of a room we are in or when someone blows a horn near you in traffic. In this case, **exogenous orienting** is captured without direct influence of volition. These two different ways of directing attention interact constantly, allowing us to keep our mental resources, which are limited (see Carrasco, 2011), in what is important for our current goals while also keeping track of sudden events that might be important.

1.1 HOW ATTENTION HAS BEEN INVESTIGATED?

In 1980, Michael I. Posner, a Northern American psychologist, described a novel behavioral task, the covert orienting of attention task (Posner, 1980), that allowed the detailed investigation of these types of orienting and that still is very influential on how attention is experimentally studied to these days. By using it, he showed that selective attention could be investigated in humans by using a visuospatial task in a computer screen.

In a typical Posner task (see Chica et al., 2014 for a review) the subjects are exposed to several trials in a session. In each trial their goal is to, whilst maintaining their eyes fixated at the center of the screen, report the appearance of a visual stimulus, referred to as "**target**", by pressing a button. These targets occur randomly in predetermined areas of the screen, typically to the left or right of the fixation point. Critically, before the target appearance, a different visual stimulus, hereinafter referred to as the "**cue**", is presented, which provides information about the future location of the target. These cues may be **valid**, when they correctly indicate target location, or **invalid**, when they incorrectly do so. Posner and many other authors showed that targets preceded by valid cues generate shorter Reaction Times (RTs) and greater accuracy when compared to targets preceded by invalid cues, indicating that the subjects oriented attention towards the place indicated by the cue, responding faster and more precisely when this information led them to the correct target location (e.g. Bartolomeo et al., 2007; Berger et al., 2005; Carrasco, 2011; Dragone et al., 2017; Folk et al., 1992; Jonides, 1981; Juola et al., 2000; Luck et al., 1996; Martín-Arévalo et al., 2013; Meyberg et al., 2015; Posner, 1980). Differences in RTs (RT in invalid trials "minus" RT in valid trials) are usually referred to as "**validity effects**" and its magnitude can be used as a proxy of the influence of attention on the task being.

The most important feature of this task is that it allows studying orienting of attention independently from the movement of the sensory surfaces – the eyes, in this case. This is why it is called a **covert attention** task, in opposition to **overt attention** tasks , where there is concomitant orienting of sensory surfaces and attention (Luck & Vecera, 2002; MacInnes et al., 2020). By controlling the position of the sensory receptors, the covert task allows ascribing any effects to attention shifts and not to changes in sensory reception. Thanks to these characteristics, this task gained

widespread popularity, enabling important advances not only in the investigation of visual attention (see Carrasco, 2011 for a review), but also in auditory attention (e.g., McDonald & Ward, 1999; Mondor & Zatorre, 1995; Spence & Driver, 1994), tactile attention (e.g., Spence & Gallace, 2007; Tassinari & Campara, 1996) and also in the study of cross-modal attention shifts (e.g., Spence et al., 2000; Störmer, 2019; Tassinari & Campara, 1996).

The task allows fine-tuned assessment of different aspects of attentional orienting, including its time course, predictability of the pending events and types of cues.

The **Stimulus-Onset Asynchrony** (SOA), in this task, is the time interval between the cue onset and the target onset, such that a SOA of 0 ms means that both stimuli are presented simultaneously and a SOA of 1000 ms means that the onset of the cue precedes the onset of the target by 1000 ms. As one may suppose, it is possible to investigate the time course of orienting of attention by measuring the reaction time to the target and accuracy of performance using different SOAs (e.g., Z. Wang & Theeuwes, 2012). It is often assumed that SOAs shorter than 50 ms rarely elicit any validity effects in humans, and that longer SOAs do it. This delay in the appearance of validity effects would reflect the time required for engaging attention. Thus, when cue and target occur too close in time there is not enough time to shift the focus of attention. The way orienting of attention affects perception after these ~50 ms depends on both predictability and type of cue (see below).

Predictability refers to the likelihoods the cue informs either validly or invalidly the pending target location. When exposed to a **predictive** scheme, i.e., the percentage of valid trials is greater than that of invalid trials (e.g., 80 % valid and 20 % invalid), subjects trend to “trust” the information brought by the cue and orient spatial attention accordingly. When exposed to a **non-predictive** scheme, with the same percentage of valid and invalid trials (i.e., 50 % valid and 50 % invalid), subjects tend to “ignore” the cues, at least relative to spatial signaling, since the cues do not bring any relevant spatial information about the pending target location. Therefore, it seems natural to suppose that predictive schemes generate validity effects. In fact, this occurs in most of times. However, this does not necessarily mean that non-predictive schemes do not induce validity effects, since this depends largely on the type of cue.

Different types of cues, including symbolic and peripheral, have been used to investigate selective attention. **Symbolic** cues correspond to arbitrary symbols which

meaning is acquired through learning. Any symbol can be used. For instance, high-pitched beep could mean left and a low-pitched beep mean right; a square could indicate left and a circle indicate right; an “A” letter could mean left and a “B” letter could mean right; and so on. **Peripheral** cues, on the other hand, are salient stimuli which appearance, by itself, capture attention independently on the likelihood of indicating or not the pending target location. These type of cues come in many shapes and forms and usually occur in the immediate vicinity of the target. A classical visual example is the thickening of a peripheral box inside of which the target appears (e.g., Luck et al., 1996). Sounds with identifiable origin have also been used as peripheral cues (e.g., Hillyard et al., 2016; Störmer et al., 2009).

Combinations involving cue types, predictability schemes and SOAs have contributed for distinguishing different forms of orienting of attention.

For instance, in a covert orienting of attention task using symbolic auditory cues, a bilateral low-pitched beep indicates that a pending visual target, e.g., bright dots, may appear in the left visual field, and that a bilateral high-pitched beep indicates that the pending visual target may appear in the right visual field. Here, the cue is symbolic because its meaning has to be acquired (note that the association could be the reverse, i.e., low-pitch indicating right and high-pitch indicating left). If a non-predictive scheme (i.e., 50% of trials are valid and 50% of trials are invalid) is implemented using this type of cue (which spatial source is unidentifiable), no validity effect would be found. This is related to the fact that symbolic cues require learning in order to allow orienting of attention and, as the non-predictive scheme presents no pattern between cue presentation and pending target location, there is no association to be learned and, thus, no attentional effects are found (Jonides, 1981; Luck & Vecera, 2002).

Differently, a predictive scheme using symbolic auditory cues and visual targets should produce validity effects, since there would be a consistent pattern between the beeps and the location of dots. For instance, a high-pitched beep preceding a bright dot on the left in most trials, would allow, after some trials, prediction about the pending target location and thus orienting of attention to the cued side. This would render response to the target faster and more precise when the cue is valid, as compared to when it is invalid. This improved performance in valid trials is sometimes called **facilitation.** It has been considered an **endogenous** orienting of attention because it depends on the subjects' internal expectations about the meaning of the stimuli, which

is formed through learning (Chica et al., 2014; Luck & Vecera, 2002). These validity effects usually begin around SOAs of 150 ms and last for several seconds (Doallo et al., 2004; Posner, 1980; Remington & Pierce, 1984; Shulman et al., 1979).

Another possible combination of cues and targets may involve peripheral auditory cues, for instance either low-pitched or high-pitched beeps, laterally and randomly presented close to the location where the visual target later appears. Differently from the symbolic non-predictive cues, peripheral stimuli do generate validity effects even when half of the trials are valid and the other half are invalid (i.e. they are non-predictive). Presentation of the cue peripherally, near the location of the pending target, captures attention in an **exogenous** manner (Chica et al., 2014; Luck & Vecera, 2002). This facilitation effect appears at SOAs shorter (of about 50 ms) than those observed for endogenous orienting of attention, leading to the interpretation that exogenous orienting of attention is quicker. This exogenous capture of attention, however, seems transient and last for about 100 ms, waning shortly after. Therefore, peripheral non-predictive cues seems to promote “automatic” capture of attention due to cue saliency, showing facilitation effects at short SOAs (Posner & Cohen, 1984; Wright & Richard, 2000). Interestingly, under certain circumstances, non-predictive peripheral cues associated with longer SOAs (about 300 ms) may reverse the validity effect, i.e., reaction times in trials using invalid cues are shorter as compared to those seen in trials using valid trials (Posner & Cohen, 1984; Wright & Richard, 2000). This effect, known as **Inhibition Of Return** (IOR), demonstrated both for cues and targets of the same sensory modality and cues and targets of different sensory modalities (Spence et al., 2000; Spence & Driver, 1998), is thought to promote attention orienting towards novel spatial locations by inhibiting orienting towards recently attended locations (Martín-Arévalo et al., 2013).

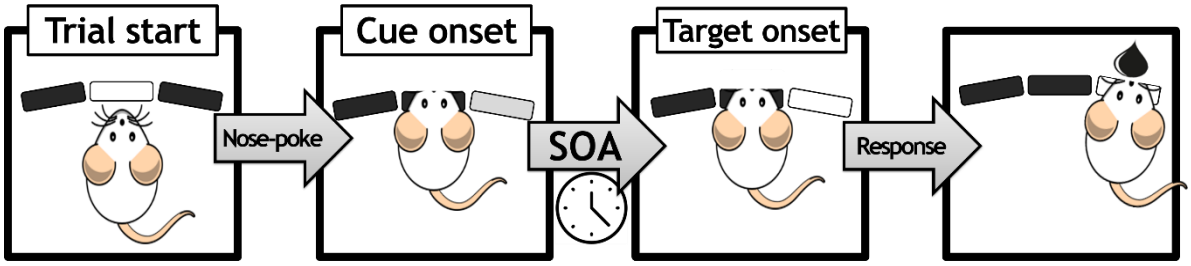
Finally, peripheral cues associated with predictive schemes promote, as expected, facilitation effects at short SOAs related to capture of exogenous attention by peripheral cues, and also endogenous orienting of attention at longer SOAs, associated with the predictability of the scheme (Bartolomeo et al., 2007; Luck & Vecera, 2002).

1.2 THE USE OF RATS IN THE INVESTIGATION OF ATTENTION

The Posner task has been adapted for non-human primates (Bowman et al., 1993), fish (Gabay et al., 2013), chicken (Sridharan et al., 2014), mouse (Li et al., 2021; L. Wang & Krauzlis, 2018) and rats (Rosner & Mittleman, 1996; Ward & Brown, 1996). These adaptations have allowed further investigations on the mechanisms and neural substrates of attention in ways not possible with humans.

Rats have been widely employed as experimental models in science, mainly due to their relative similarity to humans, small size, ease of maintenance, short life cycle, and relatively complex behavioral repertoire (Aitman et al., 2016; Ellenbroek & Youn, 2016). It is not surprising, therefore, that it was one the first animal models to be adapted to attention research. The Posner task for rats involves an operant conditioning chamber (OCC) with at least three holes in a wall, each hole equipped with a light source on its end (see Figure 2 for more details); these holes serve as "fixation point" (central hole) and targets (lateral holes). A typical trial (Figure 1) involves the animal nose-poking the central hole, receiving a visual cue (a dim light) through the lateral holes, keeping the nose poke for a SOA and then making a lateralized response to the target (a bright light) presented in one of the lateral holes (Rosner & Mittleman, 1996; Ward & Brown, 1996).

Figure 1 – Typical trial stages in a covert orienting of attention task in rats.



Source: Mateus Torres Cruz.

Early studies with rats employing the adapted Posner task investigated the role of different brain regions, or neurotransmitter systems, on orienting of attention, including the parietal posterior cortex (Rosner & Mittleman, 1996; Ward & Brown, 1997), striatal dopamine (Ward & Brown, 1996), thalamic reticular nucleus (Weese et al., 1999), cholinergic neurotransmission (Phillips et al., 2000) and the subthalamic nucleus (Phillips & Brown, 2000). Even though none of these studies investigated the phenomenology of attentional orienting, data allowed evaluation of hypothesis related

on how rats orient attention. Collectively, these studies suggest that rats, similarly to humans, exhibit validity effects when exposed to the task, responding faster and more accurately in valid trials, as compared to invalid trials. Further, when the peripheral cues were non-predictive, validity effects did not appear at SOAs longer than 400 ms (Phillips et al., 2000; Weese et al., 1999), whilst validity effects for predictive cues extended to SOAs up to 1500 ms (Rosner & Mittleman, 1996; Ward & Brown, 1997).

Marote and Xavier (Marote & Xavier, 2011) were the first to directly investigate the phenomenology of attentional orienting in rats. Their results corroborated previous hypotheses that predictive cues tend to generate facilitation at longer SOAs, as compared to non-predictive cues. These authors suggested that, similarly to humans, rats exhibit short-lived validity effects for peripheral non-predictive cues interpreted as exogenous orienting of attention and more persistent validity effects for peripheral predictive cues interpreted as “endogenous-like” orienting of attention. They did not risk to refer to this latter effect as properly endogenous because when using peripheral cues, exogenous effects are always present which may confound with endogenous orienting of attention at intermediate SOAs. To have an unequivocal answer related to endogenous orienting of attention in rats, it would be necessary to employ symbolic predictive cues, that do not capture exogenous attention.

In order to evaluate the effectiveness of symbolic cues for promoting orienting of endogenous attention in rats, a task using auditory cues and visual targets was implemented (Cruz, 2017). In contrast to previous studies where dim lights were used as visual cues, in this novel task pure-tone sound beeps with different frequencies were employed as symbolic auditory cues. When beeps predicted the location of the visual target, there were validity effects. In other words, when a high-pitched beep preceded a visual target to the right in 80% of trials and a low-pitched beep preceded a visual target to the left in 80% of trials, subjects improved their performance (there was a reduction in reaction times and an increase in accuracy) in valid trials as compared to invalid trials[1]. Differently, when beeps were presented non-predictively to a different group of animals, no validity effects were seen. These results showed that rats do orient attention in a purely endogenous manner.

[1] The frequency of the beeps and the side they indicated for the impending visual target were counterbalanced, such that it is not possible to explain the results solely based on the frequencies employed.

1.3 THE SCOPE OF THIS WORK

Our previous study (Cruz, 2017) also included independent groups of subjects exposed to predictive and non-predictive peripheral auditory cues. While validity effects were seen for subjects exposed to peripheral predictive cues, there were no validity effects for subjects exposed to peripheral non-predictive cues. Together, these results suggested that peripheral auditory cues do not lead to capture of visual attention exogenously. Since results involving validity effects when using either symbolic or peripheral auditory cues were very similar, the observed validity effects were all ascribed to endogenous orienting of attention.

Some hypotheses were advanced for the lack validity effects when using non-predictive peripheral auditory cues. First, that rats are not able to orient visual attention exogenously when the cues are auditory. Second, that the location of the beep-releasing speakers relative to the location of the nose-poke device where visual targets were presented were to distant in space, thus limiting the occurrence of validity effects.

The first explanation seems farfetched, since identification of the origin of sounds is supposed to play an important role in orienting towards visual stimuli in mammals (R. S. Heffner & Heffner, 1992). The second hypothesis seemed more likely.

Hypothetically, several factors may have contributed for the lack of validity effects when using peripheral non-predictive auditory cues. For instance, the SOAs employed (200, 400, 800 and 1200 ms) may have not been short enough to allow detection of exogenous capture of attention, which usually is detectable at shorter SOAs (see above). It is possible that exogenous attention was captured by the non-predictive auditory cue, but, because of its transient nature, this attentional effect had dissipated at the SOAs employed. Another possible factor may be related to prolonged practice. Human beings exposed to this type of task exhibit reductions of attentional effects (Lupiáñez et al., 2001; Weaver et al., 1998). If a similar overtraining phenomenon occurs in rats, this may have contributed for the lack of validity effects because the animals had been exposed to more than 50 testing sessions. The last factor may be related to a possible spatial dissociation between the cue and target, an effect known to reduce attentional effects in humans (Spence, 2013). That is, the beep sounds were released from speakers located laterally to the place where the visual

targets were presented (see Figure 3). This supposed spatial dissociation could have diminished the validity effect. Perhaps releasing both the cue and the target from the same location, i.e., inside the hole where the visual target was presented, could increase the validity effect.

This thesis reports two experiments that aimed at evaluating these hypotheses.

Experiment 1 evaluated if cross-modal, auditory-visual exogenous orienting of attention using peripheral non-predictive cues associated with shorter SOAs promotes the appearance of the validity effect in rats. That is, the two initial factors discussed above were evaluated by exposing the subjects to shorter SOAs and by reducing the amount of repetitive training. Results confirmed that rats do exhibit validity effects when orienting attention to visual targets using auditory cues, in a manner consistent with exogenous orienting of attention.

Experiment 2 investigated if a closer spatial origin of auditory cues and visual targets contributes for increasing the validity effect. Although this hypothesis was initially raised because of the lack of exogenous orienting of attention for non-predictive peripheral cues (Cruz, 2017), the experiment aim was broader. In addition to evaluating exogenous orienting of attention by non-predictive peripheral stimuli when cue and target have the same spatial origin, endogenous orienting of attention was also investigated using predictive peripheral auditory cues. A possible prediction was that a closer spatial auditory cue and visual target origin would enhance the validity effect, similarly to what happens in humans (Spence, 2013; Spence & McDonald, 2004). Surprisingly, we found that this closer spatial cue and target proximity had little or no effect in orienting of attention in rats.

2 Objectives

The general goal of this study was to investigate to which extent rats employ auditory cues to orient spatial visual attention. Taking into account previous hypotheses advanced by Cruz (2017), described above, our specific objectives included:

1. Investigate the time course of orienting of attention, particularly at short time intervals (SOAs < 300 ms), when using peripheral non-predictive auditory cues and visual targets;
2. Evaluate attentional effects in rats when using a conditioning protocol that avoids prolonged practice with task stimuli, and;
3. Test to which extent a closer spatial origin of auditory cues and visual targets stimuli interfere with either endogenous or exogenous orienting of attention, employing peripheral predictive and non-predictive auditory cues in independent groups of subjects.

3 Experiment 1 – Do rats orient attention exogenously when non-predictive auditory cues indicate the pending visual target location?

3.1 MATERIAL AND METHODS

3.1.1 Subjects

Twenty male Wistar rats (*Rattus norvergicus*), 90 days old at the beginning of the experiments were used. They were housed in standard polypropylene cages, either 2 or 3 animals per cage. The animal facility was maintained at 23ºC ± 5 and was illuminated with artificial white lights, that were switched on from 7:00 to 19:00. Experiments were run in the light phase of the light/dark cycle, usually 5 days a week.

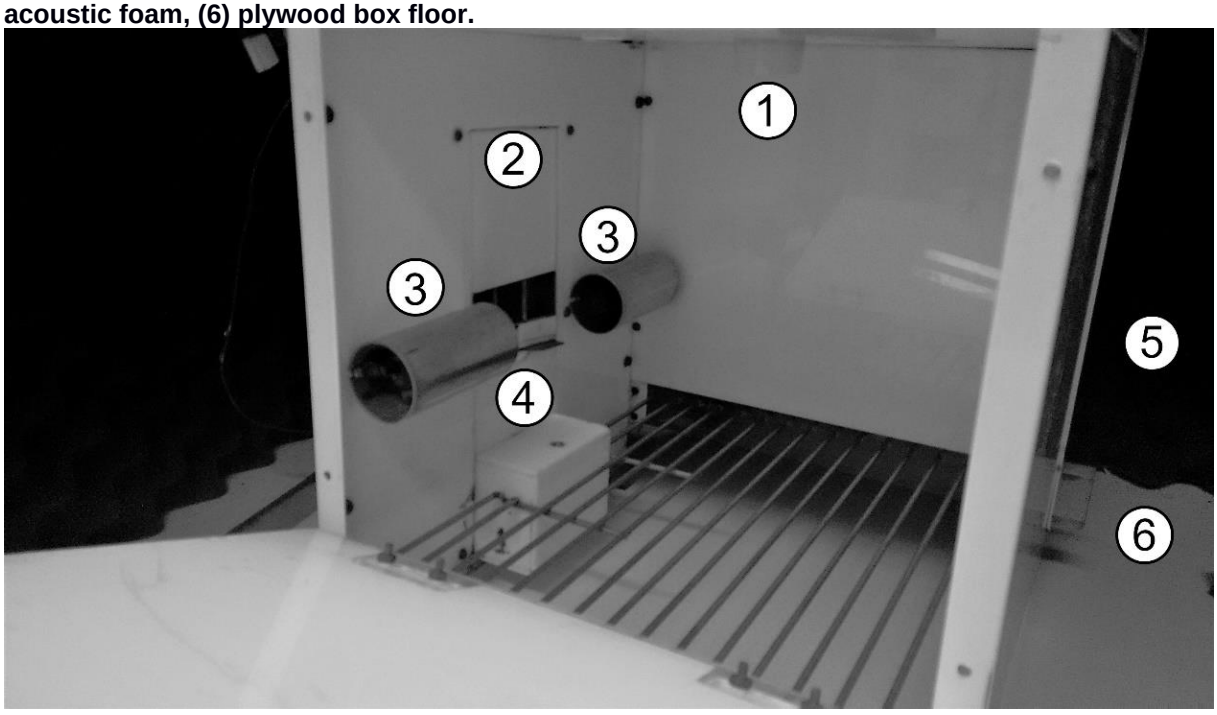
The animals were subject to a food deprivation regimen starting the week before the beginning of conditioning (see below). That is, the subjects had access to standard laboratory rat food pellets (Nuvilab®, Quimitia S/A) during 3 contiguous hours per day, starting immediately after each experimental session. When an experimental session was planned not to occur in the following day, the subjects were given *ad lib* access to food. In these cases, food was removed from the cage at least 24 h prior to the next experimental session. The body mass of the subjects was monitored in order to make sure it remained above 90% of their mass before the food control began. Water was provided *ad lib*.

All proceedings were carried out according to the protocol approved by the Animal Care and Use Committee of the Institute of Biosciences, University of Sao Paulo (Comissão de Ética no Uso de Animais do Instituto de Biociências, Universidade de São Paulo, protocol #307/2017).

3.1.2 Equipment

Seven similar Operant Conditioning Chambers (OCC; Figure 2) custom-made by Prof. Gilberto Fernando Xavier were used. Each of them measured 26 x 26 x 21 cm (width x length x height). The walls and ceiling were made of white opaque 3.0 mm thick acrylic sheets. The floor was made of parallel 3.1 mm diameter stainless steel rods with a 13 mm space between them. On the center of the ceiling there was a houselight, i.e., a 10 mm white LED calibrated to emit 15 lux of light intensity (as measured on the box's floor, directly under the LED). Each chamber was equipped, in one of its internal walls, with a nose poking device, a pair of speakers, and a drinking device (see below). Externally to the chamber there was a microprocessor-controlled system, which supported the chamber's functionalities (see below).

Figure 2 - Picture showing the setup of the chambers. (1) houselight, (2) poking device (see Figure 3, for details), (3) stainless steel tubes containing speakers, (4) drinking device, (5) acoustic foam, (6) plywood box floor.

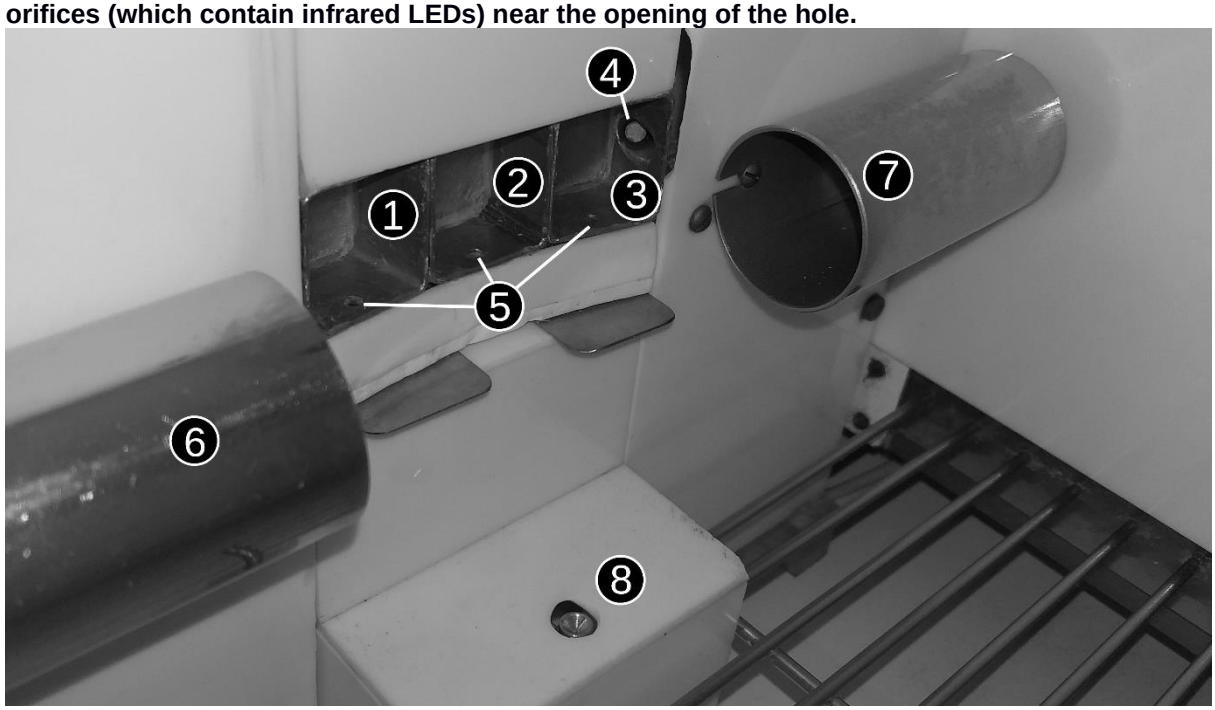


Source: Mateus Torres Cruz.

The poking device was located at the center of one the OCC's walls, approximately 10 cm above the floor, and consisted of a set of three horizontally adjacent recessed holes made of stainless-steel square tubes measuring 2.5 cm of side and 4.5 cm long (Figure 3). At the far end of each hole there was a 5 mm white

LED that projected its light towards a translucent acrylic shield located 2 cm from the hole's entrance such that the light was not perceived as a focal point, but as filling the entire hole (the exact luminance was later calibrated for each subject – see below). A photocell located in the superior wall of each square tube, 5 mm from the entrance of the hole, vertically aligned with an infrared beam in the opposite hole's wall, allowed detection of nose-pokes within the hole.

Figure 3 - Picture showing the poking and drinking devices. (1) Left hole, (2) central hole, (3) right hole, (4) the additional speaker used in experiment #2 (see below) inside of the lateral hole, (5) small orifices (which contain infrared LEDs) near the opening of the hole, (6) left main speaker tube, (7) right main speaker tube, (8) drinking device. Note the translucent shields and the small orifices (which contain infrared LEDs) near the opening of the hole.



Source: Mateus Torres Cruz.

Piezo ceramic sound emitters installed inside stainless steel lateral tubes (one piezo per tube) which exit was approximately 2 centimeters away from the nearest lateral hole, emitted sounds towards the location in front of the poking device, corresponding to the subject's head position during the experiments. This arrangement allowed the release of either unilateral or bilateral 60 to 75 dB sounds, with frequencies ranging from 0.5 to 42 kHz.

The drinking device allowed releasing 20 µL of 10% sucrose solution used as reward. The drinking device dispenser consisted of a small electric motor which axis was perpendicularly welded to a metal rod that had, at one of its ends, a 20 µL metal cup, counterweighted on the opposite end. Once activated by a ~20 ms pulse, the

motor submerged the metal cup within a flask containing the sucrose solution. Upon the motor inactivation, the metal cup returned to the drinking position filled with sucrose solution. The whole device was located outside the chamber, below the center hole of the poking device. The subjects had access to the metal cup through a hole located in a 5-cm-wide horizontal platform protruding from the wall and 4.5 cm above the floor (see Figure 3).

All LEDs, photocells, speakers and drinking device were connected by an interface to an Arduino Mega 2560 board (Arduino, 2017) running custom made code that controlled the stimuli presentation, their duration, the nose poke responses and the release of the reward, according to predefined and balanced experimental conditions (see below), and recorded the subjects' responses in a Micro SD card.

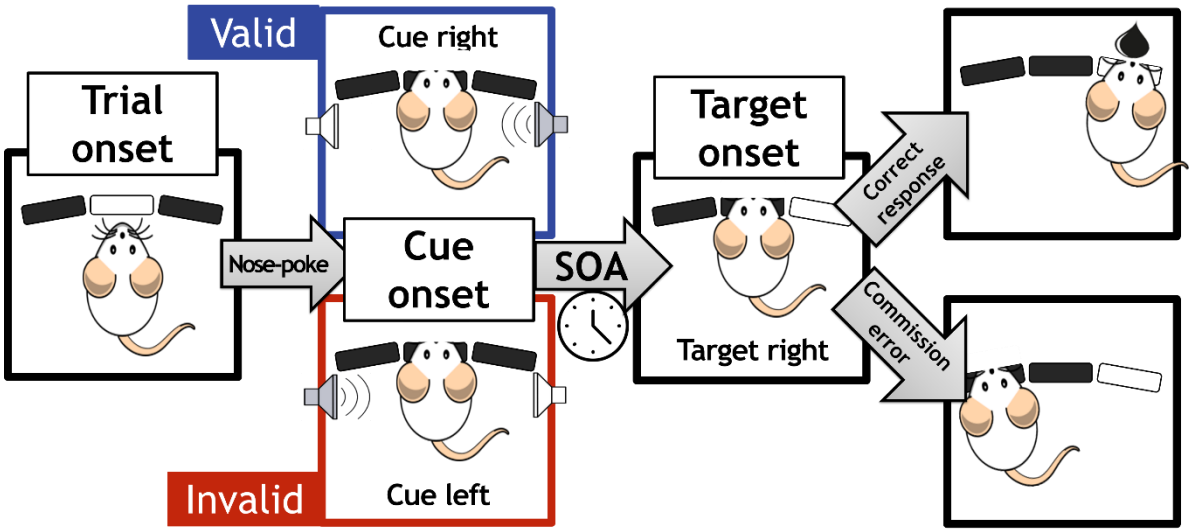
Each OCC was located inside a larger sound-attenuating plywood box which internal walls (except the floor) were covered by 2-cm thick acoustic egg crate foam studio panels. A fan installed in the center of its roof promoted a continuous flow of air inside this box and, in addition, generated a ~60 dB white noise.

3.1.3 Behavioral task

The behavioral procedure followed the general guidelines reported in previous studies of attention involving rats (e.g. Marote & Xavier, 2011; Rosner & Mittleman, 1996). A session consisted of a succession of trials. Each trial followed the steps described below, illustrated in Figure 4.

1. A light appeared in the central hole. The rat had to insert its nose within this hole;
2. Immediately after the nose-poke, the central hole light was turned off and an auditory cue (see below) was presented for 100 ms. The animal had to maintain its nose within the central hole until the appearance of a 100-ms-duration light target within one of the lateral holes. The time interval between the beginning of the sound and the beginning of the target light, named Stimulus Onset Asynchrony (SOA), ranged from 50 to 1200 ms;
3. The animal had to remove its nose from the central hole and introduce it within the lateral hole where the target was presented.

Figure 4 - Graphical depiction of the steps of a single trial.



Source: Mateus Torres Cruz.

When the animal performed the task as just described, the response to the target produced the release of a reward. Then, after an Intertrial Interval (ITI) of 250 ms, the next trial started. Each session included 600 trials.

Auditory cues consisted of either 5 kHz or 8 kHz pure-tone beeps presented either on the left or the right piezo, at a sound pressure level of 65-70 dB. These frequencies were chosen relying on the audiogram for the species (H. E. Heffner et al., 1994) aiming at employing clearly distinguishable frequencies for which rats have similar sensitivities. The side of occurrence of this cue, regardless of its frequency, indicated the likely location of the pending target.

The frequency and location of each auditory cue was random but counterbalanced.

The SOA used in a given trial was randomly chosen between 5 possible intervals including 50, 150, 300, 900 or 1200 ms. Each of these SOAs was used in 1/5 of trials within a session; the sequence of their occurrence varied randomly. The trials using the 4 shorter SOAs proceeded exactly as described above. Differently, SOAs of 1200 ms were used in catch trials, i.e., trials on which there was presentation of the auditory cue, but no visual target was presented. In catch trials the subject was rewarded soon after the end of the SOA, i.e., as a reward for maintaining its nose within the central hole all along the 1200 ms. This type of trial was included in the present experiment in order to stimulate maintenance of the nose within the central

hole and only remove it upon the visual target presentation. In experiments where no catch trials are included, rats trend to remove their noses from the central hole before the end of the SOA, thus exhibiting premature responses (Klein, 2000), which strongly interferes with performance, particularly at longer SOAs. Catch trials reduce the probability of these premature responses even when they correspond to only 20% of trials within a session.

The luminance of the targets was individually calibrated to be the lowest possible light intensity that produced an overall accuracy of at least 75%; during this calibration no auditory cue was presented. Therefore, no specific spatial orienting of attention was stimulated (see below).

On considering the aim of investigating exogenous orienting of attention in the present experiment, valid trials consisted of presenting both the auditory cue and the visual target at the same side; in contrast, in invalid trials, the auditory cue was presented in one side and the visual target was presented in the opposite side. Valid auditory cues corresponded to 50% of the trials and invalid auditory cues corresponded to the remaining 50% of trials (excluding catch trials). Therefore, there was no engagement of endogenous orienting of attention. In both cases the auditory cues and visual targets were equally distributed to the left and right sides.

If the subject did not perform as required it did not receive reward at the end of the trial. In addition, it was exposed to a “punishment” involving a 5000 ms timeout period - the houselight was switched off and the subject was maintained in complete darkness. After this timeout period the task proceeded to the next trial. Three different types of errors were recorded:

- Anticipation errors - the subject removed its nose from the central hole either before the end of the SOA or up to 80 ms after the beginning of the visual target presentation, thus indicating a premature response.
- Commission errors - the rat nose-poked the hole opposite to that where the target was presented, thus indicating that the target was incorrectly detected; and
- Omission errors - the subject did not respond to the visual target up to 1000 ms after its onset, thus indicating that the target was not detected.

3.1.4 Groups

The initial plan was to run four experimental groups, each of them with 6 subjects, including two groups exposed to auditory symbolic cues, one predictive and another non-predictive, and two groups exposed to Peripheral auditory cues, being one predictive and another non-predictive (total of 24 subjects). However, due to the exclusion of 11 subjects during conditioning (41% of subjects - see details in Experiment 1), we decided to allocate all the remaining 13 subjects to the peripheral non-predictive group, because our main hypothesis depended more heavily on the results of this experimental manipulation. However, this was decided after 4 animals had already been allocated to other groups. As the previous training history of the animal in this task can affect the learning of a new scheme and impact the results in unpredictable ways, we decided to not change the experimental groups of these animals. Therefore, the results presented below come from 9 subjects that were tested using peripheral non-predictive cues.

3.1.5 Conditioning

In order to condition the subjects in this behavioral task, 9 successive phases were implemented, as it follows.

1. Reward familiarization. In order to familiarize the subjects with the sucrose solution to be later used as reward, a drinking tube filled with 100 ml of the 10% sucrose solution was inserted in the subjects' cage for 5 days before the first exposure to the OCC.
2. Reward freely available at the OCC. Groups of either 2 or 3 subjects were inserted within the OCC, with the drinking device freely releasing reward at every 3000 ms. A single 30-minute session was run.
3. Nose-poke conditioning. Subjects individually inserted within the OCCs were free to explore it without any intervention from the experimenter, along a 40-minute session. A few drops of sucrose were placed at the entrance of the holes before the start of the experimental session to further motivate nose-pokes. The LEDs of all three holes were switched on. Then, upon a nose poke in any of the

holes, the LEDs of all holes switched off and the reward was released. After a 250-ms ITI, another trial started with the LEDs of all holes being switched on. When the subject managed to obtain more than 100 rewards within a session, it was exposed to additional sessions without sucrose drops at the hole's entrance, up to obtaining at least 200 rewards. Then, it was promoted to the next phase.

4. Center-to-lateral nose-poke conditioning. Subjects were trained to insert their noses within the illuminated hole, starting by the central hole followed by one of the lateral holes. In other words, at the beginning of every trial only the central hole LED was switched on. When the animal nose poked it, its LED was switched off and the LED of one of the lateral holes (hereinafter referred to as "target") was switched on. Nose poking the target hole released the reward. In this phase, there was no time limit to nose poke the target hole and no timeout was applied when non-illuminated holes were nose-poked. From this phase on trials were shuffled in a pseudo-random schedule, randomizing trial order in order to avoid repetitions of either more than five consecutive trials with a target on the same side or five consecutive catch trials. Sessions usually lasted 30 to 45 minutes. Subjects were transferred to the next phase after achieving at least 100 rewards per session along at least five consecutive sessions.
5. SOA introduction. Subjects were then trained to wait for variable amounts of time until the presentation of the target. This was achieved by adding a delay between the central hole nose-poke and the onset of the target. Initially the delay was 100 ms, but it increased by 50 ms, to a maximum of 1200 ms, every time the animal responded correctly to six consecutive trials. If the subject committed an error in three consecutive trials the delay was decreased by 50 ms, to a minimum of 150 ms. Only commission errors were considered in this phase. Its occurrence triggered a 5000 ms timeout period. Timeout periods were imposed throughout the experiment whenever an error occurred. In this phase there was no time limit for responding to the target. In addition, removal of the nose from the central hole during the SOA restarted the trial. Each session started with the SOA that the animal reached at the end of the previous session. The subjects were promoted to the next phase when both they achieved at least three consecutive sessions starting with a SOA of 1200 ms

and their mean accuracy (percentage of correct trials relative to total trials in the session) was equal or higher than 75%.

6. All errors considered. From this phase on, anticipation and omission errors, in addition to the already considered commission errors, generated a timeout. Furthermore, the target duration time was set to 300 ms, i.e., the target light was switched on for 300 ms and then switched off. Nose poking the hole when the light was still on, turned the light off. Nose poking the correct hole triggered the release of the reward, both before and after its LED was switched off. If the animal did not respond up to 1000 ms of the target's onset, an omission error was registered. A 1200 ms SOA was employed in all trials. Twenty percent of trials were catch trials; therefore, in these trials, no target was presented and the subject was rewarded for sustaining the nose poke within the central hole all along the SOA duration. Subjects were promoted to the next phase after achieving accuracies equal or higher than 75 percent in three consecutive sessions.
7. All SOAs included. In this phase each planed SOA, including 50, 150, 300, 900 and 1200 ms, was used in 20% of trials randomly distributed along the session training; the side of target presentation, either left of right, was also counterbalanced with the different SOAs. As explained above, in catch trials the SOA was 1200 ms and there was no target stimulus presentation. After achieving 3 consecutive sessions with accuracy equal or higher than 75 percent, the subjects were promoted to the next phase.
8. Reduction of target duration. The target duration was reduced from 300 to 100 ms. Subjects were promoted after exhibiting at least 75 percent of accuracy in 2 sessions.
9. Threshold detection. In order to increase the attentional demand for performance of the task, the threshold for the visual target luminance detection was individually identified. This was achieved by reducing the visual target luminance in steps of 10 lux in consecutive sessions until the subject's accuracy became lower than 75 percent. Then, in the following session the target luminance was increased by 5 lux. If accuracy was still lower than 75 percent, the target luminance was further increased by 5 lux. For subjects which accuracy was greater than 75% even when the visual target reached intensities lower than 10 lux, the target luminance was reduced to 5 lux, then to 3 lux and

then to 2 lux. These steps of target luminances were also used when it was necessary to increase target light intensities in order to maintain accuracy of performance just above 75%. The remaining sequence of events and conditions relative to previous phases were maintained the same. In other words, these procedures were maintained until the lowest target light intensity generating accuracy higher than 75 percent was found for each subject. This target light intensity for each subject was then employed in its testing sessions.

10. Testing sessions. In testing sessions auditory cues were introduced soon after the subject inserted its nose within the central hole. At this point the task events occurred following the order described above in the behavioral task section (see above). Each subject performed at least twenty testing sessions, being one testing session per day.

3.1.6 Measured Variables and Data Analysis

Reaction time (RT) and movement time (MT) were measured for each trial in which the subject's response was correct. The RT corresponded to the time interval between target onset and the removal of the nose from the central hole. The MT corresponded to the time interval between removal of the nose from the central hole and its insertion within the target hole. When the subject did not perform correctly, the type of error (see above) was recorded.

Scores calculated for each subject, in each testing session, included median RT, median MT, number of correct responses, and anticipation, commission and omission errors, as a function of Validity (trials using either valid or invalid cues, except for anticipation errors, see below) and SOA (50, 150, 300 and 900). Then, the proportion of both correct responses (accuracy) and types of errors relative to the total number of trials (excluding the catch trials) were also calculated.

Data from catch trials were not included in this analysis, since there was no presentation of the target stimulus and the subjects did not exhibit any reaction.

The analysis of anticipation errors was only done as a function of SOA and not validity. It did not make sense to include Validity in comparisons involving this score since this kind of error happens before the appearance of the target.

These median RTs and MTs, proportions of correct responses, and proportions of anticipation, commission and omission errors scores, as a function of Validity (when applicable) and SOA (50, 150, 300 and 900), per session, per subject, were then used to calculate general **mean scores** per subject, involving distinct combinations of testing sessions (see below). A first combination of testing sessions to calculate these mean scores included all twenty sessions. Additional combinations of testing sessions included subsets of initial (sessions 1 to 5), intermediate (sessions 8 to 12) and final (sessions 16 to 20) sessions, in order to evaluate the effects of accumulation of experience in the task performance on orienting of attention.

Separate Repeated Measures Analyses of Variance (ANOVA) were then run for each dependent variable, including means of RT, means of MT, means of response accuracy, and means of commission and omission errors, having SOA and Validity as independent within-subjects factors.

Possible violations of ANOVA basic assumptions were evaluated using (1) Q-Q plot of residuals and Shapiro-Wilk test for normality of residuals, (2) plot of residuals versus fitted values for homoscedasticity assessment, and (3) detection of extreme outliers using boxplots [extreme outliers are either values higher than the third quartile plus three times the interquartile range (IQR) or values lower than the third quartile minus three times the IQR].

When tests 1 and 2 indicated violations of ANOVA assumptions, an additional analysis was run using transformed data, for instance, natural log for RT and MT and arcsine (Ahrens et al., 1990) for either accuracy or types of errors proportions. When violations were still present, other methods were employed (see below). When test 3 indicated extreme outliers, their origin was further investigated and the outcomes were discussed in light of what was found.

Since data were balanced, ANOVA results used type II sum of squares. Degrees of freedom were corrected using the Greenhouse-Geisser method when Mauchly's test was significant. We employed generalized eta squared (η^2_G) to compute effect sizes. *Post hoc* analysis, when required, involved pairwise comparisons tests with Tukey correction.

Data that violated ANOVA assumptions were fit using General Linear Mixed Models (GLMM) estimated using Maximum Likelihood and Nelder-Mead optimizer. All GLMMs used validity and SOA as fixed effects and subject as random effect. For RT, Gamma GLMM was first used with a log link function. When its diagnostics revealed

an improper fit, an inverse link function was used instead. When this latter transformation also did not fit, non-parametric methods were used (see below). For response analyses, a Poisson GLMM with log link function was used, employing the number of occurrences of that response, instead of the percentage, as dependent variable.

Log link model diagnostics involved a simulation-based approach, similar to the Bayesian p-value or the parametric bootstrap, that transforms the residuals to a standardized scale (Hartig, 2021), which allowed testing normality of simulated residuals, measuring dispersion and identifying outliers. The fit of models using inverse link we evaluated (1) whether the random effects were normal, (2) whether a scatterplot of predicted versus Person residuals was homogeneous, and (3) whether the relation between the dependent variables and Pearson residuals was linear.

Type II Wald chi-square tests were used to obtain ANOVA-like tables with *p* values for *omnibus* tests from the GLMMs. *Post hoc* analysis, when required, involved pairwise comparisons tests with Tukey correction. We used R^2 to calculate the amount of variance that is explained by each fit model[2] (Nakagawa & Schielzeth, 2013).

Data that violated ANOVA assumptions or revealed improper GLMM fit were analyzed using what we will refer to as “the non-parametric approach”. Non-parametric analysis involving responses included number of occurrences, instead of percentages, to preserve the original format of the data. Wilcoxon signed rank tests paired by SOA (Holm corrected) were run to compare the dependent variable results on valid and invalid trials. Additionally, we used the Friedman’s Test to analyze a main effect of SOA (in this case we combined the scores for valid and invalid trials and pooled them by SOA). In this case, Kendall’s W was employed for obtaining effect size measurements. The Friedman’s Test was followed by a Wilcoxon Signed Rank test with Holm correction when a multiple comparisons test was necessary.

[2] The coefficient of determination, R^2, is a summary statistic that quantifies the goodness-of-fit of a model by providing an index of the amount of variance explained by the model factors. It is subdivided into two types, marginal R^2, which is related to the variance explained by fixed effects (validity and SOA), and conditional R2, which is related to the variance explained by both fixed (validity and SOA) and random effects (subject).

The only exception to this workflow was the analysis of anticipation errors. A correlation test, using Kendall's method, between the number of correct responses and anticipation errors was run.

For all analysis, $p < .10$ was considered to be marginally significant and $p < .05$ to be significant.

Summary statistic values, when inserted in the text below, used the notation "median (interquartile range)" when reporting non-parametric analysis and "mean (standard error of the mean)" when reporting ANOVAs and GLMMs.

The software R 4.0.5 (R Core Team, 2021) was employed. Data importation, organization and transformation was run using the aid of "tidyverse" 1.3.0 package (Wickham et al., 2019). The package "rstatix" 0.7.0 (Kassambara, 2021) was employed to simplify the process of running ANOVAs and non-parametric tests. The "lme4" 1.1-26 (Bates et al., 2015) and "lmerTest" 3.1-3 (Kuznetsova et al., 2017) packages were employed for adjusting models GLMMs, "car" 3.0-10 (Fox & Weisberg, 2019) for most statistical tests on GLMMs, "emmeans" 1.5.5-1 (Lenth, 2021) for multiple comparisons tests and "report" 0.3.0 (Makowski, 2018) for obtaining R-squared. GLMM diagnostics were performed using "DHARMa" 0.4.0 (Hartig, 2021) and "sjPlot" 2.8.7 (Lüdecke, 2021). Data visualizations employed "ggplot2" 3.3.3 (Wickham, 2016), "ggpubr" 0.4.0 (Kassambara, 2020), and "ggsignif" 0.6.1 (Ahlmann-Eltze & Patil, 2021) packages. Additionally, we used "rmarkdown" 2.7 (Allaire et al., 2021; Xie et al., 2018, 2020) for internal reporting.

3.2 RESULTS

This experiment started with twenty-four rats. While thirteen subjects completed all twenty testing sessions, eleven did not and were excluded. One these latter subjects was excluded because it got ill. The remaining ten subjects failed to progress beyond the “SOA introduction” phase of conditioning (they performed at least 40 sessions in this phase without progressing). As explained above (see “**3.1.4 Groups**” subsection in the material and methods section) the results presented here come from the nine subjects that were tested using peripheral non-predictive cues.

Data will be reported separating the two analyses proposed, one of them including all sessions and the second analysis including subsets of sessions (initial, intermediate and final subsets, corresponding, respectively, to sessions 1-5, 8-12 and 16-20). While the first analysis provides an overall view of the subjects’ performance, the second analysis provides a clearer view of changes of performance along testing sessions, zooming into specific periods of test.

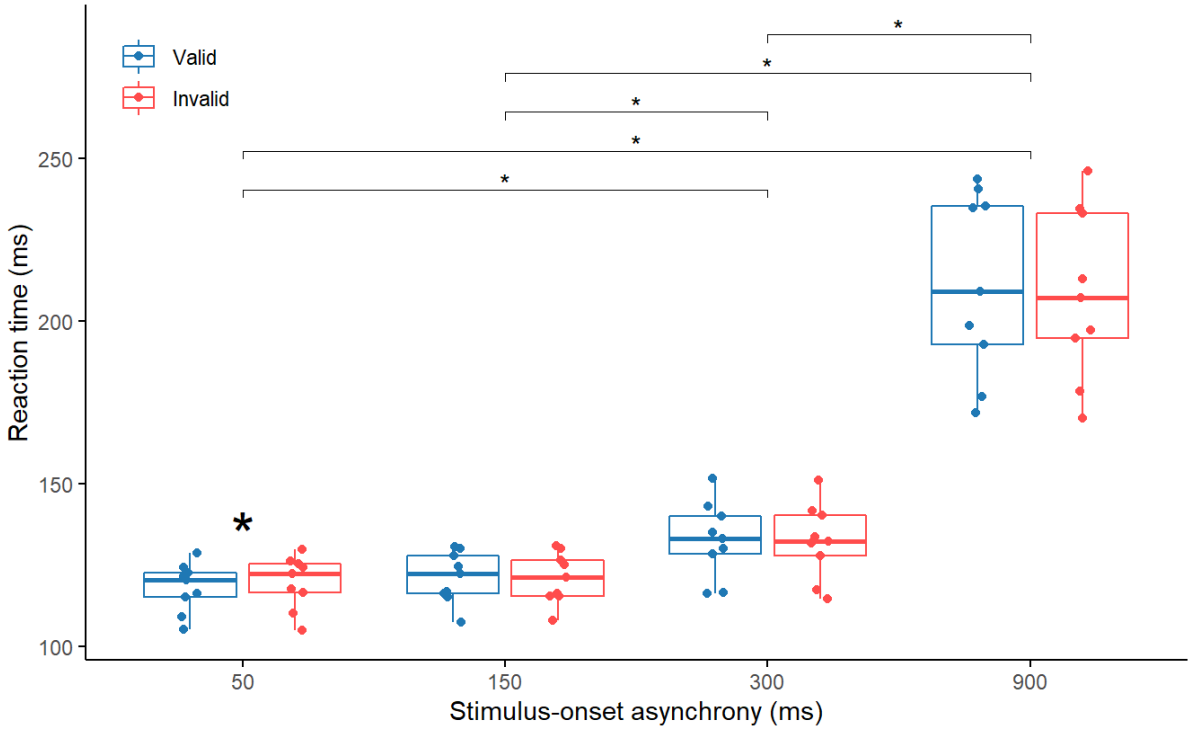
3.2.1 Analyses including all sessions pooled

3.2.1.1 Reaction times

RT data were analyzed using non-parametric methods. Paired Wilcoxon Tests allowed comparing RTs in valid and invalid trials at each SOA, revealing a significant difference at the SOA 50 ms *(p* = .047; Figure 5). This indicates that RT *[Median (*first quartile – third quartile*)*[3] = 120.6 (115.4 – 122.7) ms] in valid trials were significantly shorter as compared to those seen in invalid trials [122.4 (116.8 – 125.6) ms].

[3] We will use the notation “median (first quartile – third quartile)” when reporting non-parametric analysis and “mean (standard error of the mean)” when reporting ANOVAs and GLMMs.

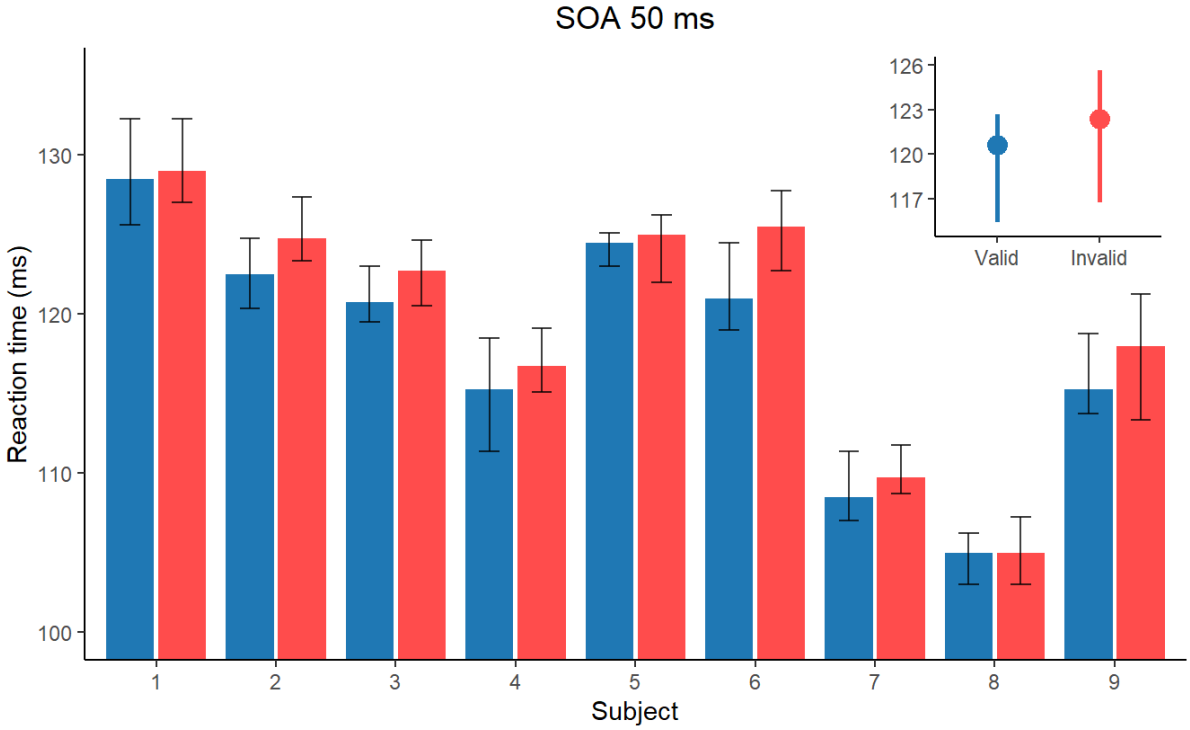
Figure 5 - Reaction Times (ms) for valid and invalid trials at all SOAs in Experiment 1. Data analysis involved Friedman and Wilcoxon signed rank tests. * without a guide line indicates a significant difference between valid and invalid conditions at the corresponding SOA. * indicates significant differences between SOAs connected by the bracket.



Although the difference between valid trials and invalid trials at SOA 50 ms is statistically significant and 8 out of 9 subjects have lower RTs on valid compared to invalid (visual inspection; Figure 6), the median difference (1.8 ms) seems too small to be biologically relevant.

Data also revealed an increase in RTs as the SOAs increased (Friedman $X^2_F(3) = 23.8$, $p < .001$, *Kendall W* = .88, indicating a strong association). A *post hoc* analysis showed that TRs at SOAs 50 [121.2 (115.7 – 124.4) ms], 150 [121.9 (115.8 – 127.7) ms] and 300 [132.9 (128.1 – 140.3) ms] were smaller as compared to those seen at SOA 900 ms [208.1 (193.4 – 234.6) ms; all $p = .023$], and that TRs at SOAs 50 and 150 ms SOAs were smaller as compared to those seen at SOA 300 ms (both $p = .023$).

Figure 6 - Median Reaction Times (ms) for valid and invalid trials at the SOA 50 ms for each subject, in Experiment 1. Colored bars with error bars represent the median ± interquartile range for individual animals. The insert on the upper right shows medians and interquartile ranges of all animals pooled.

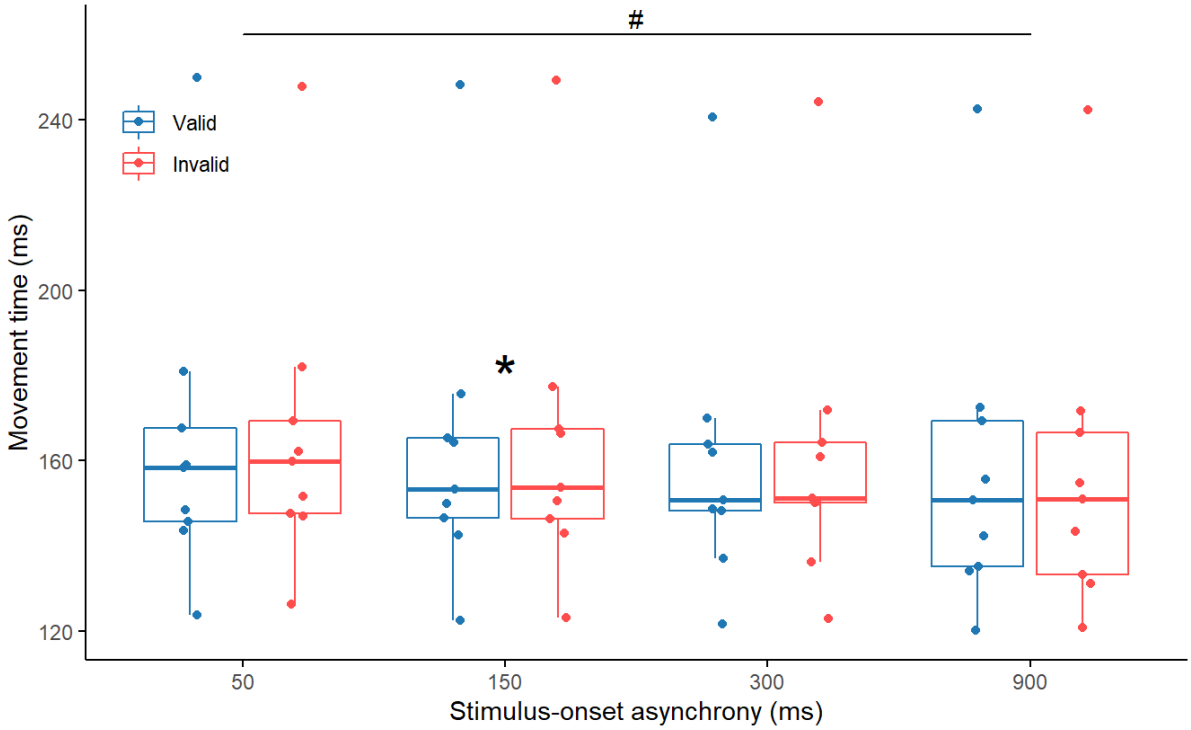


3.2.1.2 Movement times

The MTs in both valid and invalid trials, as a function of SOA, are presented in Figure 7.

Non-parametric statistics involving paired Wilcoxon Tests looking at possible valid and invalid MT differences, revealed that MTs in valid trials are 0.6 ms faster than MTs in invalid trials at SOA 150 ms [v: 153.2 (146.7 – 165.3); i: 153.8 (146.4 – 167.6); p = .031].

Figure 7 – MTs for valid and invalid trials, as a function of SOA, in Experiment 1. # indicates a significant Friedman result in the statistical analysis, indicating an effect of SOA in the results, without the occurrence of significant differences in a *post hoc* analysis. All other conventions are the same as in Figure 5. Data analysis involved Friedman and Wilcoxon signed rank tests.



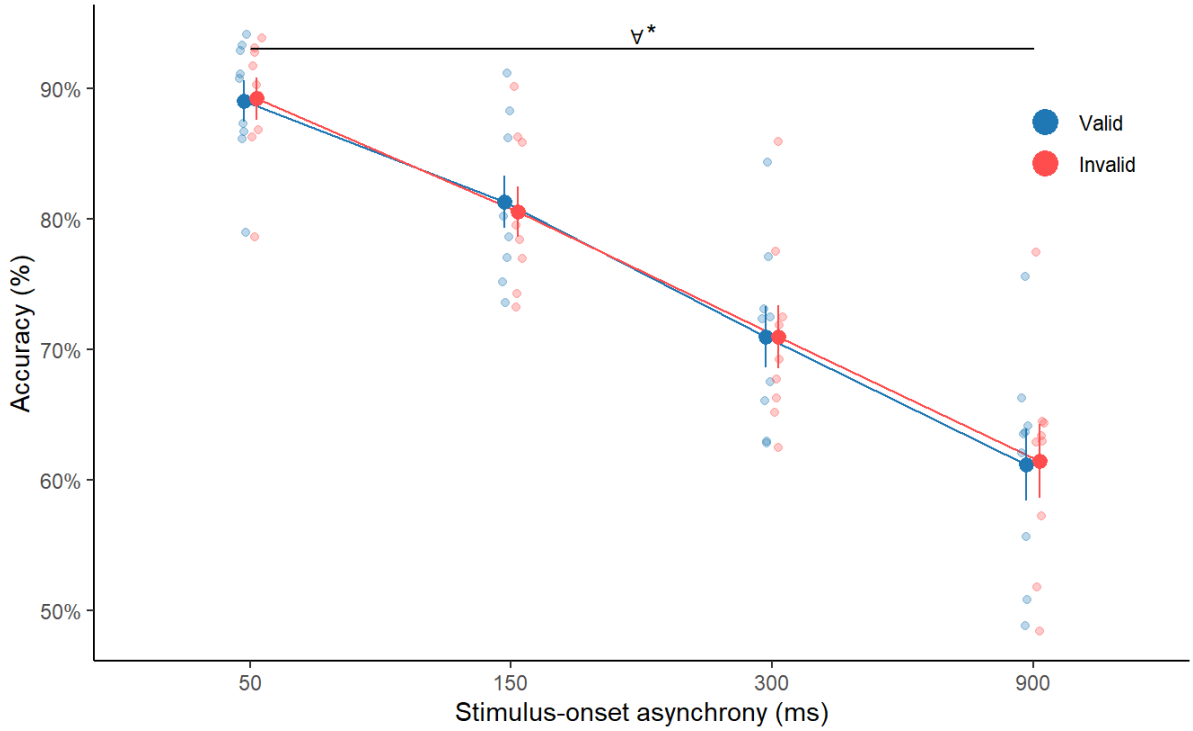
Still in relation to MTs, statistics revealed a significant decrease in MTs as the SOA increased ($X^2_F(3) = 8.73$, $p = .033$, *Kendall W* = .32), although the *post hoc* analysis showed no significant differences. This suggests that longer SOAs allow for improved motor preparation, thus generating shorter MTs.

3.2.1.3 Accuracy

The accuracy of performance in both valid and invalid trials, as a function of SOA, is presented in Figure 8.

The ANOVA for accuracy showed a significant main effect of SOA ($F_{(1.26, 10.1)} = 64.1$, $p < .001$, $\eta^2_G = .73$), indicating a drop in accuracy as the SOAs increased, ranging from 89.2% at SOA 50 ms to 61.2% at SOA 900 ms. P*ost-hoc* analysis indicated that accuracy at each SOAs is different for that seen in every other SOA (in all comparisons $p < .01$). No effect involving Validity was observed in accuracy data.

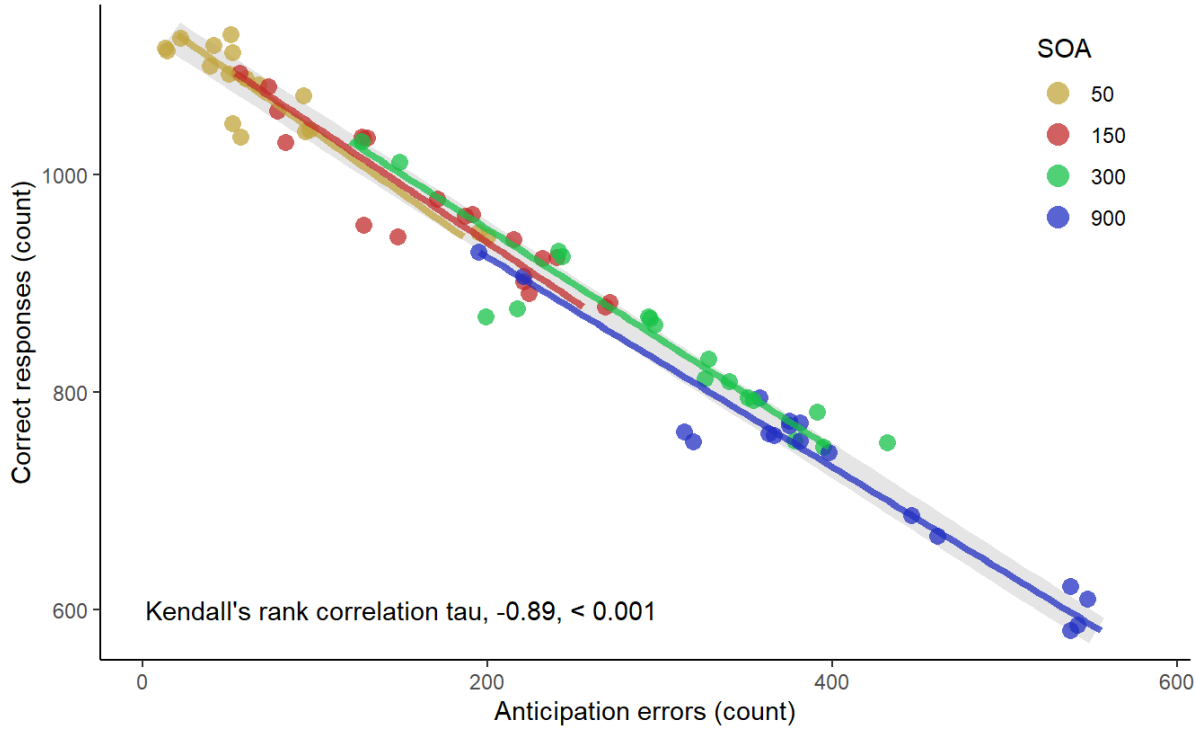
Figure 8 – Accuracy for valid and invalid trials as a function of SOAs in Experiment 1. Smaller colored dots indicate individual animals. The "∀*" symbol indicates that all comparisons between SOAs (not referring to validity) are statistically significant. Data analysis involved ANOVA.



The Accuracy of performance as expressed by the general mean percentage of correct responses was 75.6 % ± 3.9[4] and the most common error was Anticipation [19.5 (3.8)[5]], ranging from 5.9% at the shortest SOA to 33.1% at the longest SOA. We found a strong negative correlation between Accuracy and Anticipation Errors (*Kendall's tau* = -0.89, $p < .001$, Figure 9), which seems to be consistent throughout the SOAs (*Kendall's tau* of per-SOA analysis: 50 ms = -0.71, 150 ms = -0.79, 300 ms = -0.89, 900 ms = -0.71, all $p < .001$). This indicates that the drop in Accuracy is highly correlated with the increase in Anticipation Errors. This effect was also observed in prior experiments of our laboratory (e.g. Marote & Xavier, 2011) and suggests that rats have a strong tendency to anticipate their responses in longer SOAs.

[4] Standard Error of the Mean
[5] Mean (SEM).

Figure 9 - Kendall regression including counts of correct responses and ancitipation errors, showing a strong correlation between these dependent variables in Experiment 1. The thick gray transparent regression line on the backgroud represents all data. The colored regression lines and dots represent scores observed for each SOA.



3.2.1.4 Omission errors

The number of Omission Errors, in both valid and invalid trials, as a function of SOA is presented in Figure 10.

The Poisson GLMM for Omission Errors (conditional R^2 = .80; marginal R^2 = .32) revealed a significant interaction between SOA and Validity ($X^2(3) = 9.12$, p = .027). *Post hoc* analyses indicated that the number Omission Errors is lower in valid [3.45 (0.97) errors] as compared to invalid trials [6.23 (1.79) errors] at SOA 150 (p = .008). This effect could indicate that the subjects oriented attention to the side indicated by the cue, missing a smaller number of trials (approximately half less) when the cue was valid in comparison to when it was invalid (Figure 10).

Figure 10 - Number of omission errors in valid and invalid trials as a function of SOA in Experiment 1. All conventions are the same as in Figure 5. Data analysis involved a Poisson GLMM.

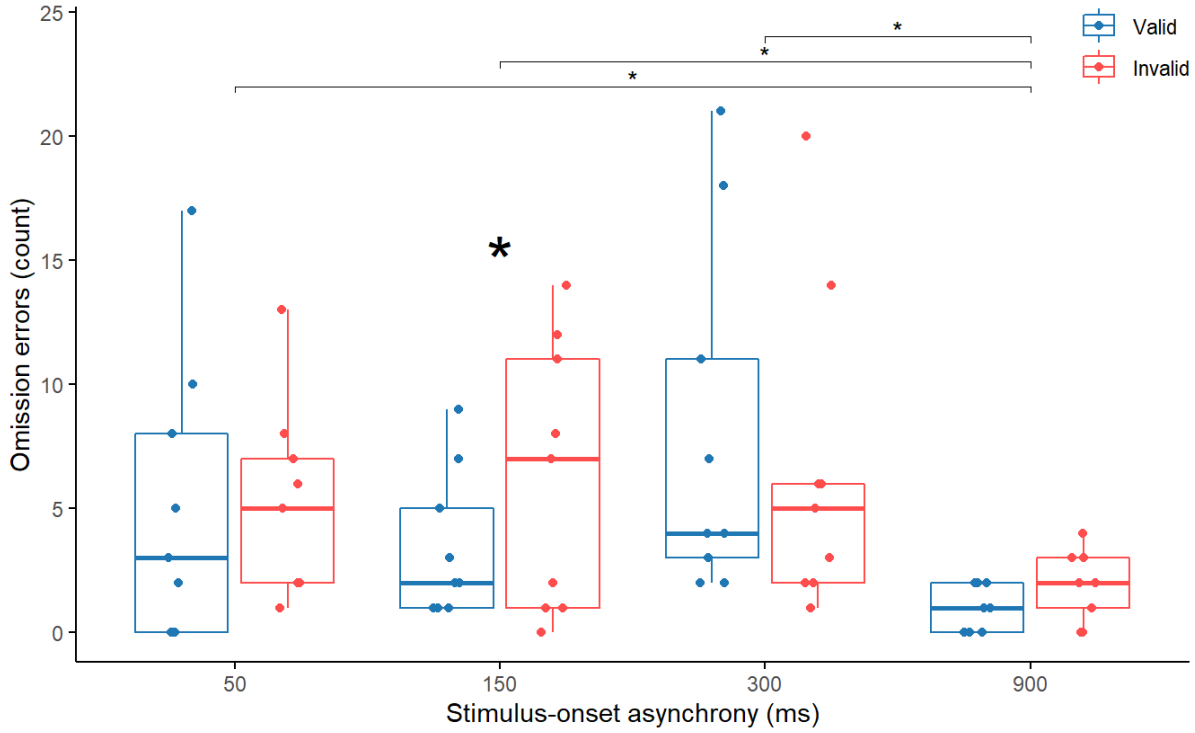
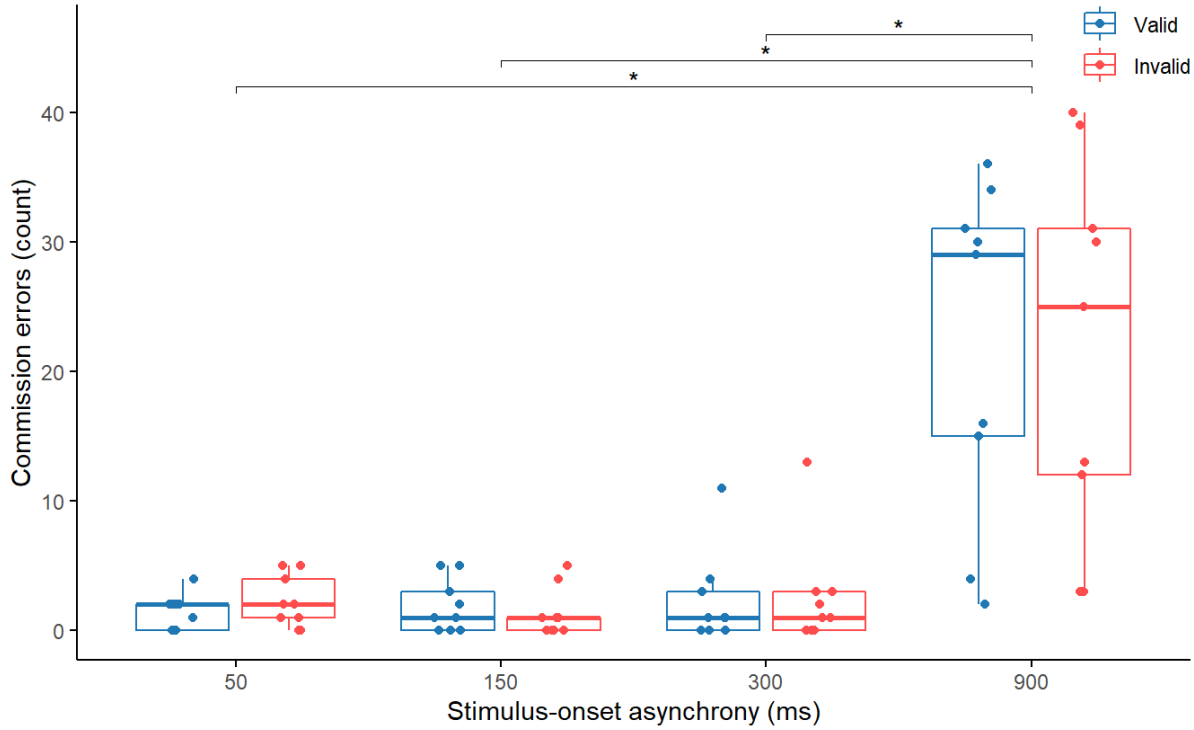


Figure 10 also shows that there was a significantly smaller number of Omission Errors at the 900 ms SOA, in both valid [1.11 (0.31) errors] and invalid [1.89 (0.45) errors] trials, as compared to those seen at SOAs 50 ms [valid: 5.00 (1.92), invalid: 5.11 (1.29) errors], 150 ms [v: 3.44 (0.97), i: 6.22 (1.79) errors] and 300 ms [v: 8.00 (2.38), i: 6.56 (2.12) errors]. This smaller number of omission errors as the SOA increases may be related to the increase in the probability of appearance of the pending target as the SOA progressively increases. In other words, given the repetitive experimental schedule, having elapsed 300 ms after the auditory cue presentation, the probability of target appearance increases to 50% (since the only additional possibility is a catch trial). This could increase attention just prior the likely moment of target presentation and motivation for responding to that specific moment in time, which also corresponds to the last opportunity to obtain the reward in that specific trial. This would decrease their probability of omitting the response at SOA 900 ms, as compared to other SOAs, thus decreasing the number of omission errors.

3.2.1.5 Commission errors

The number of Commission Errors as a function of Validity and SOA are presented in Figure 11.

Figure 11 - Number of commission errors in valid and invalid trials as a function of SOA in Experiment 1. All conventions are the same as in Figure 5. Data analysis involved a Poisson GLMM.



The Poisson GLMM for Commission Errors (conditional R^2 = .92; marginal R^2 = .52) revealed a main significant effect for SOA ($X^2(3) = 480.6$, $p < .001$). *Post hoc* tests showed that the number of errors at SOA 900 ms [21.8 (3.14) errors] were significantly greater as compared to those seen at SOAs 50 [1.83 (0.40) errors], 150 [1.61 (0.45) errors] and 300 [2.4 (0.87) errors] ms (all $p < .001$). These figures indicate a rise in Commission Errors at the longest SOA. It is interesting to note that this increase in the number of commission errors at the longer SOA occurs concurrently with a reduction of the number of Omission Errors (Figure 10). Thus, apparently, the subjects seem less likely to omit their response at the SOA 900 ms but this occurs in association with a lower accuracy of the response since their number of commission errors substantially increase (Figure 11). It is important to note, however, that the reduction of the number

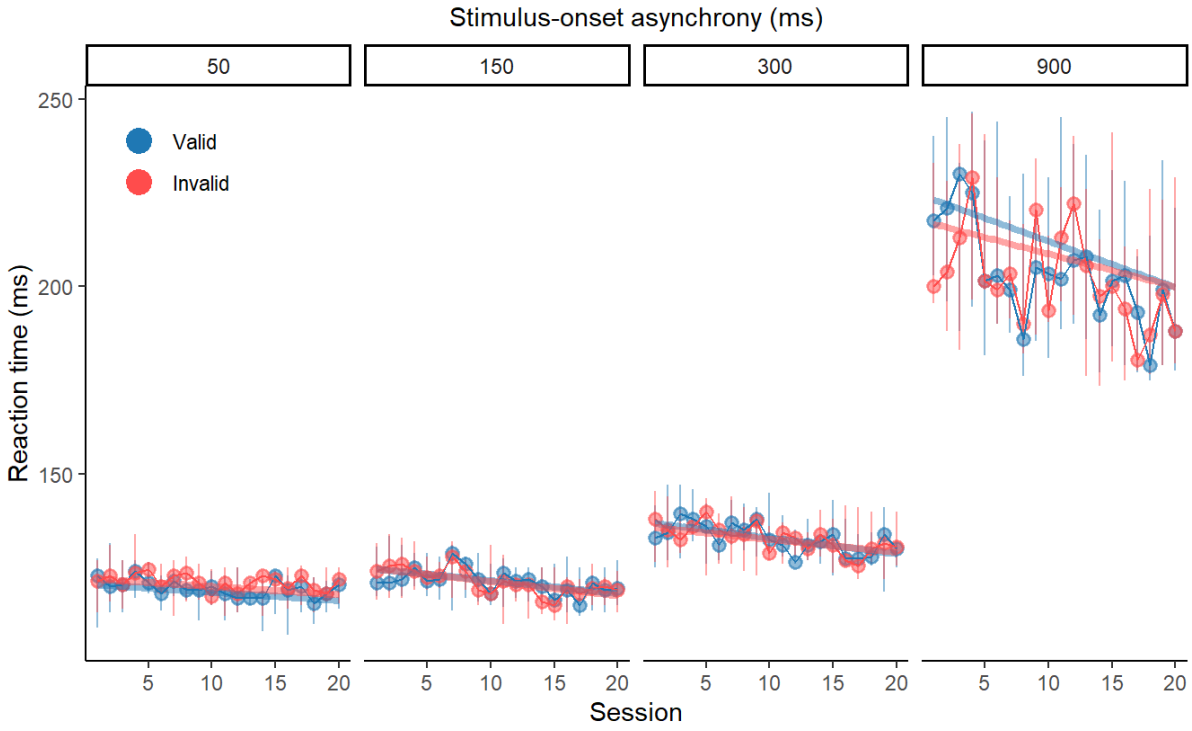
of omission errors is not in the same order of magnitude as the increase of the number of commission errors, because the latter is more pronounced.

3.2.1.6 Summary

Overall, analysis including data of all sessions pooled revealed only two pieces of evidence that rats orient attention exogenously when auditory cues indicated the likely pending visual target location. The first piece of evidence is the significantly lower amount of omission errors in valid as compared to invalid trials at SOA 150 ms. Note that this effect did not occur concurrently with a significantly higher accuracy in valid as compared to invalid trials at this SOA (see Figure 10), suggesting that the magnitude of the observed validity effect is small. The second piece refers to the shorter RT observed in valid trials as compared to invalid trials at SOA 50. Even though the difference is statistically significant and 8 out of 9 subjects have lower RTs on valid compared to invalid (visual inspection; Figure 6), the median difference (1.8 ms) seems too small to be biologically relevant.

One has to consider that this effect appeared for data involving all sessions pooled. Polling groups of sessions may be a strategy adequate for revealing general effects, but that may hide some specific subtleties of specific effects along sessions. For instance, an exploratory plot of the testing data is presented in Figure 12. It suggests that there were changes in performance along testing sessions which are particularly visible when the SOA was 900 ms. There have been reports involving humans indicating that spatial attention effects may wane throughout testing sessions (Lupiáñez et al., 2001; Pratt & McAuliffe, 1999; Weaver et al., 1998). Thus, by pooling data of all twenty testing sessions one could be either “diluting” or “hiding” possible attentional effects. A possibility to reveal if this was the case would be to include the factor “Session” in the ANOVAs and GLMMs (or group data in the case of a non-parametric approach). Another possibility to reveal if this effect occurred in the present set of data would be to pool subsets of sessions (e.g., first five, intermediate five and last five sessions) thus rendering analysis easier to understand and run.

Figure 12 – Exploratory plot showing RTs in valid and invalid trials per session as a function of SOA in Experiment 1. Each vertical panel shows a different SOA. The colored lines crossing the dots indicate linear regressions. The vertical lines indicate the interquartile range.



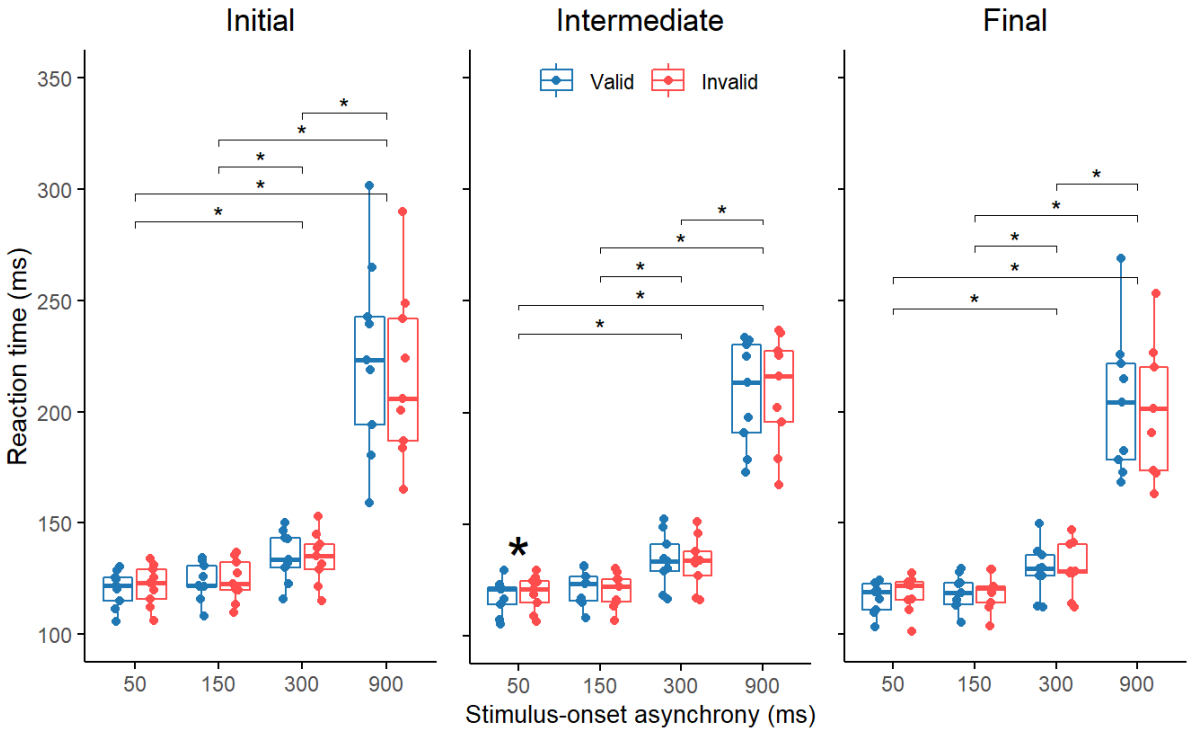
3.2.2 Subsets of initial, intermediate and final sessions pooled

3.2.2.1 Reaction times

Figure 13 shows RTs for the initial (sessions 1 to 5), intermediate (sessions 8 to 12) and final (sessions 16 to 20) subsets of sessions as a function of Validity and SOA.

Non-parametric statistics involving paired Wilcoxon Tests looking at possible valid and invalid RTs differences, revealed lack of significant differences for any of the scores of both initial and final pooled testing sessions, independently on the SOA (all $p > .11$). Differently, RTs for valid trials of intermediate sessions [121.0 (114.1 – 121.8) ms] were significantly shorter as compared to the corresponding scores of invalid trials [121.2 (114.9 – 124.7) ms; $p = .047$] at the SOA 50 ms, but not for the remaining SOAs ($p > .22$) (Figure 13).

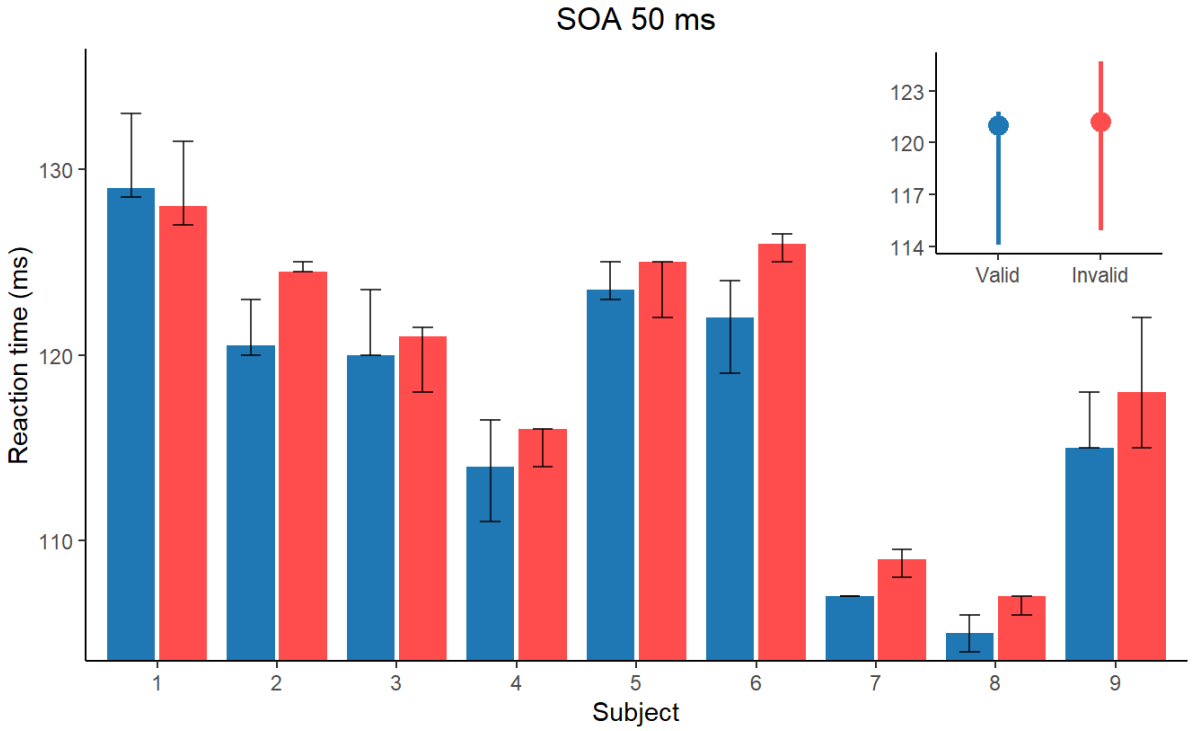
Figure 13 – RTs in initial (sessions 1 to 5), intermediate (sessions 8 to 12) and final (sessions 16 to 20) subsets of sessions, for valid and invalid trials, as a function of SOA, in Experiment 1. All conventions are the same as in Figure 5. Data analysis involved Friedman and Wilcoxon signed rank tests.



A detailed analysis of this effect revealed that even though the median RTs in valid and invalid trials exhibit a very small difference (perhaps, biologically speaking, not critical), it was consistent since for 8 out of 9 animals RTs in valid trials were smaller when compared to the subject's RTs in invalid trials (Figure 14).

Still in relation to RTs as a function of SOA in initial, intermediate and final subsets of testing sessions (Figure 13), statistics revealed a substantial increase in RTs as the SOA increased (initial: $X^2_F(3) = 21.9$, $p < .001$, *Kendall W* = .81; intermediate: $X^2_F(3) = 23.1$, $p < .001$, *Kendall W* = .86; and final: $X^2_F(3) = 23.1$, $p < .001$, *Kendall W* = .86). In all *post hoc* analyses, RTs at the SOA 900 ms were longer as compared to all other SOAs (all $p = .023$). In addition, RTs at SOA 300 ms were longer as compared to corresponding scores at SOAs 50 and 150 ms in all subsets of testing sessions (all $p = .023$).

Figure 14 – Reaction Times (ms) for valid and invalid trials at the SOA 50 ms of the intermediate subset of testing sessions of the Experiment 1. Conventions are the same as in Figure 6.



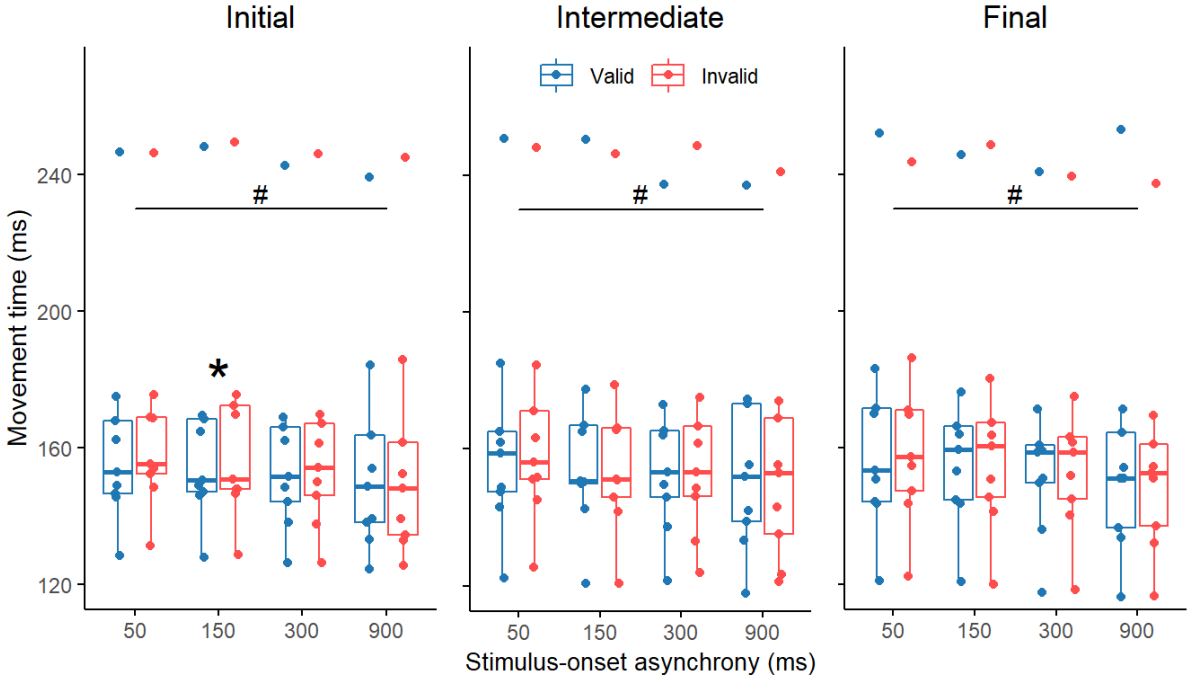
Overall, analyses involving subsets of initial, intermediate and final testing sessions generated results similar to those observed for RTs when using all sessions pooled (compare Figure 13 and 5).

3.2.2.2 Movement times

The MTs in both valid and invalid trials, as a function of SOA, for the initial, intermediate and final subsets of sessions are presented in Figure 15.

Non-parametric statistics involving paired Wilcoxon Tests looking at possible valid and invalid MT differences, revealed that, in the initial subset of sessions, MTs in valid trials are 2.5 ms faster than MTs in invalid trials at SOA 50 ms [v: 152.8 (146.4 – 167.8); i: 155.3 (152.3 – 168.9); p = .031]. Differently, in the intermediate and final subsets of sessions, there were no significant differences involving validity.

Figure 15 - MTs in initial, intermediate and final subsets of sessions, for valid and invalid trials, as a function of SOA, in Experiment 1. All conventions are the same as in Figure 5 and 7. Data analysis involved Friedman and Wilcoxon signed rank tests.

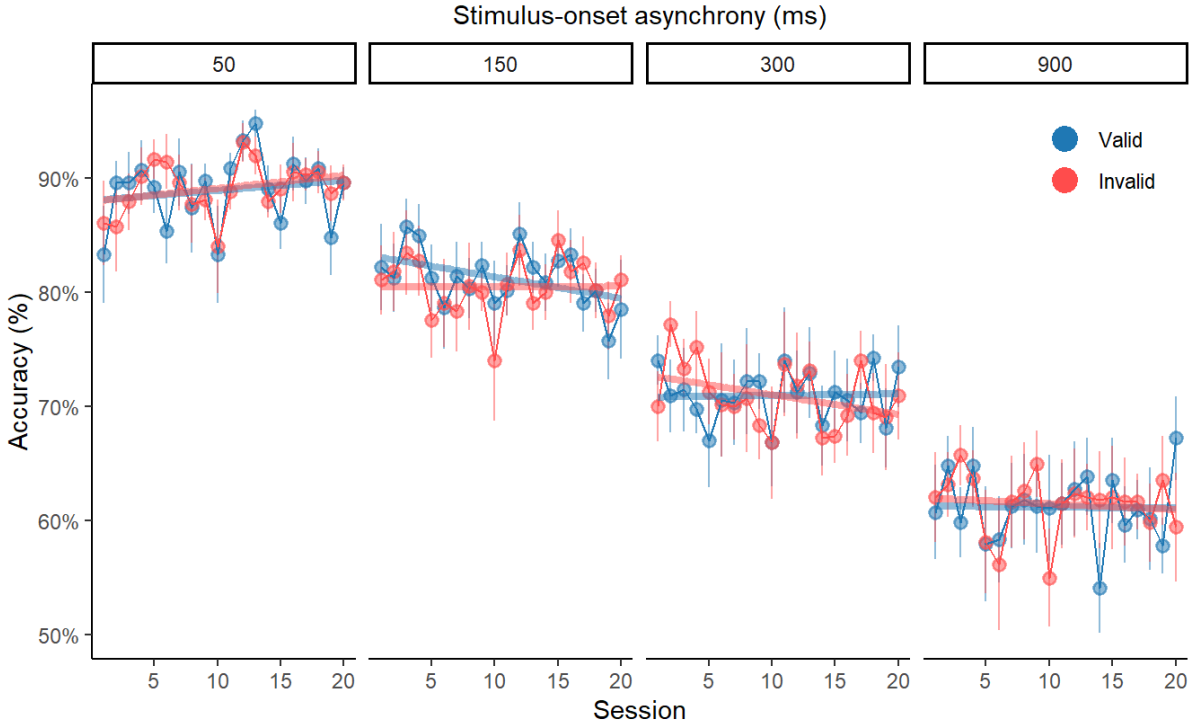


Still in relation to MTs, the statistical analysis revealed a significant decrease in MTs as the SOA increased in all subsets of sessions (**initial**: $X^2_F(3) = 8.06$, $p = .044$, *Kendall W* = .30; **intermediate**: $X^2_F(3) = 8.33$, $p = .040$, *Kendall W* = .31; **final**: $X^2_F(3) = 12.3$, $p = .006$, *Kendall W* = .46), although none of the *post hoc* analysis showed significant differences. This effect was also observed in the analysis including all sessions pooled, what further suggests that longer SOAs allow for a slightly improved motor preparation, thus generating shorter MTs.

3.2.2.3 Accuracy and Errors

A general exploratory plot of accuracy of responses in valid and invalid trials all along the testing sessions as a function of SOA is presented in Figure 16. Similarly to what is seen for RT (Figure 12), there seems to be changes in Accuracy involving Validity along the sessions, especially at SOAs 150 and 300 ms.

Figure 16 - Exploratory plot showing the means (± S.E.M.) of accuracy of responses in valid and invalid trials all along 20 testing sessions as a function of SOA. Each vertical panel shows a different SOA. Colored horizontal/diagonal thicker lines represent linear regressions and vertical lines represent S.E.M.

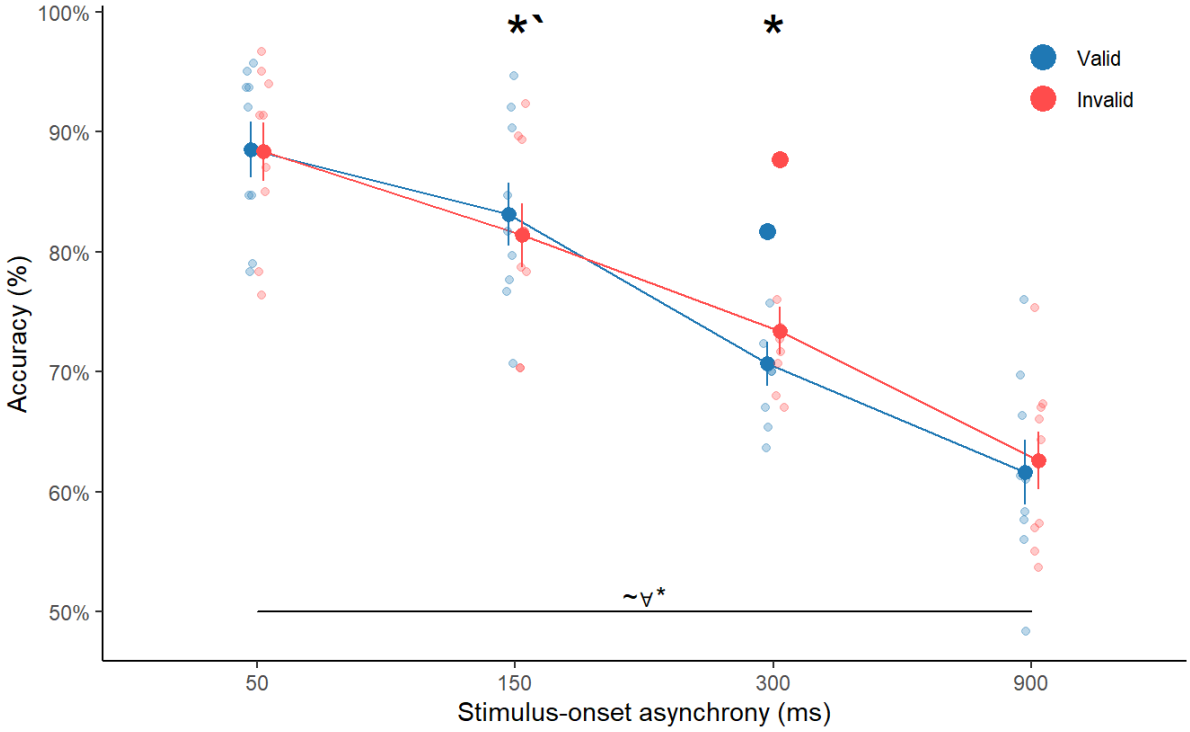


The accuracy of performance in the initial subset of testing sessions as a function of Validity and SOA is presented in Figure 17.

The ANOVA for accuracy at the initial testing sessions revealed a significant interaction effect involving SOA and validity ($F_{(3, 24)} = 3.61$, $p = .028$, $\eta^2_G = .014$). *Post hoc* analysis showed that accuracy is poorer in valid [70.7 (1.83) %] as compared to invalid trials at SOA 300 ms [73.4 (2.01) %; $p = .007$]. In addition, there was a marginally significant difference in accuracy of performance in valid and invalid trials at SOA 150 ms ($p = .075$), indicating better accuracy in valid [83.1 (2.65) %] as compared to invalid trials [81.4 (2.67) %].

These results may be indicating that despite the modality difference involving auditory cue and visual target, animals did orient attention exogenously, improving detection accuracy at SOA 150 ms and showing an inversion of the validity effect, due to inhibition of return, at SOA 300 ms.

Figure 17 – Accuracy (Mean ± S.E.M.) of performance in the initial subset of testing sessions, in valid and invalid trials, as a function of SOA, in Experiment 1. *` indicates a marginally significant difference between valid and invalid at that SOA. ~∀* indicates significant differences for all pairwise comparisons between SOAs (not Validity), except scores of valid trials at SOA 50 ms compared to valid trials at SOA 150 ms. The two larger colored dots at SOA 300 ms indicate outliers of the same subject, in valid and invalid trials. All other conventions are the same as in Figure 5. Data analysis involved ANOVA.



However, one has to be considered that our analysis detected two outlier points, both at SOA 300 ms one valid and the other invalid from the same subject, that could be potentially biasing our analysis. They are indicated by the larger colored dots in the Figure 17.

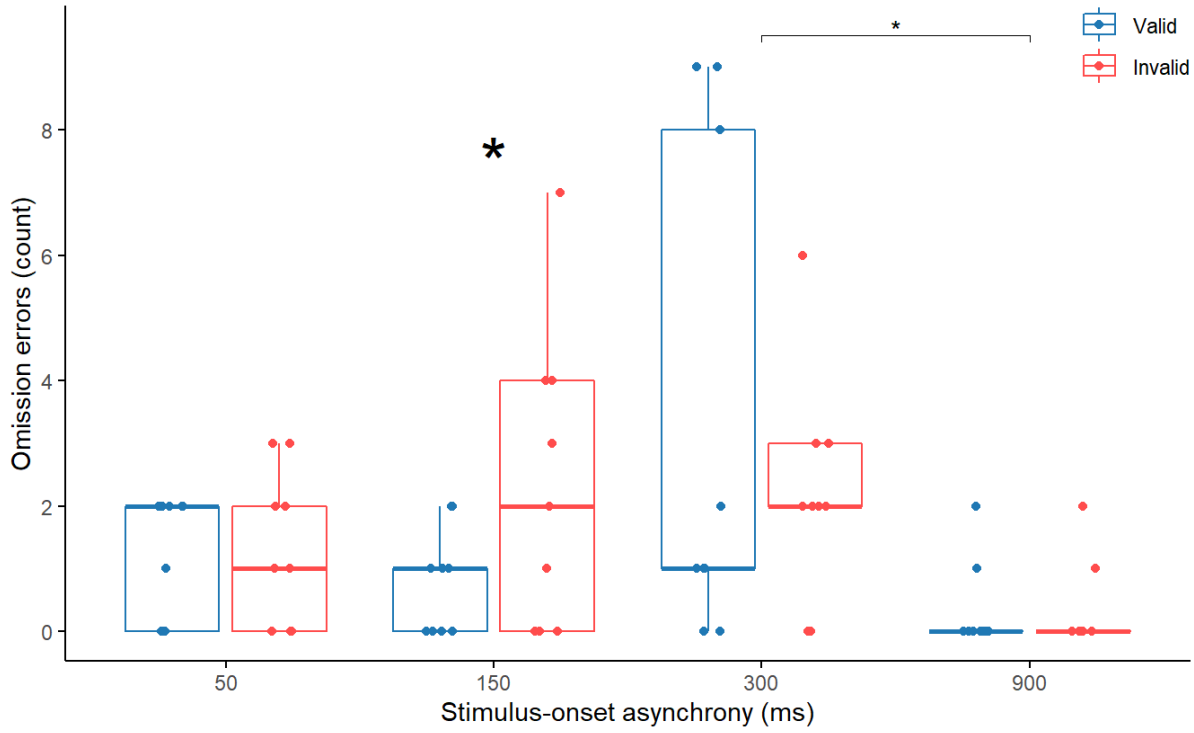
An ANOVA after removing data of the subject that exhibited the referred outliers reveals that the interaction between "SOA" and "validity" is marginally significant ($F_{(3, 21)} = 2.53$, $p = .085$, $\eta^2_G = .015$). The *post hoc* analysis[6], still indicated the existence of a significant difference between scores in valid [69.3 (1.37) %] and invalid [71.6 (1.06) %] trials at SOA 300 ms ($p = .032$).

[6] Formally, a *post hoc* analysis should not be run in these cases when the interaction is not significant. The intention of showing it here was to compare ANOVAs with and without an outlier, considering that it could be informative.

Therefore, the ANOVA revealed a significant difference between valid and invalid accuracy at SOA 300 ms when the outliers were included in the analysis, and a marginally significant difference when the outliers were excluded from the analysis. In both conditions *post hoc* analysis revealed significant differences. These analyses including and excluding the outliers seems to suggest that the difference in valid and invalid trials is consistent and not related either to measurement errors or to the outliers. Thus, this animal seems in fact to exhibit an overall accuracy greater than the other subjects at SOA 300 ms, but is not the main responsible for the observed significant effect. This issue will be discussed additionally below.

The number of omission and commission errors in the initial subset of testing sessions, in valid and invalid trials, as a function of SOAs, is presented in Figures 18 and 19, respectively.

Figure 18 - Number of Omission Errors in valid and invalid trials as a function of SOA in the initial subset of testing sessions in Experiment 1. All conventions are the same as in Figure 5. Data analysis involved a Poisson GLMM.



The Poisson GLMM for omission errors for the initial subset of testing sessions (*conditional/marginal R2* = .66/.38) revealed a significant interaction effect involving SOA and validity ($X^2(3) = 8.92$, $p = .030$). *Post hoc* analysis revealed a smaller number of omission errors in valid trials [0.78 (0.28) errors] compared to invalid [2.34 (0.80)

errors] only at SOA 150 ms (p = .011) (Figure 18). This shows that subjects exposed to valid trials reduce their number of omission errors as compared to their own performance when exposed to invalid trials, when the SOA is 150 ms. This result is fully congruent with the results observed for accuracy of responses at the same SOA (Figure 17). Together, these results indicate that rats did orient attention towards the validly cued side, at the SOA 150 ms, omitting less targets in comparison to when they orient attention to the opposite side of target presentation, in the initial subset of sessions.

Similar to results seen for all sessions pooled, the number of omission errors in both valid and invalid trials at SOA 300 ms [valid: 3.44 (1.32), invalid: 2.22 (0.59) errors] was higher as compared to the corresponding results at SOA 900 ms [**v**: 0.33 (0.23) errors, $p < .001$; **i**: 0.34 (0.23) errors, $p = .010$]. Although scores at SOAs 50 and 150 ms did not differ significantly from those at SOA 900 ms, this effect seems similar to that observed for data including all sessions pooled, indicating a drop in omission errors at the last SOA.

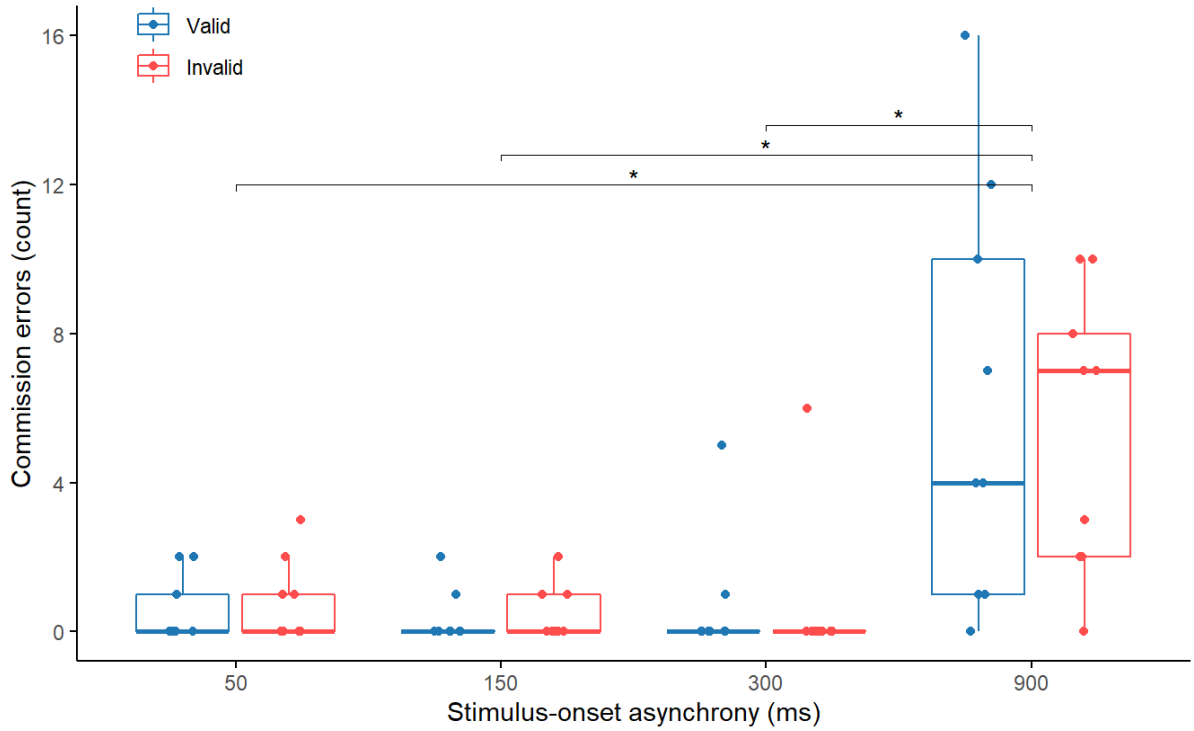
The number of commission errors as a function of Validity and SOA is presented in Figure 19.

The Poisson GLMM for commission errors involving the initial subset (*conditional/marginal* R^2 = .79/.41) revealed a main effect of SOA ($X^2(3) = 127.5$, $p < .001$). *Post hoc* analysis revealed that the number of commission errors at SOA 900 ms [5.78 (1.09)] were higher than those in seen at SOAs 50 ms [0.67 (0.29)], 150 [0.39 (0.16)] and 300 ms [0.67 (0.42); all $p < .001$]. In practical terms, this effect is identical to that seen for analyses including all sessions pooled (compare Figure 11 and 18).

Face to data of response accuracy showing a negative validity effect at SOA 300 ms, one expected a difference involving either the number of Omission or Commission Errors in valid and invalid trials at SOA 300. However, none of these effects was found.

Scores at intermediate and final subsets of testing sessions for Accuracy and errors were also analyzed. Only scores which statistics revealed significant differences were reported below.

Figure 19 - Number of commission errors in valid and invalid trials as a function of SOA involving the initial subset of testing sessions, in Experiment 1. All conventions are the same as in Figure 5. Data analysis involved a Poisson GLMM.



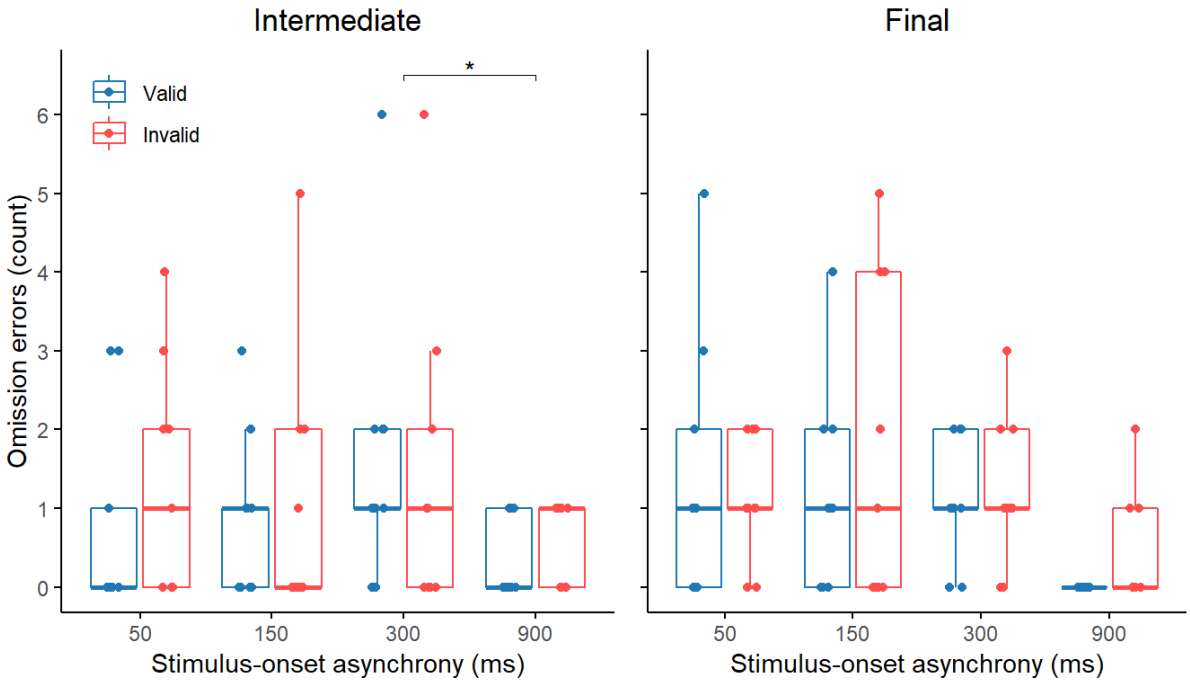
Separate ANOVAs involving Accuracy of intermediate and final subset of testing sessions (data not shown) revealed a SOA main significant effect ($F_{(1.46,\ 11.67)}$ = 73.2, p < .001, η^2_G = .67 and $F_{(1.28,\ 10.23)}$ = 53.9, p < .001, η^2_G = .72, respectively), indicating a drop on overall Accuracy as the SOAs increase, identically to that observed for scores of all testing sessions pooled.

Similarly, separate analysis including Commission Errors at intermediate and final subsets of testing sessions revealed main significant SOA effects [7] (Intermediate (Friedman): $X^2_F(3)$ = 22.2, *p* < .001, *Kendall W* = .82; Final (GLMM): $X^2(3)$ = 85.6, *p* < .001, *conditional/marginal* R^2 = .73/.31). *Post hoc* analyses indicated that all SOAs differed from SOA 900 ms (all *p* at least < .045). No significant effects involving Validity were found (data not shown).

Figure 20 shows the number of Omission Errors in valid and invalid trials as a function of SOA in the intermediate (left panel) and final (right panel) subsets of testing sessions.

[7] The GLMM for the intermediate sessions showed poor fit, hence we used non-parametric methods for this subset of sessions. Both initial and final subset fits were OK.

Figure 20 - Number of omission errors in valid and invalid trials as a function of SOA, in the intermediate and final subsets of testing sessions in Experiment 1. All conventions are the same as in Figure 5. Data analysis involved Poisson GLMMs.



Different from the initial subset of testing sessions, the analysis including Omission Errors at the intermediate and final subsets of testing sessions revealed no significant effects involving the Validity main factor. The Poisson GLMM for the intermediate subset of testing sessions (*conditional/marginal* R^2 = .50/.15) revealed a significant main effect of SOA ($X^2_{(3)}$ = 10.4, p = .015). *Post hoc* analysis showed that the number of Omission Errors at SOA 300 ms [1.56 (0.44) errors] was higher when compared to the corresponding score at SOA 900 ms [0.45 (0.12) errors], an effect similar to the one observed for the analysis including all sessions pooled. Interestingly, the scores at the final subset of testing sessions (Poisson GLMM; *conditional/marginal* R^2 = .98/.97) did not exhibit a main effect of SOA ($X^2_{(3)}$ = 5.59, p = .133). This latter lack of significant effects may be related to an overall low number of Omission Errors in the final subset of testing sessions (mean = 1.03), given the fact that subjects were extremely proficient in the task by the end of the experiment and omitted less responses (Figure 20).

3.3 PRELIMINARY DISCUSSION

The main hypothesis investigated in Experiment 1 was that auditory peripheral non-predictive cues would lead rats to orient exogenous attention towards the location where each auditory stimulus was presented thus improving, at certain SOAs, detection of visual targets presented at the same location. A secondary hypothesis was that attentional effects, if existent, would be stronger on initial testing sessions as compared to later testing sessions. Departing from these hypotheses, the main predictions were that:

A. RTs following valid auditory cues would be shorter than those following invalid auditory cues, particularly at shorter SOAs (50 and 150 ms). In parallel, higher numbers of Omission and Commission Errors were expected in invalid trials as compared to valid trials, at these shorter SOAs. These figures, if confirmed, would lend support to cross-modal, auditory/visual, effects of exogenous capture of attention;
B. No differences between performance in valid and invalid trials were expected at longer SOAs. This result, if confirmed, would emphasize that the result predicted in “A”, involving shorter SOAs, would be an effect of exogenous orienting of attention;
C. Alternatively, at longer SOAs, there could be a reversion of the effect predicted in “A”, that is, shorter RTs associated with a lower number of Omission and Commission Errors in invalid trials as compared to RTs seen in valid trials. These figures would indicate the occurrence of inhibition of return; and
D. The differences between valid and invalid cues involving RTs, accuracy and error data predicted in “A” would wane throughout testing sessions, thus confirming that attentional effects diminish when the subject is repeatedly exposed to testing.

This section will comment on whether the observed results corroborate these predictions or not.

An analysis involving the initial subset of testing sessions (initial five testing sessions) revealed that Accuracy of responses at SOA 150 ms was higher for visual targets preceded by valid auditory cues as compared to those preceded by invalid auditory cues (Figure 17). This effect was only marginally significant. Relating to that,

analyses involving Omission Errors revealed significantly greater numbers of errors in invalid as compared to valid trials at that same SOA (Figure 18). Taken together these results indicate the occurrence, at the SOA 150 ms, of exogenous capture of attention towards the side indicated by the auditory cue. This capture of attention either (1) enhances detection of the visual targets when the trials are valid as compared to when the trials are invalid, (2) impairs detection of the visual target when the trials are invalid as compared to when the trials are valid, or (3) both. It is important to note that this effect involving Omission Errors was promoted by a peripheral auditory cue presented in a non-predictive manner. In addition, the effect was transient, i.e., restricted to the SOA 150 ms – a hallmark of exogenous capture of attention. These figures partially corroborate prediction “A” referred above.

Relative to predictions “A” involving RTs, there were significant validity effects at the SOA of 50 ms both when all testing sessions were included in the analysis (Figures 5 and 6) and when only the intermediate five testing sessions were included (Figures 13 and 14). However, the differences were so small (1.8 and 0.2 ms, respectively) that they may seem biologically irrelevant.

In this context, it is important to also consider the MT results. As this variable is thought to measure the time to perform the motor response to the correct target once the subject has already decided which side to choose, we did not expect it to show any differences between valid and invalid trials. However, contrary to that, we found positive validity effects for MT at SOA 150 ms in the analysis including all sessions pooled and at SOA 50 ms in the analysis including a subset of the initial sessions. Both, similarly to the differences observed in the RT analysis, are also very small (0.6 and 2.5 ms, respectively). Although the validity effect for MT found in the initial sessions occurs concomitantly with a validity effect for RT at SOA 50 ms, even if we sum both figures, resulting in a “total response time” validity effect of 2.7 ms, its magnitude is in a time frame that hardly seems biologically relevant. On the other hand, since the subjects underwent extensive training (over 9 months) to perform the task, and motor responses were highly consistent, it could be argued that even such small RT and MT differences could be suggesting attentional orienting. Given the small magnitude of the validity effect, however, it seems difficult to decide among these possibilities.

Results of the present experiment involving accuracy at the initial subset of testing sessions revealed signs of a negative validity effect for accuracy (i.e., higher accuracy in invalid as compared to valid trials) at the SOA of 300 ms (Figure 17). This may indicate the occurrence of cross-modal inhibition of return (IOR), thus corroborating, at least partially, the prediction “C” outlined above. However, there was no statistical evidence that this effect is related to either omission errors (Figure 18) or commission errors (Figure 19).

In the present experiment, validity effects were revealed when analysis involved a pool of 20 testing sessions (Figure 10), and also when analysis focused on initial (Figures 17 and 18) subsets of testing sessions, but never when analysis focused on the final subset of testing sessions. These figures indicate that repeated testing in rats lead to a decrease in the attentional response, as it has been reported for humans (Lupiáñez et al., 2001; Pratt & McAuliffe, 1999; Weaver et al., 1998). This confirms prediction “D” outlined above.

Overall, the results of Experiment 1 partially corroborate predictions of the hypothesis that rats have their exogenous attention captured for a place where a non-predictive auditory cue is presented thus interfering with their reaction to visual targets presented in the same location. This effect wanes with repeated testing.

4 Experiment 2 – Does spatial superposition of auditory cues and visual targets interfere with endogenous and exogenous cross-modal orienting of attention?

Although results from Experiment 1 do provide evidence supporting the hypothesis that rats exhibit exogenous capture of attention by a non-predictive auditory cue, thus improving detection to a visual target presented at the same side, important differences were found relative to studies employing non-predictive visual cues preceding visual targets (e.g. Marote & Xavier, 2011). In these latter studies, differences in accuracy of responses in valid and invalid trials are usually larger than 5% (e.g. Marote & Xavier, 2011), that is, twice as large as the ones reported in Experiment 1 of the present study. Furthermore, the validity effects for RT (RT in invalid trials minus RT in valid trials) were bigger than 30 ms and, in other studies, even larger than 100 ms (e.g. Rosner & Mittleman, 1996), whereas our results revealed differences smaller than 3 ms. The origin of these differences is not clear.

One possibility is that they are related to the sensory modality of stimuli used as cues and targets in the experiments. That is, while the present experiments employed auditory stimuli as cues and visual stimuli as targets, Marote and Xavier's (2011) and Rosner and Mittleman's (1996) studies used visual stimuli as cues and targets.

Another possibility is that, while in these latter studies the cue and the target stimuli were presented exactly at the same location of space (i.e. they were spatially superposed), this was not exactly the case for the Experiment 1 (see Figure 21, for a schematic representation of the auditory cue and visual target spatial location). In fact, previous studies involving humans show that the spatial superposition of auditory and visual stimuli is important for attentional orienting (Spence et al., 2000).

In order to evaluate this latter possibility, the architecture of the operant conditioning chambers was changed such that the auditory cue and visual target were released exactly from the same hole (see Figure 21). Thus, a first objective we had with Experiment 2 was to investigate the effect of releasing cue and target from the same spatial location on cross-modal attention orienting in rats.

A second objective we had with this experiment was to investigate orienting of attention using peripheral **predictive** cues.

In Experiment 1, we implemented a protocol that did not expose the animals to the auditory cues before the testing sessions. This allowed us to show that, as long as repetitive training in the task is avoided, exogenous orienting of attention in rats is possible using auditory peripheral non-predictive cues. The finding corroborates similar reports in humans (Lupiáñez et al., 2001; Pratt & McAuliffe, 1999; Weaver et al., 1998) and suggests that our previous study (Cruz, 2017) may not have revealed exogenous orienting of attention due to overtraining.

Although we had planned to investigate the effect of overtraining on peripheral predictive cues in Experiment 1, this was not possible. As several animals were unable to learn the task and had to be excluded from the study, we decided to restrict our experiment to exogenous orienting of attention, using only peripheral non-predictive cues.

In Experiment 2, by using an improved conditioning protocol to train the animals, there was a substantially increase in the number of animals able to perform the task. This allowed us to implement a group exposed to peripheral predictive cues, enabling the investigation of whether overtraining also affects attention when both endogenous and exogenous interact.

Thus, in Experiment 2 we investigated:

1. The effect of releasing the auditory cues and visual targets from the same spatial location on both endogenous and exogenous cross-modal orienting of attention, employing both predictive and non-predictive peripheral cues; and
2. Whether overtraining also affects attention when both endogenous and exogenous interact.

4.1 METHODS

Most of the methods used in Experiment 2 were the same as in Experiment 1. Thus, this section will report only differences between Experiments 1 and 2.

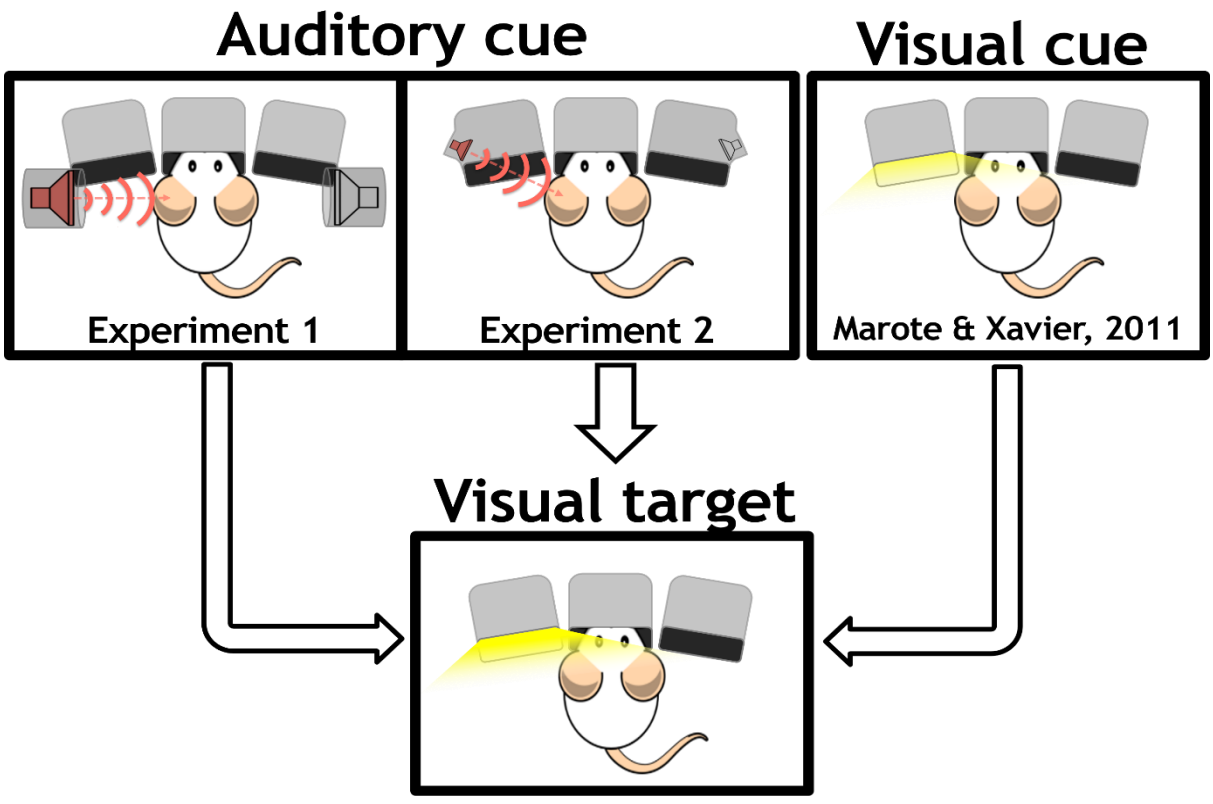
4.1.1 Subjects

Twenty-seven male Wistar rats (*Rattus norvergicus*) were employed. An improved animal facility, with temperature at 23±2 °C, was used for maintaining the subjects of this experiment. All other conditions were identical to those of Experiment 1.

4.1.2 Equipment

Eleven nearly identical operant conditioning chambers as those used in Experiment 1 (Figure 2) were employed in the present experiment.

Figure 21 - Comparison of the position of different spatial cues (top row), both auditory (present work) and visual (previous studies), in relation to the visual targets employed in these tasks (bottom row). Note the difference of speaker positions between Experiments 1 and 2. The rose arrow and arcs depict the path that the sound travels from the left speaker to the animal's ears. Also note how in experiments employing visual cues, both cue and target come from the same source.



Source: Mateus Torres Cruz.

The chambers' specifications and features remained essentially the same as reported in Experiment 1, with the difference that an additional speaker (Philips MDR-EX15LP) was installed in each of the lateral holes such that the auditory cue and the visual target were released from approximately the same location, i.e., from inside the hole. That is, each speaker was installed within a recess added to the external lateral wall of the lateral holes, 10 mm from its entrance and in an angle of approximately 30° relative to the wall, such that its sound was released inside the lateral hole being directed towards the corresponding rat's ear when nose poking the central hole (Figure 21, see also Figure 3, for a photo). These newly installed speakers were the only ones used for presenting the sound cues in the present experiment. The speakers used in Experiment 1 were removed from the chambers. Each novel speaker was calibrated to emit ~65 dB sound pressure level. The position of the new speakers allowed presenting cues and targets at the same side (as with the previous speakers), and originating within the same hole, thus increasing their spatial superposition.

4.1.3 Behavioral task

The behavioral task was essentially the same as in Experiment 1, with small differences. Anticipation Errors, involving removal of the nose from the central hole either before the end of the SOA or up to 80 ms after target onset, similarly to Experiment 1, were "punished" with a timeout period. Differently, however, instead of skipping the trial, it was reinitiated, that is, after the timeout the animals had to nose-poke the central hole again, hear the same sound cue, and maintain their noses within the central hole until at least more than 80 ms had elapsed after the target onset. In other words, testing proceeded to the next trial following either a correct response, an Omission or a Commission Error, but not after an Anticipation Error.

This strategy of repeating a trial when the subject exhibited an Anticipation Error was implemented to increase the number of trials available for data analysis, since Anticipation is the most common error and its occurrence, unlike Commission and Omission Errors, is not informative about orienting of attention. Thus, a positive side effect of this procedure was the increase in the total number of rewards received by

the subjects within a session, since they could retry to perform correctly trials that were anticipated, differently from Experiment 1.

Another difference relative to Experiment 1 was that a session included 750 trials, instead of 600 trials. This allowed collecting data of a larger number of trials per condition, thus increasing the reliability of data for each condition. The ITI was reduced from 250 to 100 ms, and the timeout from 5000 to 2000 ms. These changes accelerated the sessions whilst keeping the rat's motivation high.

4.1.4 Groups

Similar to Experiment 1, this experiment employed both 5 and 8 kHz pure-tone beeps, individually presented either at the left or right speaker, i.e., peripheral auditory cues. Each cue frequency was presented an equal number of times at each side, and in random order, such that it provided no information about the pending target.

In order to valuate endogenous and exogenous orienting of attention, the effects of cue predictability were investigated in independent groups of subjects. One group of subjects was exposed to predictive (P) cues, involving 80% of valid trials (the cue was presented at the same side as that of the target) and 20% of invalid trials (cue presented at the opposite side as that of the target). The second group was exposed to non-predictive (NP) cues, involving 50% of valid and 50% of invalid trials.

Since 5 subjects were excluded from the experiment because they did not learn the task, by the end of Experiment 2 each group included 11 subjects (see below).

4.1.5 Conditioning

Conditioning steps in Experiment 2 followed the guidelines employed in Experiment 1, but with some differences reported below.

The major modifications involved the repetition of trials in which the subjects exhibited errors of Anticipation (see above) and the reduction of both the timeout period and the ITI, thus rendering the task less punitive and more agile. In addition, criteria for promotion towards the next phase were also changed. That is, instead of

requiring three sessions (in most of phases) with at least 75% of performance accuracy, a plateau of performance was demanded. This plateau was defined as a stabilization of the value of one or more variables being measured in a given conditioning phase (e.g., accuracy, number of “C” commission errors; see below) for three consecutive days, as estimated by the visual inspection of the variable as a function of session. These procedural changes substantially reduced number of sessions required for task acquisition by the rats in order to start testing (see results section).

Phases 1-3 were similar to those of Experiment 1, except for the ITI (reduced from 250 to 100 ms) and the timeout (reduced from 5000 ms to 2000 ms). These novel ITI and timeout period were used all along the training and testing phases. In addition, during phase 2, involving free availability of sucrose solution independently on the subject’s response, the drinking device was activated every 5000 ms, instead of every 3000 ms, in order to increase the subject’s engagement. The changes made to the following phases are described below.

4. Center-to-lateral nose-poke conditioning. Similar to Experiment 1, the goal here was to shape the nose-poking behavior acquired in the previous phase in order to teach the subject to always poke the hole with the LED switched on. At the beginning of every trial only the central hole’s LED was switched on. When the animal nose poked it, the LED switched off and, in 80% of the trials, the visual target appeared in one of the lateral holes. Nose poking the hole’s target led to the release of the reward; there was no time limit to poke it. The remaining 20% of trials were catch trials, i.e., there was no target and the reward was released immediately after nose poking into the central hole. There were no timeout periods upon nose pokes into holes with the LED switched off; even though, when these responses occurred, they were recorded. For other responses, two kinds of errors were distinguished. They included “C” Commission Errors, when the animal nose poked a lateral hole having the central hole with its LED switched on, i.e., a central nose poke was required, and “L” Commission Error, when the animal nose poked either the central or a lateral hole which LEDs were switched off, i.e., a specific lateral nose poke was required. The number and types of errors were used to calculate accuracy of performance. From this phase on, trials were shuffled in a pseudo-random way. That is, the order of trials was randomized such that it avoided repetitions of more than five

consecutive trials with a target on the same side or five consecutive catch trials. Sessions usually lasted 30 minutes and animals were moved to the next phase after reaching a plateau in accuracy and number of each type of error per trial.

5. "L" commission error punishment. This novel phase was introduced in Experiment 2. By analyzing Commission Errors in phase 4, it was noticed that even though the subjects responded correctly to the central hole (thus exhibiting a reduction number of "C" Commission Errors throughout the sessions), their number of "L" Commission Errors did not decrease substantially along the sessions. This phase was introduced in an attempt to reduce this type of error. Thus, whenever the animal nose poked a hole with its LED switched off, a timeout period was initiated. This stimulated the subjects to avoid "L" commission. Additionally, if a subject, holding its nose within the central hole, introduced one of its paws into one of the lateral holes, the trial was reinitiated after a 2000 ms delay. Differently from the timeout, however, during this delay the houselight remained on to indicate to the subject that the delay is related specifically to the introduction of the pawn in the hole and not to other types of error. Everything else was identical to the previous phase. After reaching a plateau in Accuracy and numbers of each error, subjects were promoted to the next phase.
6. SOA's insertion. This phase corresponded to an adaptation of phase 5 of Experiment 1. It added an adjustable SOA between the central hole nose-poke and either the onset of the visual target, in targeted trials, or the release of the reward, in catch trials. Initially, the adjustable SOA was 0 ms (i.e., there was no delay). However, it increased by 50 ms steps every time the animal responded correctly in six consecutive trials, until a maximum of 1200 ms. It also decreased by 50 ms steps, to a minimum of 50 ms, if the subjects committed a Commission Error (either "L" or "C") in three consecutive trials. When the adjustable SOA reached the length of one of the SOAs planned to be used in the task, that pre-defined SOA was randomly presented within the same session, interspersed with the adjustable SOA. For example, the SOAs of 50, 150, 300, 900 and 1200 ms, were planned to be used in this experiment. Thus, when the animal reached an adjustable SOA of 100 ms, 20% of trials employed the pre-defined SOA of 50 ms and the remaining trials employed the adjustable SOA, i.e., 100 ms.

Similarly, when the animal reached an adjustable SOA of 200 ms, 20% of the trials employed the pre-defined SOA of 50 ms, 20% employed the pre-defined SOA of 150 ms and the remaining trials employed the adjustable SOA. And so on. When the subject reached an adjustable SOA of more than 900 ms, i.e., all pre-defined SOAs planned to occur in targeted trials were already occurring as planned, only catch trials occurred in the 20% remaining trials using the adjustable SOA. Misses and hits in trials using the pre-defined SOAs were not considered for increasing or decreasing the adjustable SOA. Anticipation errors were considered in this phase. If the subject removed its nose from the central hole before completion of the SOA for that trial, the reward was not released and the trial was repeated after a timeout. The number Anticipation Errors were recorded, although they did not count towards increasing or decreasing the adjustable SOA. Differently from previous phases, “C” Commission Errors restarted the current trial, after a timeout period (similarly to trials with anticipation errors). This prevented the subjects’ trend to nose poke randomly after Anticipation Errors, a behavior that increased “C” Commission Errors. Each session started using the adjustable SOA that the animal had reached at the end of the previous session. The subjects were promoted to the next phase after reaching the maximum SOA (1200 ms) and after their accuracy and number of anticipation errors by trial plateaued.

7. Reduction of the target duration. This phase corresponded to phase 8 of the Experiment 1. In all previous phases, the LED of the target hole remained switched on indefinitely, until a nose poke occurred, such that there was no time limit to respond. From this phase on the target duration was limited to 100 ms. Even though the animals could respond up to 1000 ms after target onset. This served to two purposes. First, reduction of target light duration stimulated the animal to respond as fast as possible. Second, this allowed the insertion of the Omission Error, i.e., if the animal responded 1000 ms or more after the target onset, a timeout period was triggered. Everything else was identical to the previous phase, except that the SOAs were maintained stable in the pre-defined durations, i.e., 50, 150, 300 and 900 ms in trials with a target and 1200 ms in catch trials. The criterion for promotion to the next phase was to reach a plateau in Accuracy and within each type of error.

8. Individual target threshold identification. This phase corresponded to phase 9 of Experiment 1. An adaptive procedure was employed to identify the target luminance threshold for each subject. This identification of the threshold occurred in an independent manner for targets presented in the left and in the right holes. The procedure applied here was based on both the Fixed Step-Size Staircase (García-Pérez, 1998, 2000) and Unforced Weighted Up–Down (Kaernbach, 2001) procedures. The general logic behind these procedures was to reduce the light intensity of the lateral hole's LEDs after correct responses and to increase it after incorrect responses, until an 80% level of accuracy was reached. At the beginning of the session the LEDs' intensity was 50 lux and the step size for either decreasing or increasing the LEDs' intensity was 5 lux. The step sizes, however, were reduced along the session based on the number of reversals. A reversal was defined as whenever a correct response was followed by an incorrect response and vice-versa. At every second reversal, the step sizes were halved, down to a minimum that depended on the direction of the step. The minimum step size for increasing LED intensity was 1.75 lux and for decreasing LED intensity was 0.5 lux. Usually, a single experimental session was run (see below). This session ended after 60 reversions for each side had occurred. The individual target threshold was calculated for each side of each subject, corresponding to the mean of luminance employed just before each reversal, excluding the first and second reversals. An additional session was run for each animal using the calculated target luminance. If accuracy of response was about 80%, they proceeded to the testing phase. If accuracy was still higher than 80%, the subject was submitted to an additional session for target threshold identification.
9. Testing. Peripheral auditory cues were inserted according to the experimental group (either predictive or non-predictive) and visual targets luminance for each side of each rat was set to the values calculated in phase 8. Then, the task events followed the order presented in the behavioral task section. Each subject performed at least 20 testing sessions.

4.1.6 Measured variables and data analysis

The variables measured and data analyses were similar to those employed in Experiment 1, with adaptations for the novel procedures and factors introduced in this experiment.

In the present experiment, whenever the subject made an anticipation error the trial was reinitiated. In order to evaluate whether the subject's responses were different in such reinitiated trials we ran each analysis twice, one including only data of non-anticipated trials, and another including all trials, i.e., both anticipated (and reinitiated) and non-anticipated trials.

Similar independent variables as those used for analyzing data of Experiment 1 were included in the present experiment. However, data analyses here also included a between-subjects independent variable, named Predictability, in order to compare performance of subjects exposed to the predictive condition (P; 80% of valid trials and 20% of invalid trials) relative to performance of subjects exposed to the non-predictive condition (NP; 50% of valid trials and 50% of invalid trials). When a non-parametric approach was used, separate Wilcoxon signed rank and Friedman tests were run for each group. A Wilcoxon Rank Sum test (equivalent to the Mann Whitney U) was employed to compare the results between groups (Predictability) using data pooled across all levels of SOA and Validity for each level of Predictability.

Box-Cox transformation (Box & Cox, 1964), instead of natural logarithm for RT data, was used to improve ANOVA fits.

Finally, Binomial GLMMs (with logit link), instead of Poisson GLMMs, were used for analyzing accuracy and number of errors, when appropriate. This approach was required because P and NP groups, by their nature, included different numbers of valid and invalid trials. Dependent variables included the percentage of responses in a given condition relative to the total number of trials in that condition (percentage of successes). The number of trials in that condition was used as weights (total number of cases) when defining the GLMMs.

All other procedures and conventions were the same as those used in Experiment 1.

4.2 RESULTS

Twenty-two rats, out of twenty-seven that were initially included in the experiment, completed all twenty testing sessions. Thus, five subjects were excluded, being one for complete inactivity within the conditioning chamber and four that did not reach the level of accuracy required to progress to the testing sessions. The final number of subjects per group (P and NP) was eleven animals.

Similar to Experiment 1, two different analyses were run, one including data from all testing sessions pooled and another including data of subsets of testing sessions, including initial (sessions 1 to 5), intermediate (sessions 8 to 12) and final (16 to 20) testing sessions.

4.2.1 Analyses including all testing sessions pooled

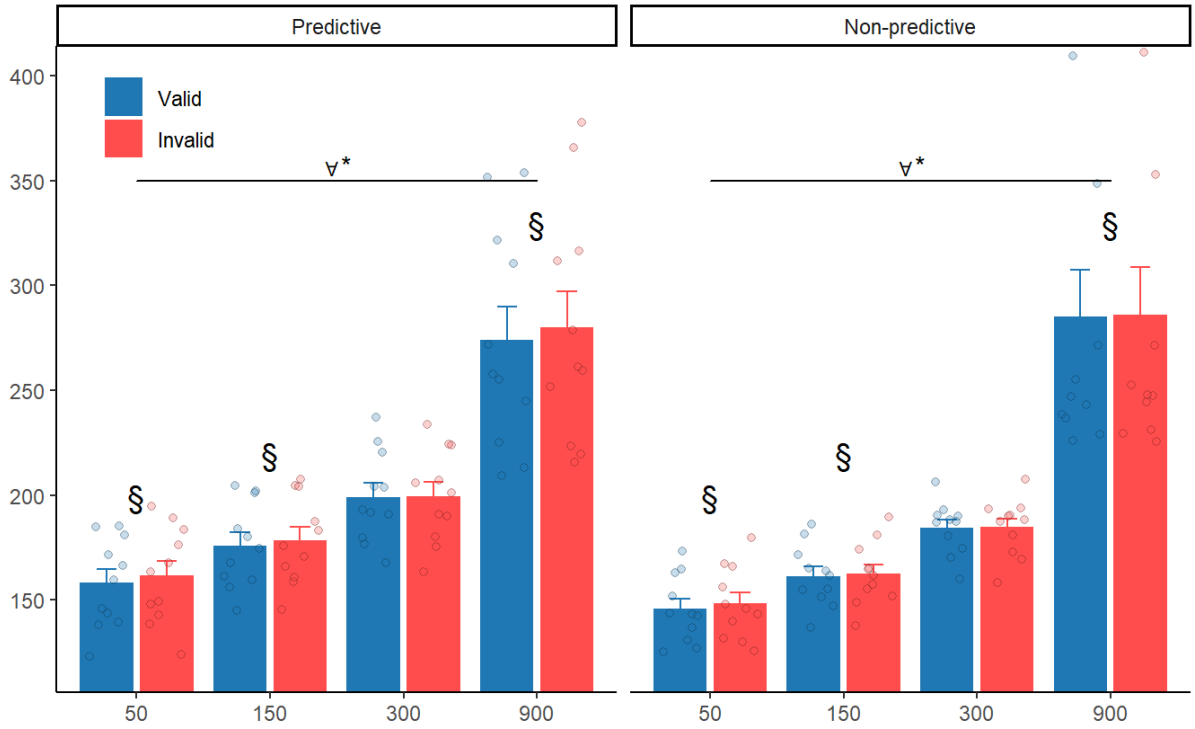
4.2.1.1 Reaction Times

Figure 22 shows the RTs in valid and invalid trials, for subjects trained using either P or NP peripheral auditory cues, as a function of SOA.

ANOVA employing Box-Cox transformed data revealed significant main effects for SOA ($F_{(1.19, 23.9)} = 123.2$, $p < .001$, $\eta^2_G = .732$) and Validity ($F_{(1, 20)} = 57.7$, $p < .001$, $\eta^2_G = .002$), and a significant interaction effect for SOA and Validity ($F_{(3, 60)} = 12.1$, $p < .001$, $\eta^2_G = .001$). It also revealed a marginally significant Predictability X Validity interaction ($F_{(1, 20)} = .3.12$, $p = .093$, $\eta^2_G < .001$). There was no effect of Predictability ($F_{(1, 20)} = 2.46$, $p = .132$, $\eta^2_G = .063$), nor interactions between Predictability and SOA ($F_{(1.19, 23.9)} = 1.36.$, $p = .262$, $\eta^2_G = .030$), or Predictability, Validity and SOA ($F_{(3, 60)} = 1.32$, $p = .275$, $\eta^2_G = < .001$). *Post hoc* analyses showed that RTs in invalid trials, as compared to corresponding RTs in valid trials, were longer at SOAs of 50 [**v**: 151.8 (4.15), **i**: 154.9 (4.45) ms, $p < .001$], 150 [**v**: 168.6 (4.00), **i**: 170.4 (4.14) ms, $p < .001$] and 900 ms [**v**: 279.5 (13.5), **i**: 283.0 (13.9) ms, $p = .005$], but not at the SOA of 300 ms, independently on predictability. Although these differences seem small (about 3 ms) to be considered biologically relevant, the statistical effects did reveal a validity effect at these SOAs, suggesting that there was either capture or orientation of

attention towards the cued side, leading either to faster responses in valid trials or to slower responses in invalid trials, or both. Additionally, the RT at each SOAs differs from all other SOAs (all $p < .001$), indicating a strong raise in RT throughout SOAs.

Figure 22 - Reaction Times in valid and invalid trials, for subjects trained using either P or NP peripheral auditory cues, as a function of SOA, in Experiment 2. § indicates significant differences between valid and invalid trials as shown by the *post hoc* analysis of a significant validity X SOA interaction (no effect involving predictability was found). The "∀*" symbol indicates that all comparisons between SOAs are statistically significant. Data analysis involved an ANOVA with Box-Cox transformed data.



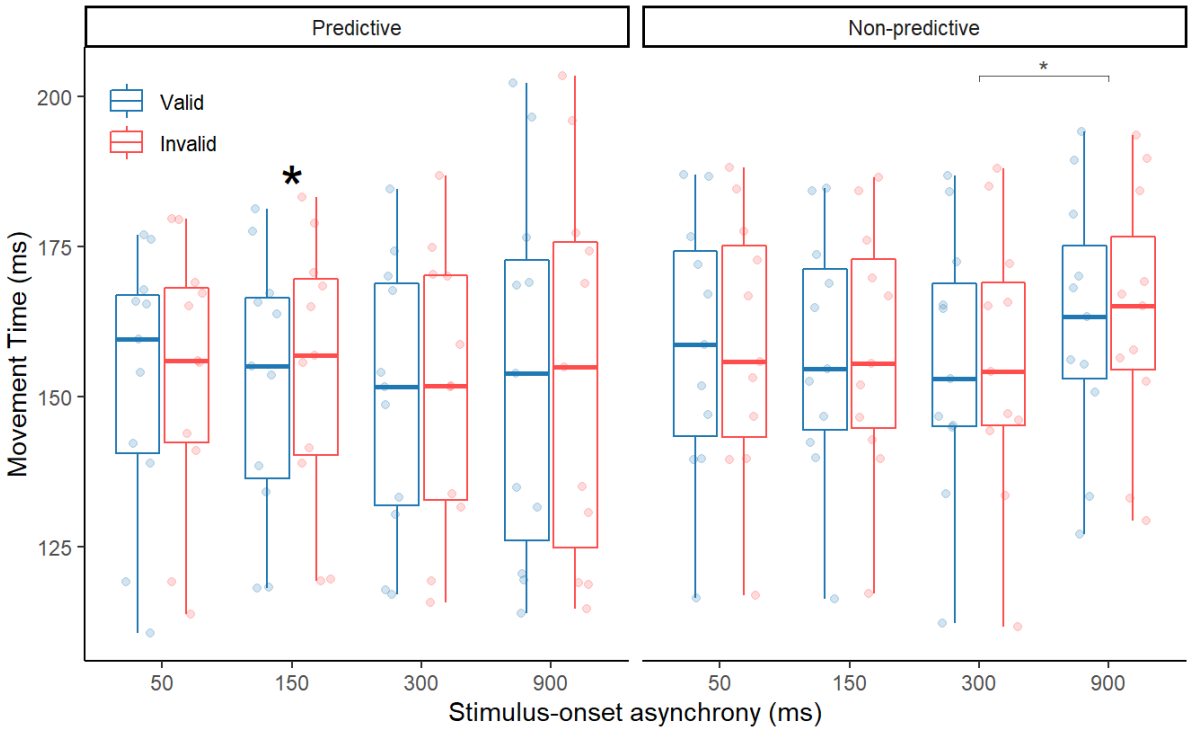
4.2.1.2 Movement times

The MTs in both valid and invalid trials, as a function of SOA and group, are presented in Figure 23.

Non-parametric statistics involving paired Wilcoxon Tests looking at possible valid and invalid MT differences, revealed that MTs in valid trials are 2.8 ms smaller than MTs in invalid trials at SOA of 150 ms of the P group [**v**: 155.0 (136.3 – 166.4); **i**: 156.8 (140.2 – 169.5) ms; p =.004] (Figure 23). Additionally, the analysis revealed a significant rise in MTs as the SOAs increase in the NP group ($X^2_F(3) = 13.4$, $p = .004$, *Kendall W* = .41), but not in the P group ($X^2_F(3) = 3.33$, $p = .344$, *Kendall W* = .10). The

post hoc analysis indicated that, in the NP group, MTs at the SOA 900 ms [164.2 (153.3 – 177.7) ms] are longer than at SOA 300 ms [153.6 (144.9 – 170.5) ms]. The Wilcoxon Rank Sum test revealed no difference between groups.

Figure 23 – Movement Times for valid and invalid trials, as a function of SOA and Group, in Experiment 2. The symbols * and *` indicate, respectively, a significant and a marginally significant difference between valid and invalid conditions at the corresponding SOA. When those symbols are accompanied by a bracket, they indicate significant differences between SOAs (not Validity) connected by the bracket. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.

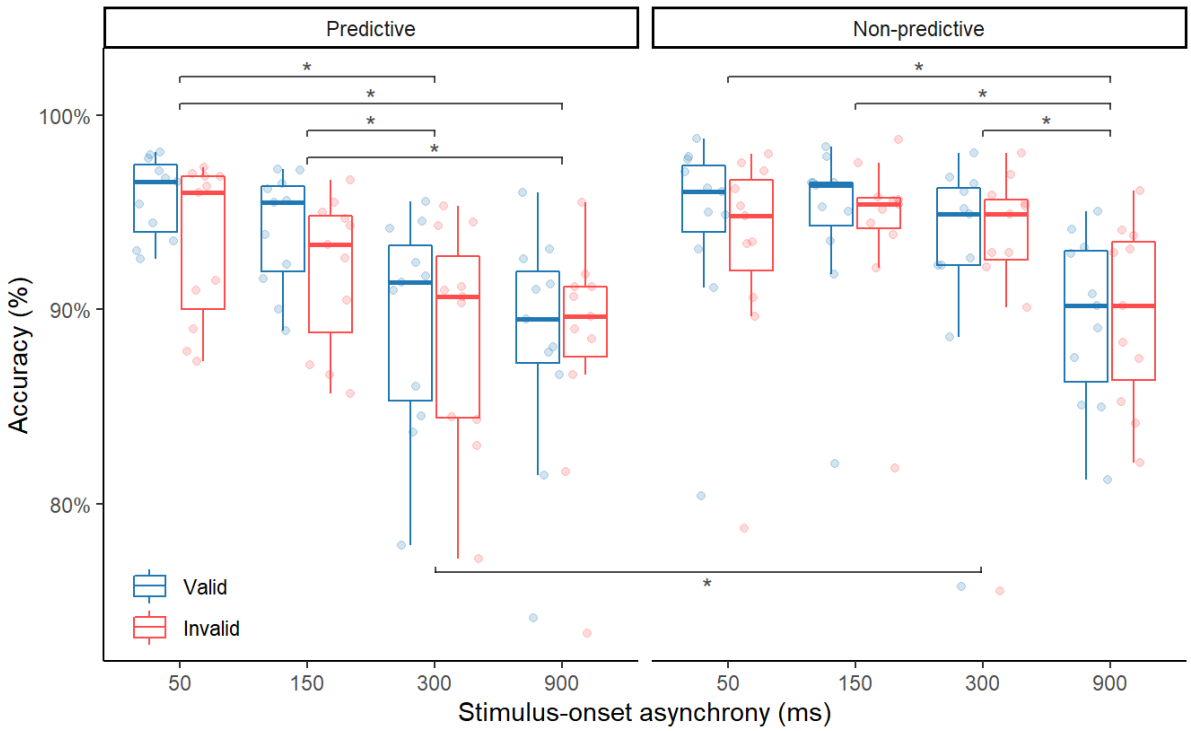


4.2.1.3 Accuracy

For the Accuracy analysis, we employed a Binomial GLMM (conditional R^2 = .04, marginal R^2 = .04). It revealed a SOA main significant effect ($X^2(3) = 84.8$, $p < .001$) and a significant Predictability X SOA interaction ($X^2(3) = 11.1$, $p = .011$). *Post hoc* analyses revealed that the Accuracy of NP subjects at SOAs of 50 [93.8 (1.11) %], 150 [94.4 (0.93) %] and 300 ms [92.7 (1.29) %] were significantly greater than those seen at SOA of 900 ms [89.6 (0.94) %; $p \leq .010$ in all comparisons], indicating that accuracy at shorter SOAs is reasonably constant, but drops at the longer SOA

(900 ms). In contrast, the Accuracy of P subjects (1) at the SOAs of 50 [94.6 (0.72) %] and 150 ms [93.0 (0.75) %] did not differ significantly among each other (p = .477), (2) decreases when one increases the SOA from 150 ms to 300 ms [89.1 (1.18) %; p = .002], and (3) at the SOA of 300 ms did not differ from that seen at SOA of 900 ms [88.2 (1.26) %] (p = .986). Thus, similarly to NP subjects, accuracy of P subjects also decreases as the SOA increases, even though this effect appears at shorter SOAs. Consequently, accuracy of P and NP subjects is significantly different at the SOA of 300 ms (p = .018), as can be seen in Figure 24.

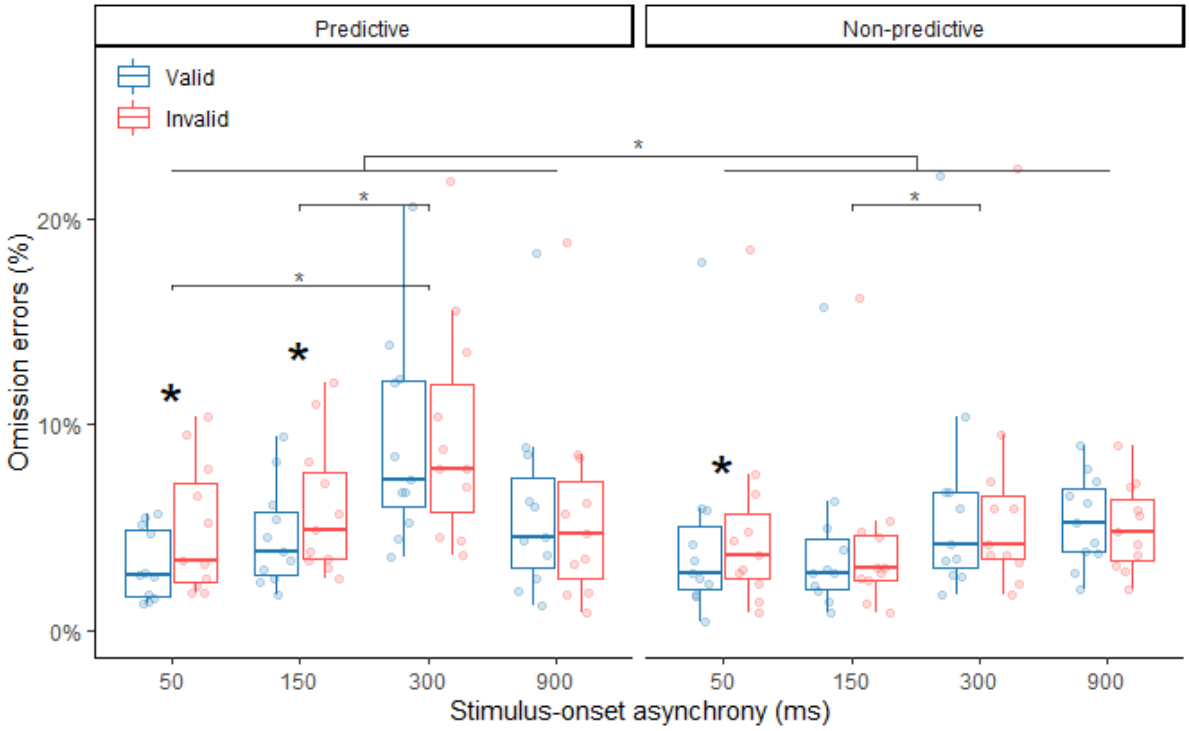
Figure 24 – Accuracy for the P and NP groups as a function of Validity and SOAs, in Experiment 2. All conventions are the same as in Figure 23. Data analysis involved a Binomial GLMM.



4.2.1.4 Errors

The percentage of omission errors in valid and invalid trials of P and PN subjects as a function of SOA is presented in Figure 25.

Figure 25 – Percentage of omission errors in valid and invalid trials of P and NP subjects as a function of SOA, in Experiment 2. A * symbol above the brackets with wide ticks indicates a significant Wilcoxon Rank Sum test indicating a difference in the general mean (without pooling data by SOA and Validity) between both groups. All other conventions are the same as in Figure 23. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



Statistics did reveal significantly higher percentages of omission errors in invalid trials, as compared to valid trials, in both P and NP subjects (Wilcoxon signed rank tests; see Figure 25). Interestingly, while P subjects revealed these differences at SOAs of 50 [**v**: 2.67 (1.60 – 4.90); **i**: 3.34 (2.34 – 7.17) %; p = .015] and 150 ms [**v**: 3.80 (2.69 – 5.75); **i**: 4.83 (3.41 – 7.67) %; p = .012], NP subjects revealed such difference at the SOA of 50 ms [**v**: 2.73 (2.00 –5.00) %; **i**: 3.60 (2.53 – 5.67) %; p = .020] (Figure 25). Thus, as expected, there were more omission errors when the subjects were exposed to invalid cues, and therefore oriented their attention towards the opposite side related to that of visual target presentation, as compared to valid cues, when the subjects oriented their attention towards the correct side related to that of visual target presentation. This effect was restricted to shorter SOAs. These figures reinforce the interpretation that the subjects did orient attention towards the location indicated by the cue. At the SOA of 50 ms, this effect appeared to both subjects exposed to the P condition and subjects exposed to NP condition. Differently, at the

SOA of 150 ms, this effect appeared for subjects exposed to P condition but not for subjects exposed to NP condition (Figure 25).

The Wilcoxon Rank Sum test comparing the percentages of omission errors between groups was statistically significant (p = .045), indicating that P subjects [4.98 (2.98 – 8.18) %] committed more errors than NP subjects [3.77 (2.65 – 6.00) %]. This suggests predictive and non-predictive cues engage different response strategies. Probably, the P group relied more in the information provided by the cue than the NP group and, thus, made more omission error when the cue was invalid.

Additionally, the Friedman test indicated a main effect of SOA in both P and NP subjects (**P**: $X^2_F(3) = 12.3$, p = .006, *Kendall W* = .372; **NP**: $X^2_F(3) = 9.76$, p = .021, *Kendall W* = .296). *Post hoc* analyses showed that for P subjects the percentage of omission errors at the SOAs of 50 [2.95 (1.91 – 5.39) %] and 150 ms [4.19 (3.08 – 6.91) %] were lower as compared to the corresponding scores at the SOA of 300 ms [7.83 (5.61 – 12.2) %; p = .002 and < .001, respectively]. For NP subjects *post hoc* analyses revealed that the percentage of omission errors at the SOA of 150 ms [2.87 (2.20 – 4.67) %] was significantly lower as compared to the corresponding result at the SOA of 300 ms [4.20 (3.30 – 6.67) %; p = .004]. These results mirror those observed for accuracy, suggesting that reduction of accuracy at the SOA of 300 ms in the P subjects is related to an increase in the percentage of omission errors.

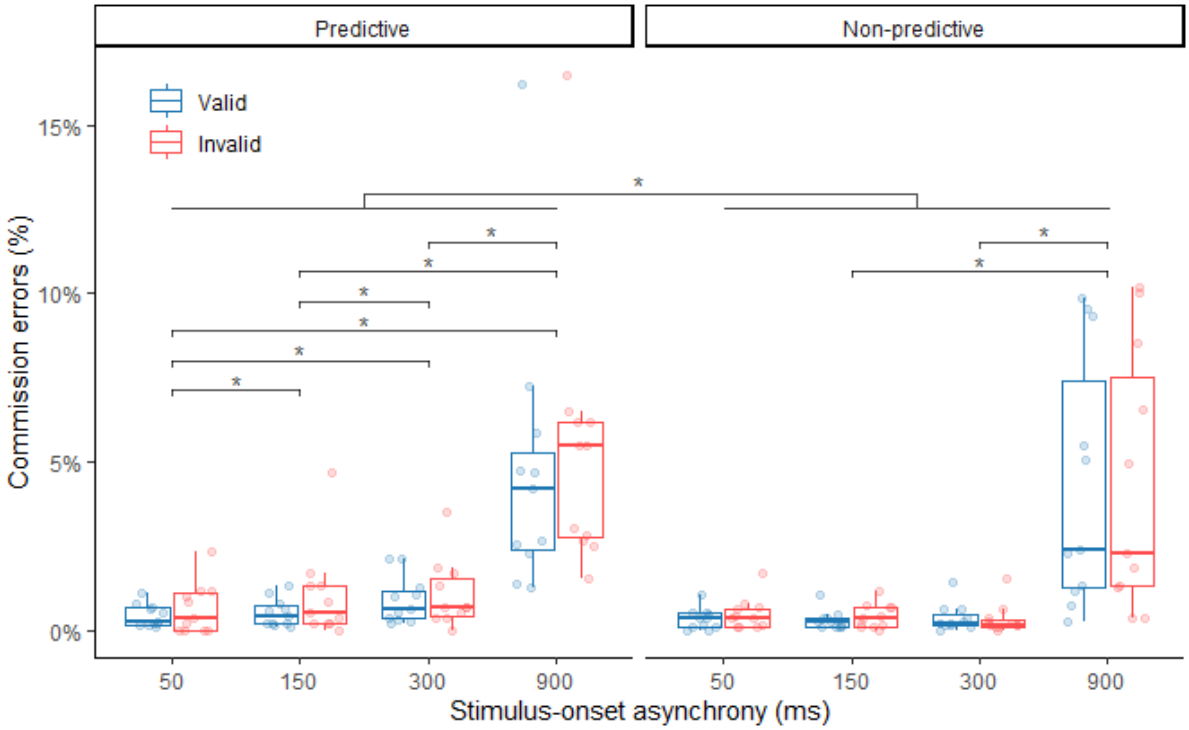
The percentage of commission errors in valid and invalid trials for P and NP subjects, as a function of SOA is presented in Figure 26.

Non-parametric statistics involving the Wilcoxon test revealed lack of significant valid “versus” invalid differences. In parallel, there was a significantly higher percentage of commission errors in P subjects [0.81 (0.25 – 2.16) %] than in NP subjects [0.34 (0.13 – 1.08) %; p = .005]. This is similar to our findings involving omission errors and further suggests that predictive and non-predictive cues engage different response strategies.

Additionally, the Friedman Test showed significant main effects of SOA (**P**: $X^2_F(3) = 29.0$, p < .001, *Kendall W* = .878; **NP**: $X^2_F(3) = 11.4$, p = .009, *Kendall W* = .346). As Figure 26 shows, the percentage of commission errors increase as the SOA increase. *Post hoc* analyses revealed that for P subjects the percentage of commission errors at each SOA differed from the corresponding scores at every other SOA (all $p \leq .042$). In contrast, for NP subjects the percentage of commission errors at SOAs 150 [0.27 (0.07 – 0.45) %] and 300 ms [0.20 (0.13 – 0.34) %] were significantly smaller as

compared to the corresponding score at the SOA of 900 ms [2.33 (1.28 – 8.03) %; both p = .018] (see Figure 26 for relevant statistical differences).

Figure 26 – Percentage of commission errors in valid and invalid trials for P and NP subjects as a function of SOA, in Experiment 2. All conventions are the same as in Figure 25. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



4.2.1.5 The effect of anticipation errors

As mentioned earlier, in the present experiment, whenever the subjects made an anticipation error the trial was reinitiated. We found that approximately 11% all trials from all our data were reinitiated at least once. We considered the possibility that the subjects' performance in these trials would be different from those of non-anticipated trials, since they might adopt a more cautious strategy to avoid anticipating again. This would possibly make mixing anticipated and non-anticipated in the same analysis inappropriate, since the animals would be assuming different strategies. In order to evaluate whether the subject's responses in these reinitiated trials interfered with the results, we repeated all the analysis already presented once more, but now removing those trials that were anticipated at least once from the data. A detailed analysis was

not presented here because, overall, results either including or excluding anticipated errors produced very similar outcomes.

The RT analysis exhibited exactly the same ANOVA results. Differently, however, the MT analysis indicated a significantly lower MTs in valid trials, compared to invalid trials, not only in P subjects [**v**: 154.8 (137.4 – 165.9) ms; **i**: 157.3 (140.9 – 168.7) ms; p = .004] – as in the analysis including both anticipated and non-anticipated trials – but also in NP subjects [**v**: 154.4 (144.7 – 172.7) ms; **i**: 155.6 (145.1 – 173.4) ms; p = .024]. Additionally, the Wilcoxon Rank Sum revealed a marginally significant MT difference between groups [**P**: 155.2 (134.0 – 168.6) ms; **NP**: 156.2 (146 – 175.1) ms; p = .095].

Relative to data of accuracy, the GLMM revealed a marginally significant interaction involving SOA and Predictability, whereas in the initial analysis including anticipation errors statistics revealed a significant effect, even though *post hoc* analysis revealed equivalent results. In the omission errors analysis the only difference we obtained was that the comparison between valid and invalid trials at SOA 50 ms of the P group became marginally significant (p = .056; it was .012 in the previous analysis). Finally, the commission errors analysis showed that only the comparisons between SOAs 150 and 300 ms were no longer significant, all other results being the same as the previous analysis.

Although the MT analysis without anticipated trials showed more significant effects in comparison to the analysis including both anticipated and non-anticipated, overall, most analysis showed only slight differences which seem to be related to a decrease in power of the tests due to removal of one tenth of the trials. Thus, this analysis was not run for data including subsets of testing sessions, except for movement times, where anticipation seemed to have an effect.

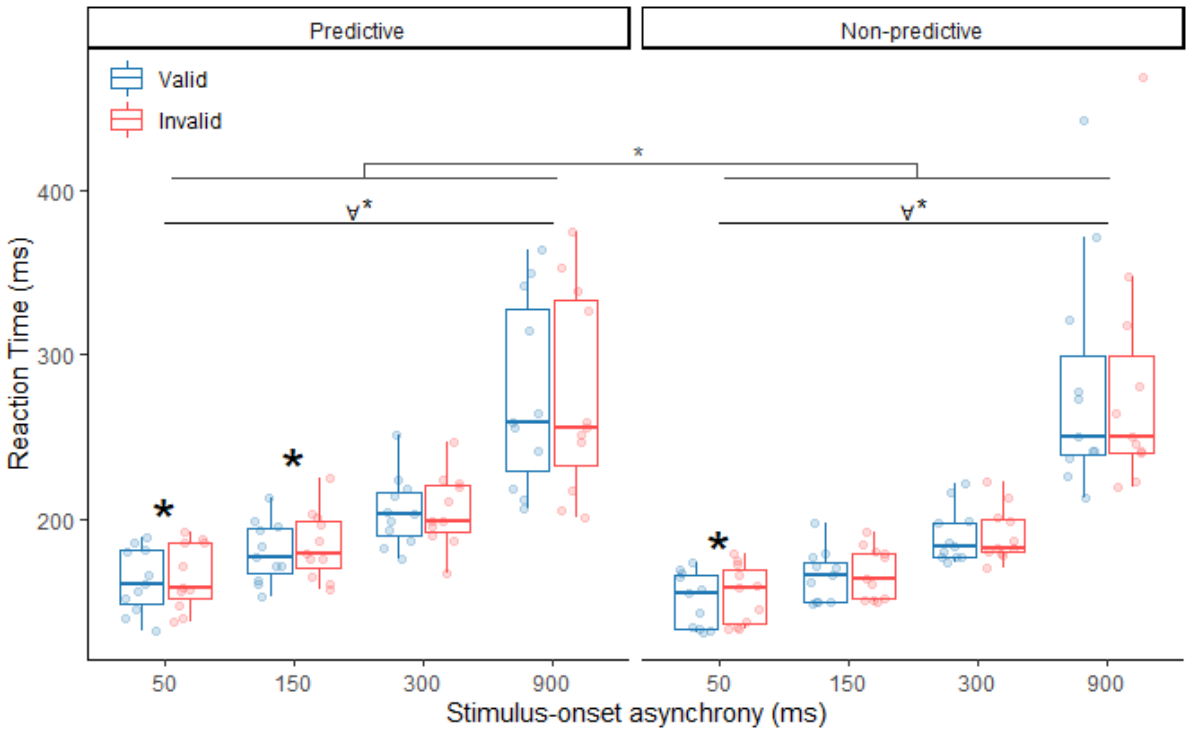
4.2.2 Subsets of initial, intermediate and final sessions pooled

4.2.2.1 Reaction times

The RTs in the initial subset of testing sessions, in valid and invalid trials, for P and NP subjects, as a function of SOA, are presented in Figure 27.

Non-parametric statistics revealed significant differences in RTs of valid and invalid trials, for P subjects at the SOAs of 50 [**v**: 160.6 (147.8 – 181.1); **i**: 158.6 (151.5 – 185.7) ms; p = .032] and 150 ms [**v**: 176.2 (166.9 – 194.4); **i**: 178.7 (170.1 – 199.0) ms; p = .020], and for NP subjects at the SOA of 50 ms [**v**: 155.0 (133.1 – 165.6); **i**: 158.6 (135.8 – 169.3) ms; p = .032] (Figure 27).

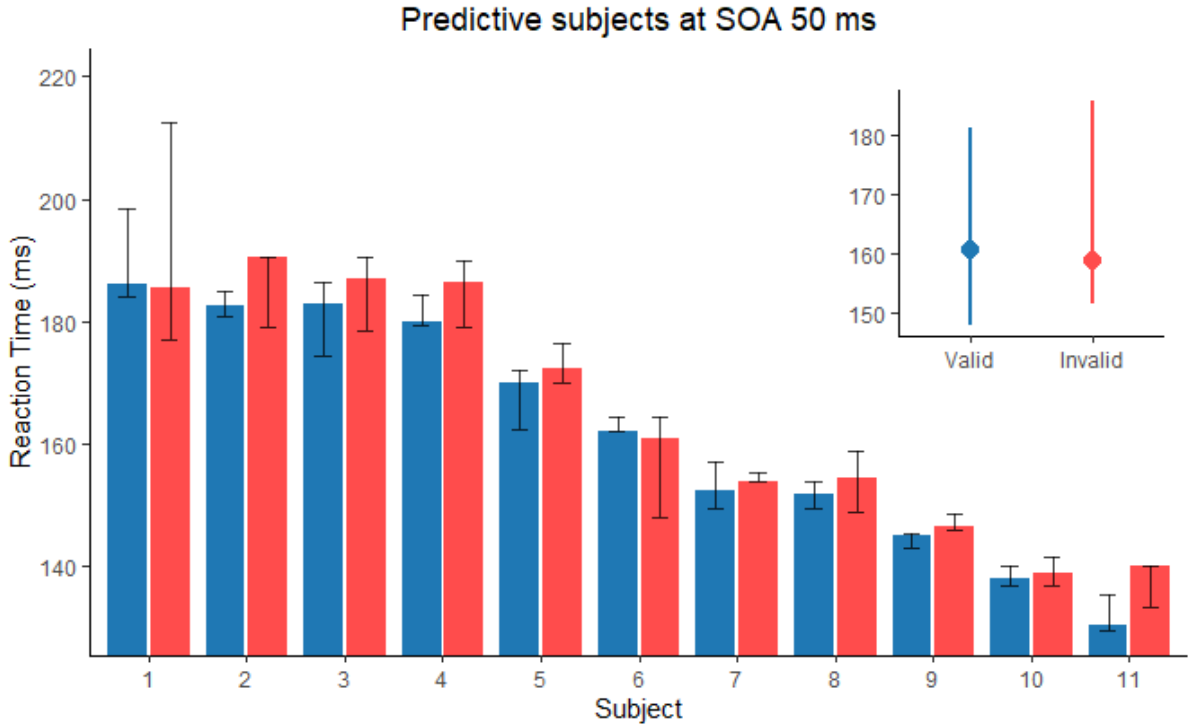
Figure 27 – Reaction Times in the initial subset of testing sessions, in valid and invalid trials, for P and NP subjects, as a function of SOA, in Experiment 2. The "∀*" symbol indicates that all comparisons between SOAs are statistically significant. All other conventions are the same as in Figure 25. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



As can be seen, at the SOA of 50 ms, both P and NP subjects, exhibited shorter RTs in valid as compared do invalid trials, thus indicating that both P and NP subjects

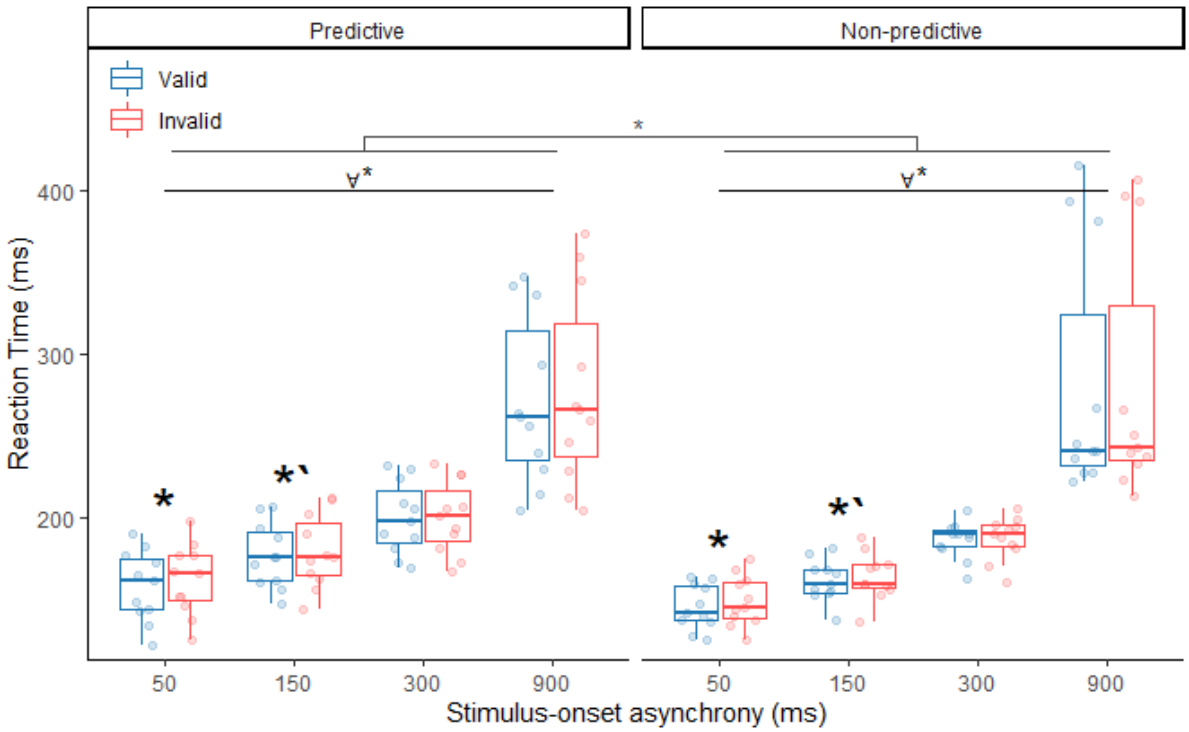
have their attention captured by the location where the auditory cue was presented thus reducing their RTs to detect the visual target presented at the same location. Note that although the median of P subjects at the 50 ms SOA is higher in valid trials, compared to invalid, the interquartile range shows that valid RTs are generally lower. In fact, 9 out of 11 P subjects exhibited shorter RTs in valid trials at this SOA, as seen in Figure 28.

Figure 28 – Reaction Times in the initial subset of testing sessions, in valid and invalid trials, for each P subject at SOA 50 ms, in Experiment 2. Colored bars with error bars represent the median ± interquartile range for individual animals. The inset on the upper right shows medians and interquartile ranges of all animals pooled.



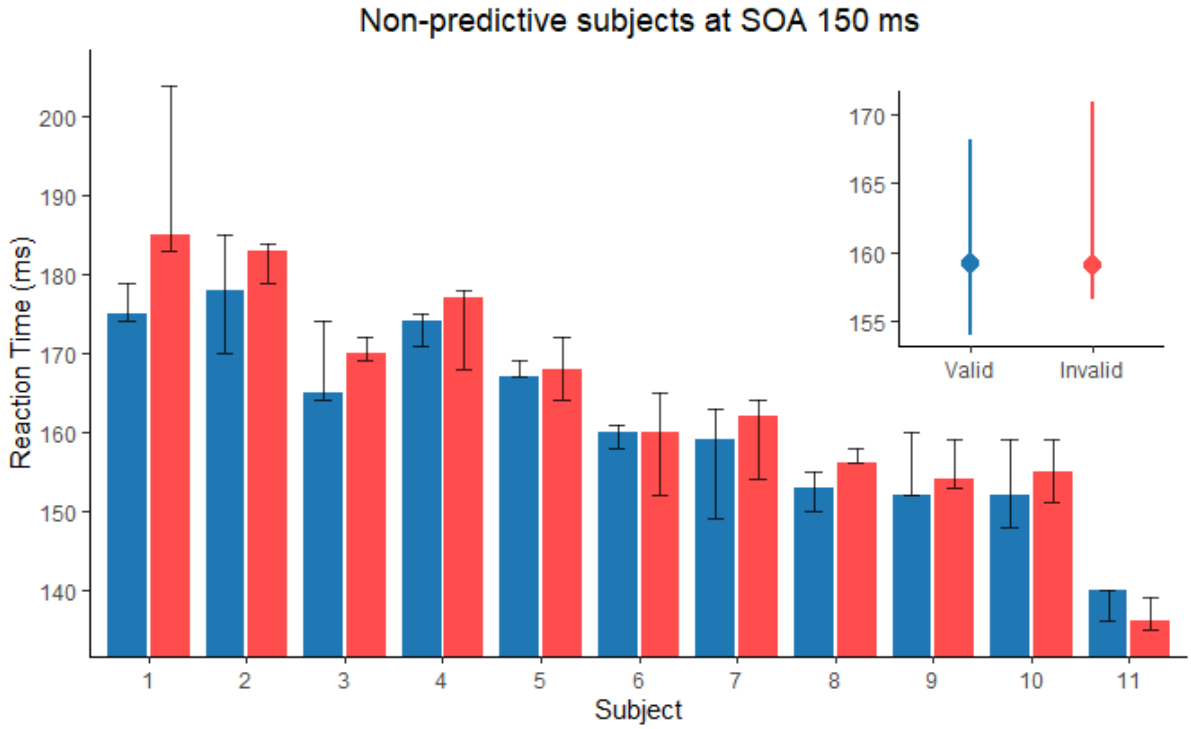
The RTs in the intermediate subset of testing sessions, in valid and invalid trials, for P and NP subjects, as a function of SOA, are presented in Figure 29.

Figure 29 – Reaction Times in the intermediate subset of testing sessions, in valid and invalid trials, for P and NP subjects, as a function of SOA, in Experiment 2. All other conventions are the same as Figure 25 and 22. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



Non-parametric statistics revealed both (1) significant differences between valid and invalid trials of P and NP subjects at the SOA of 50 ms [**P**: **v**: 161.1 (143.3 – 174.4); **i**: 165.4 (148.8 – 176.9) ms; *p* = .020; **NP**: **v**: 141.2 (137.0 – 157.8); **i**:145.2 (138.2 – 160.0) ms; *p* = .035] and (2) marginally significant differences between valid and invalid trials of P and NP subjects at the SOA of 150 ms (**P**: *p* = .097; **NP**: p = .062). In all of these comparisons RT in valid trials were shorter as compared to the corresponding RTs in invalid trials. Note that although the median of NP subjects at the SOA of 150 ms is higher in valid trials than in invalid trials, the interquartile range shows that valid RTs are generally lower. In fact, 9 out of 11 NP subjects exhibited shorter RTs in valid trials at this SOA, as seen in Figure 30.

Figure 30 – Reaction Times in the intermediate subset of testing sessions, in valid and invalid trials, for each NP subject at SOA 150 ms, in Experiment 2. Colored bars with error bars represent the median ± interquartile range for individual animals. The inset on the upper right shows medians and interquartile ranges of all animals pooled.



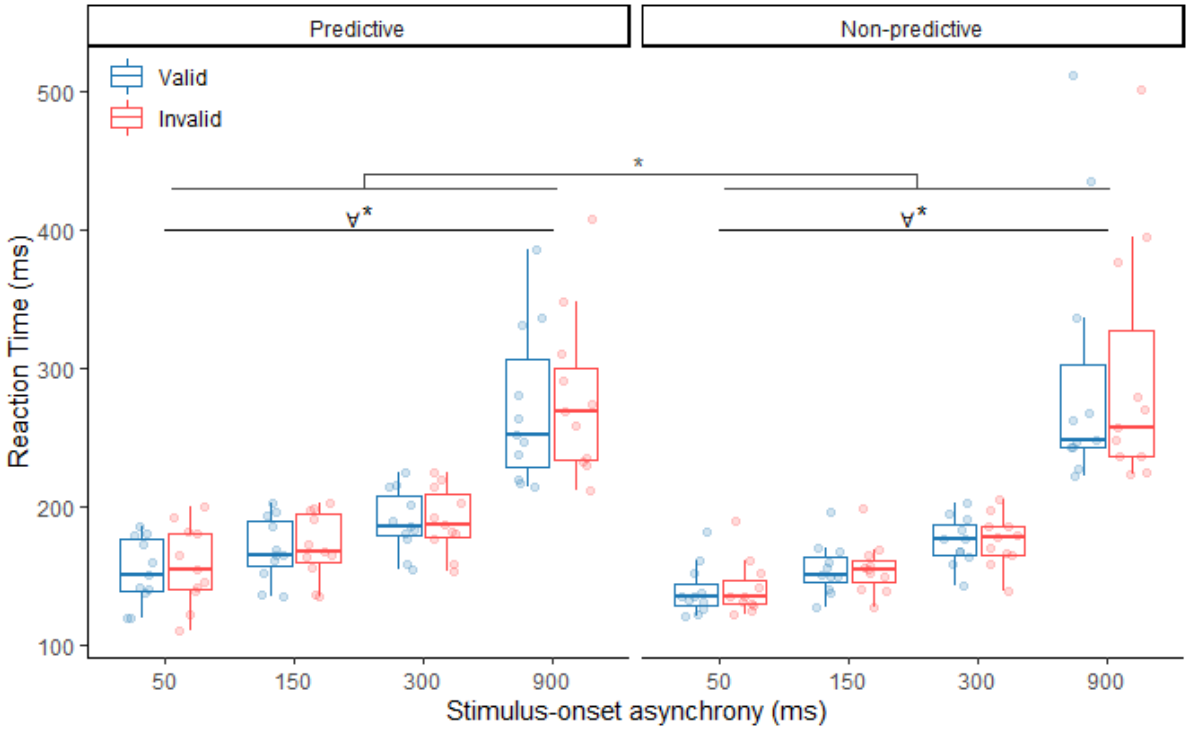
These figures suggest that the subjects oriented attention towards the location indicated by the auditory cue in the intermediate testing sessions, thus exhibiting shorter RTs, at the SOAS of 50 and, possibly, 150 ms.

The RTs in the final subset of testing sessions, in valid and invalid trials, for P and NP subjects, as a function of SOA, are presented in Figure 31.

Differently from the analysis including the initial and intermediate subsets of sessions, we found no differences between valid and invalid RTs in the final subset of testing sessions. These figures seem to indicate that the prolonged exposure to the task stimuli wanes attentional effects along sessions.

The analysis of the initial, intermediate and final subsets of sessions show that NP subjects show RT significantly lower than P subjects [**initial: P**: 194.2 (171.5 – 219.5); **NP**: 178.9 (160.3 – 219.9) ms; p = .035; **intermediate: P**: 189.8 (167.0 – 226.1); **NP**: 176.2 (156.4 – 206.9) ms; p = .042; **final: P**: 185.6 (159.4 – 217.6); **NP**: 166.3 (141.6 – 209.8) ms; p = .038]. This suggests that peripheral non-predictive cue engage a faster response mechanism than peripheral predictive cues.

Figure 31 – Reaction Times in the final subset of sessions in valid and invalid trials, for P and NP subjects, as a function of SOA, in Experiment 2. The "∀*" symbol indicates that all comparisons between SOAs are statistically significant. All other conventions are the same as in Figure 25. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



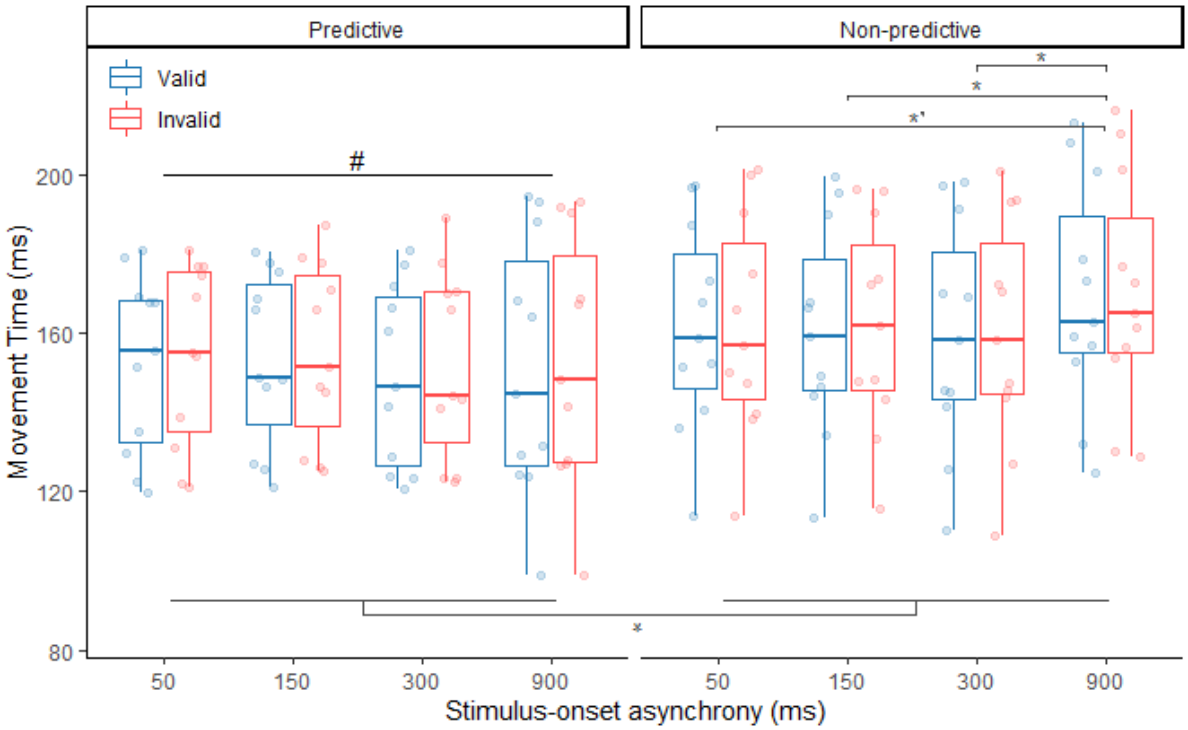
Data analyses also revealed an increase in RTs as a function of the SOAs, in both P and NP subjects in initial, intermediate and final subsets of testing sessions (in all cases $X^2_F(3) = 33.0$, $p < .001$, Kendall W = 1)[8], emphasizing the consistence of this effect. *Post hoc* analyses revealed significant effects for all comparisons between SOAs (all $p = .006$). These figures are in line with reports presented above.

[8] Exactly the same statistical results were obtained for all Friedman tests, including initial, intermediate and final subsets of testing sections. Its correctness was confirmed several times and the same tests were run using distinct software; the same results were obtained. This seems to be related to the way the analysis is computed. That is, the ranks are computed per line (i.e., per subject) and RTs were always larger in longer SOAs, as compared to RTs at shorter SOAs. Therefore, all subjects received ranks "1", "2", "3" and "4" respectively at the SOAs of 50, 150, 300 and 900 ms. This explains why the same final values were obtained in all tests (for details, see tables 12.12 through 12.15 of "Shott, S. (1991). Nonparametric statistics. In Journal of the American Veterinary Medical Association (Vol. 198, Issue 7). https://doi.org/10.4324/9781351211062-15").

4.2.2.2 Movement Times

Figure 32 shows the MTs for the initial subset of sessions as a function of Validity and SOA, for P and NP subjects.

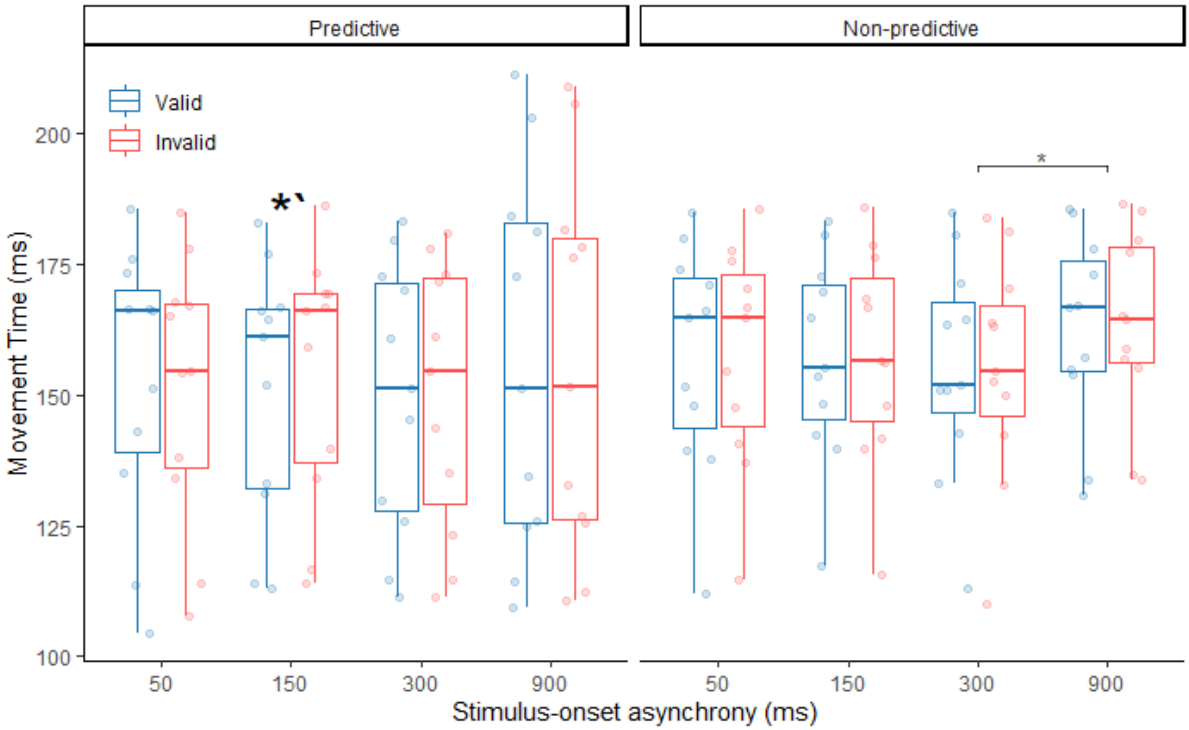
Figure 32 – Movement Times in the initial subset of testing sessions as a function of Validity and SOA, for P and NP subjects, in Experiment 2. # indicates a significant Friedman result in the statistical analysis, indicating an effect of SOA in the results, without the occurrence of significant differences in a *post hoc* analysis. All other conventions are the same as in Figure 23. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



Relative to the initial subset of testing sessions, non-parametric statistics involving paired Wilcoxon Tests revealed lack of significant differences between MTs in valid and invalid trials. The analysis, however, indicated that NP subjects exhibited significantly higher MTs than P subjects [**P**: 151.4 (128 – 172.6) ms; **NP**: 160.4 (145 – 190.1) ms; p = .014].

In contrast, the analysis including the intermediate subset of testing sessions (Figure 33) revealed a marginally significant difference between MTs in valid and invalid trials at the SOA of 150 ms, for P subjects [**v**: 161.0 (132.1 – 166.3); **i**: 166.2 (136.9 – 169.4) ms, p = .098]. The Wilcoxon Rank Sum revealed no difference between groups.

Figure 33 – Movement Times in the <u>intermediate</u> subset of testing sessions as a function of Validity and SOA, for P and NP subjects, in Experiment 2. All conventions are the same as in Figure 23. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.

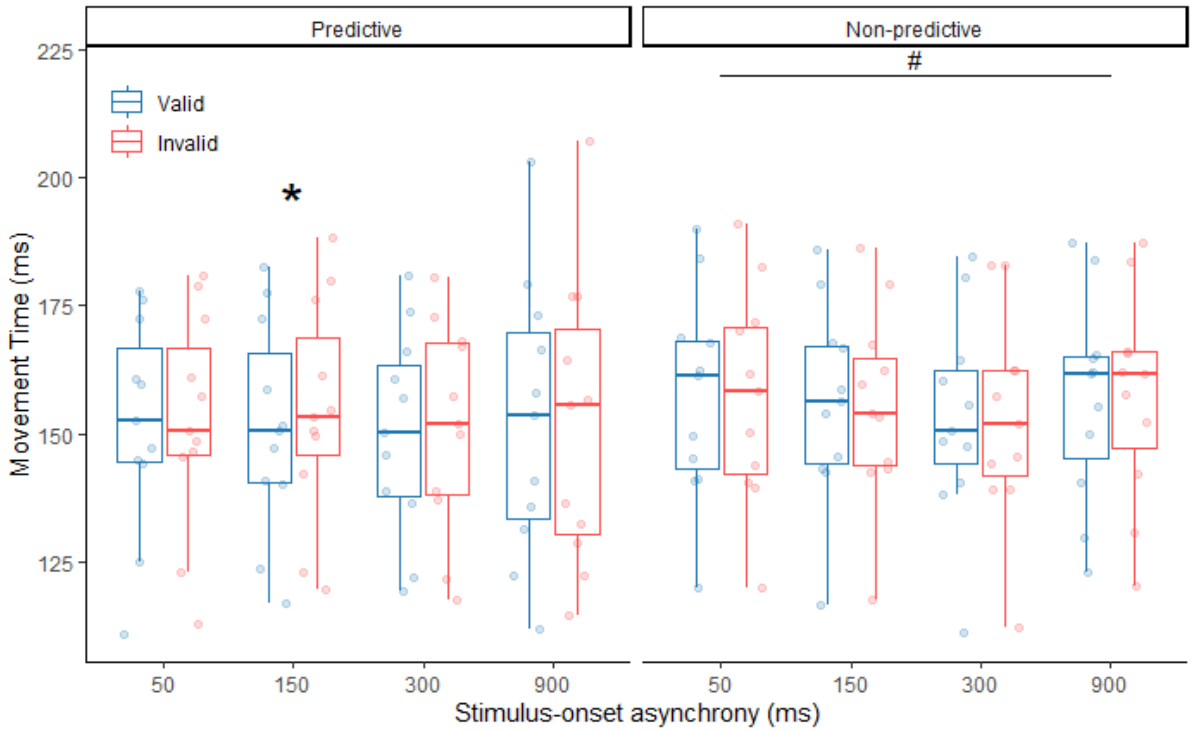


Relative to the <u>final</u> subset of testing sessions (Figure 34) the paired Wilcoxon Tests revealed that MTs are 2.9 ms significantly smaller in valid trials as compared to the corresponding scores seen in invalid trials, at the SOA of 150 ms [**v**: 150.4 (140.4 – 165.6); **i**: 153.3 (145.8 –168.9) ms, p = .027]. The Wilcoxon Rank Sum revealed no difference between groups.

The MTs in initial, intermediate and final subsets of testing sessions did not exhibit a regular pattern as a function of SOA (compare Figures 32, 33 and 34).

For instance, in the initial subset of testing sessions P subjects exhibited a significant reduction in MTs as the SOA increased ($X^2_F(3) = 8.35$, p = .039, *Kendall W* = .25), but this effect did not occur in the intermediate ($X^2_F(3) = 4.20$, p = .241, *Kendall W* = .13) and final ($X^2_F(3) = 1.8$, p = .615, *Kendall W* = .05) subsets of testing sessions. In addition, *post hoc* analyses for data of the initial subset of testing sessions did not reveal any significant difference related to SOAs.

Figure 34 – Movement Times in the <u>final</u> subset of testing sessions as a function of Validity and SOA, for P and NP subjects, in Experiment 2. A # symbol indicates a significant Friedman result in the statistical analysis, indicating an effect of SOA in the results, without the occurrence of significant differences in a *post hoc* analysis. All other conventions are the same as in Figure 23. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



In contrast, Friedman tests revealed significant SOA effects for NP subjects in the initial ($X^2_F(3) = 14.0$, $p = .003$, *Kendall W* = .42), intermediate ($X^2_F(3) = 9.87$, $p = .020$, *Kendall W* = .29) and final ($X^2_F(3) = 11.4$, $p = .010$, *Kendall W* = .34) subsets of testing sessions. The general pattern observable for these MT scores relative to the SOAs is that MTs decrease from the SOAs of 50 ms up to the SOA of 300 ms, and then increase when a SOA is 900 ms (see Figures 32, 33 and 34).

Post hoc analyses including MTs for initial and intermediate subsets of testing sessions corroborate this visually observable pattern. That is, for the initial subset of testing sessions the MT scores at both SOA of 150 ms [160.5 (145.0 – 185.9) ms] and SOA of 300 ms [158.2 (144.0 – 186.4) ms] differed from those seen at the SOA of 900 ms [163.9 (154.3 – 195.3) ms] (p = .015 and .006, respectively). For the intermediate subset of testing sessions statistics revealed that MT scores at the SOA of 300 ms [153.6 (144.4 – 168.9) ms] from those seen at the SOA of 900 ms [164.7 (155.1 – 177.8) ms] (p = .006).

4.2.2.2.1 The effect of anticipation

As mentioned earlier in the results of the analysis including all testing sessions pooled, the analysis of MTs was the only that showed a difference when including only non-anticipated trials in the analysis, as compared to when both anticipated and non-anticipated trials were included. This section will present the results of the MT analysis for subsets of initial, intermediate and final sessions when only non-anticipated trials were included in the analysis.

Relative to the initial subset of testing sessions, the analysis including only non-anticipated trials exhibited almost the same results as the analysis including all trials. The analysis also revealed a lack of significant differences between MTs in valid and invalid trials and that NP subjects exhibited significantly higher MTs than P subjects [**P**: 152.4 (127.2 – 172.5) ms; **NP**: 159.6 (144.9 – 189.3) ms; p = .013]. The difference the analysis with and without the anticipated trials is that the Friedman test, which indicates a possible main effect of SOA, was significant only for the NP subjects ($X^2_F(3)$ = 13.8, p = .003, *Kendall W* = .418), but not for P subjects ($X^2_F(3)$ = 4.2, p = .241, *Kendall W* = .127), whereas it was significant for both in the analysis including all trials. The *post hoc* analysis results for the NP subjects were identical to the analysis including all trials (Figure 32, right panel), indicating a rise in MTs throughout the SOAs.

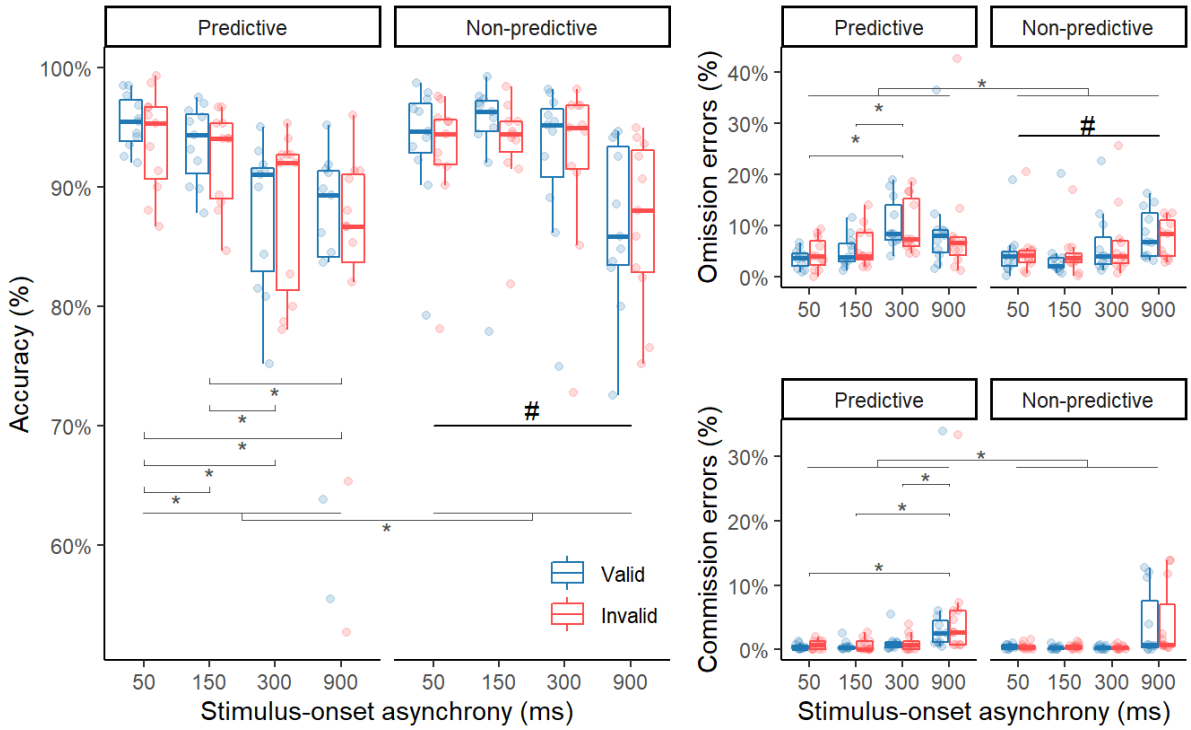
The results for the intermediate and final subsets of testing sessions including only non-anticipated trials revealed practically identical results to the analysis including both anticipated and non-anticipated trials, therefore they will not be described here.

Overall, removing the anticipated trials from the analysis of MTs did not substantially influence the results. Although the main effect of SOA observed in P subjects disappeared when including only non-anticipated trials in the analysis, such effect was already small (*Kendall W* = .252) and its *post hoc* analysis did not show differences between any SOAs. Considering that removing anticipated trials from the analysis including all testing sessions pooled (see section "**4.2.1 Analyses including all testing sessions pooled**") reveled a significant difference between valid and invalid MT at the SOA of 150 ms for NP subjects that was not present in the analysis with both anticipated and non-anticipated trials, the effect of removing anticipated trials in the present analysis is smaller.

4.2.2.3 Accuracy and Errors

Accuracy, omission errors and commission errors in the initial subset of testing sessions, in valid and invalid trials, as a function of SOA, for P and NP subjects, are presented in Figure 35.

Figure 35 – Accuracy, omission errors and commission errors in the initial subset of testing sessions, in valid and invalid trials, as a function of SOA, for P and NP subjects, In Experiment 2. A # symbol indicates a significant Friedman result in the statistical analysis, indicating an effect of SOA in the results, without the occurrence of significant differences in a *post hoc* analysis. All other conventions are the same as in Figure 25. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



Relative to accuracy, (1) the initial, intermediate and final subsets of testing sessions analyses revealed no significant effects of Validity; (2) the initial subset of testing sessions revealed that NP subjects obtained higher accuracies than P subjects [**P**: 91.9 (88.0 – 95.3) %; **NP**: 94.3 (89.9 – 96.1) %;p = .044]; (3) the intermediate subset of testing sessions (GLMM: conditional R^2 = .03; marginal R^2 = .03) revealed a marginally significant Validity X Predictability interaction effect ($X^2(1) = 3.40$, p = .065), with accuracy exhibiting larger differences between valid and invalid trials in the P subjects [**v**: 92.2 (0.75); **i**: 90.1 (0.75)%] as compared to the NP subjects [**v**: 92.4 (0.89); **i**: 92.3 (0.90)%]; and (4) the final subset of testing sessions (GLMM; conditional

R^2 = .03; marginal R^2 = .03) revealed a marginally significant main effect of Validity ($X^2(1) = 2.91$, $p = .088$), with accuracy exhibiting higher scores in valid trials [93.5 (0.51) %] as compared to invalid trials [92.6 (0.57)%].

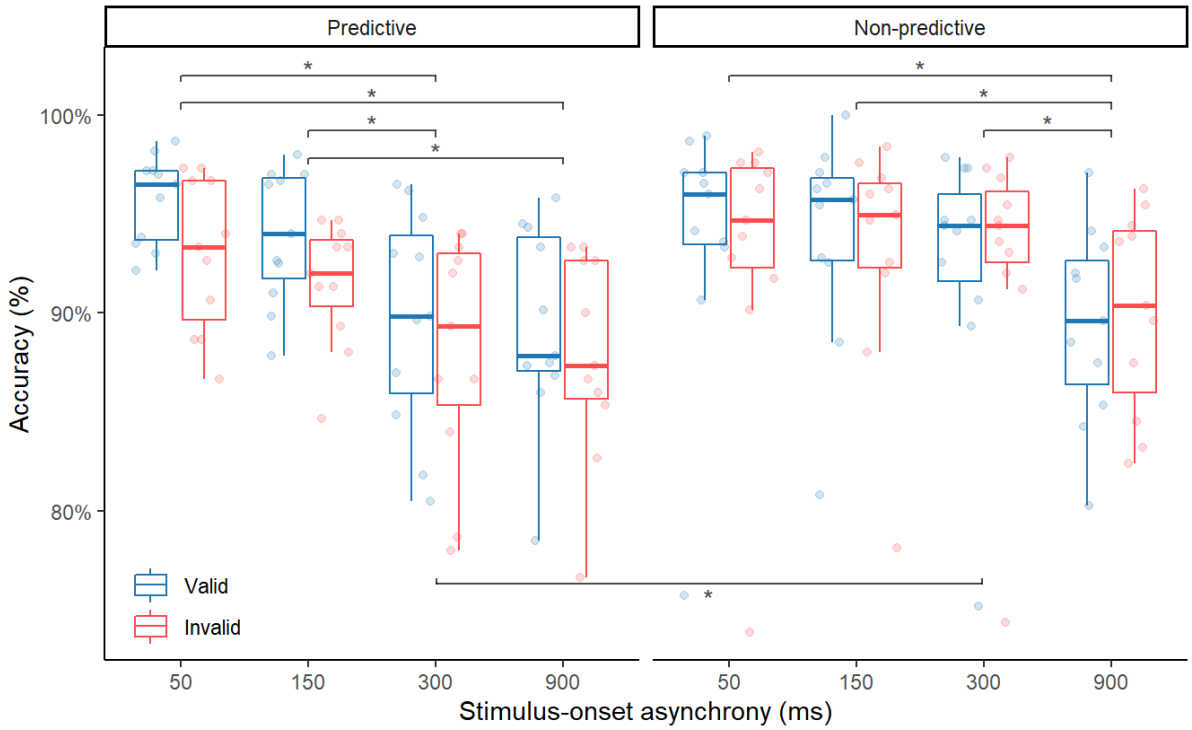
Statistics for accuracy data also revealed significant effects for SOA in initial, intermediate and final subsets of testing sessions (see Figure 35 and 36 for relevant statistical comparisons).

Relative to accuracy data in the initial subset of testing sessions, the Friedman's Test revealed a significant SOA effect both for P ($X^2_F(3) = 22.4$, p < .001) and NP ($X^2_F(3) = 10.9$, p = .012) subjects; the effect size was larger for the P subjects (*Kendall W* = **P**: .679; **NP**: .331). *Post hoc* analyses did not detect significant differences for NP subjects, but did detect significant differences for the P subjects, except between scores in the SOAs of 300 and 900 ms (all other comparisons, $p \leq .037$). These figures indicate that accuracy of P and NP subjects drop at longer SOAs, but this effect is stronger for P subjects (see Figure 35 for relevant statistical comparisons). These results were fairly similar to those seen when all testing sessions were pooled (even considering the differences between the statistical methods employed in both analyses), thus emphasizing their consistence.

Accuracy data for the intermediate subset of testing sessions (Figure 36) were similar to those seen for the initial subset of testing sessions.

The Binomial GLMM (conditional R^2 = .03; marginal R^2 = .03) revealed a significant main SOA effect ($X^2(3) = 66.4$, $p < .001$) and a significant SOA X Predictability interaction effect ($X^2(3) = 11.2$, $p = .010$). As can be seen in Figure 36, (left panel) *post hoc* comparisons revealed that the accuracy of P subjects at SOA of 300 ms was smaller compared to that seen for the corresponding NP subjects [**P**: 88.9 (1.21); **NP**: 92.7 (1.33) %; $p = .035$]. *Post hoc* statistics also revealed significant differences between accuracy scores at different SOAs. For instance, accuracy of P subjects at the SOA of 50 ms differ from that seen at both the SOA of 150 ms ($p = .020$) and at the SOA of 900 ms ($p < .001$), thus indicating a drop in accuracy as the SOAs increase. The same effect was seen for data involving all testing sessions (see Figure 24).

Figure 36 – Accuracy in the intermediate subset of testing sessions as a function Validity and SOA, for P and NP subjects, in Experiment 2. All conventions are the same as in Figure 23. Data analysis involved a Binomial GLMM.



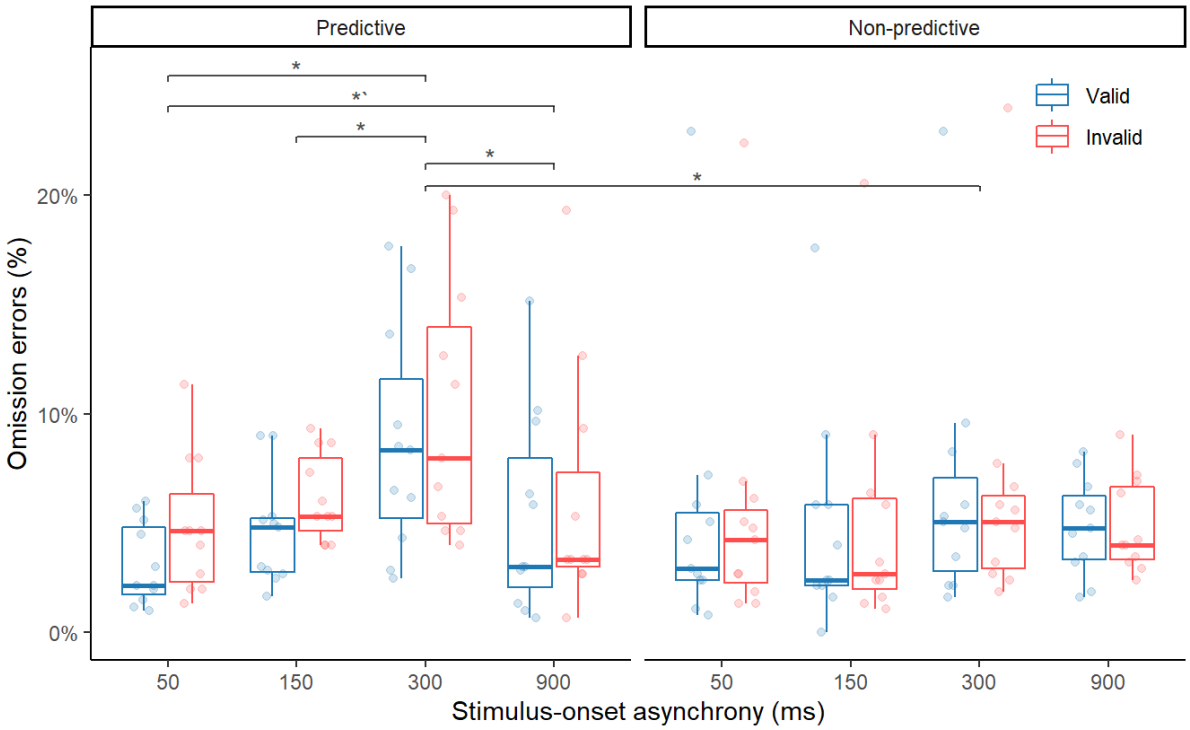
Relative to accuracy data involving the final subset of testing sessions (conditional R^2 = .03; marginal R^2 = .03) similar figures were observed in relation to corresponding scores of both the initial and the intermediate subsets of testing data (data not shown). There was a significant decrease in accuracy as the SOAs increased (main effect of SOA: $X^2(3) = 51.7$, $p < .001$). A marginally significant SOA X Predictability interaction effect was also revealed ($X^2(3) = 7.52$, $p = .057$).

The percentage of omission errors in the initial subset of testing sessions (Figure 35, top right panel) revealed lack of significant Validity effects. In contrast, NP subjects committed significantly less omission errors than P subjects [**P**: 6.00 (3.46 – 8.67] %; **NP**: 4.00 (2.87 – 6.73) %; $p = .031$]. This mirrors the results seen for accuracy and indicate that predictive and non-predictive cues engage different response strategies, similarly to results including all testing sessions pooled.

Additionally, both P and NP subjects exhibited a significant SOA effect (**P**: $X^2_F(3) = 17.5$, p < .001; **NP**: $X^2_F(3) = 13.7$, p = .003). As Figure 35 (top right panel) shows, the percentage of omission errors increased as the SOAs increased. This

effect was stronger for P subjects (*Kendall W* = **P**: .531; **NP**: .415). *Post hoc* analyses confirmed these figures. That is, there were no significant differences of the percentage of omission errors at different SOAs for NP subjects. In contrast, P subjects did exhibit smaller percentage of omission errors at the SOAs of 50 ms [3.83 (3.75) %] and of 150 ms [4.00 (5.34) %] relative to those seen at the SOA of 300 ms rates [7.83 (8.7) %, p = .006 at both comparisons]. These results mirror those seen for the initial subset of testing sessions for accuracy (Figure 35), thus indicating that the drop in accuracy is related to an increase in omission errors.

Figure 37 – Percentage of omission errors in the intermediate subset of testing sessions, for P and NP subjects, as a function of SOA and validity, in the Experiment 2. All conventions are the same as in Figure 23. Data analysis involved a Binomial GLMM.



The Binomial GLMM for omission errors for the intermediate subset of testing sessions (conditional R2 = .03; marginal R2 = .03) (Figure 37) revealed differences similar to those seen for the initial subset of testing sessions (Figure 35). The statistic test revealed a significant SOA effect ($X^2(3) = 38.2$, $p < .001$) and a significant SOA X Predictability interaction effect ($X^2(3) = 18.1$, $p = .004$) (see relevant statistical comparisons in Figure 37), but no effect involving the SOA X Predictability X Validity three-way interaction ($X^2(3) = 0.14$, $p = .979$).

The increase in the percentage of omission errors as a function of SOA seemed stronger for P subjects (Figure 37, left panel). In fact, *post hoc* analysis revealed a marginally significant difference between scores of P and NP subjects at the SOA of 300 ms [**P**: 9.48 (1.17); **NP**: 6.41 (1.26) %; p = .065]. In addition, the percentage of omission errors by P subjects is greater at the SOA of 300 ms as compared to the remaining SOAs (all p < .014).

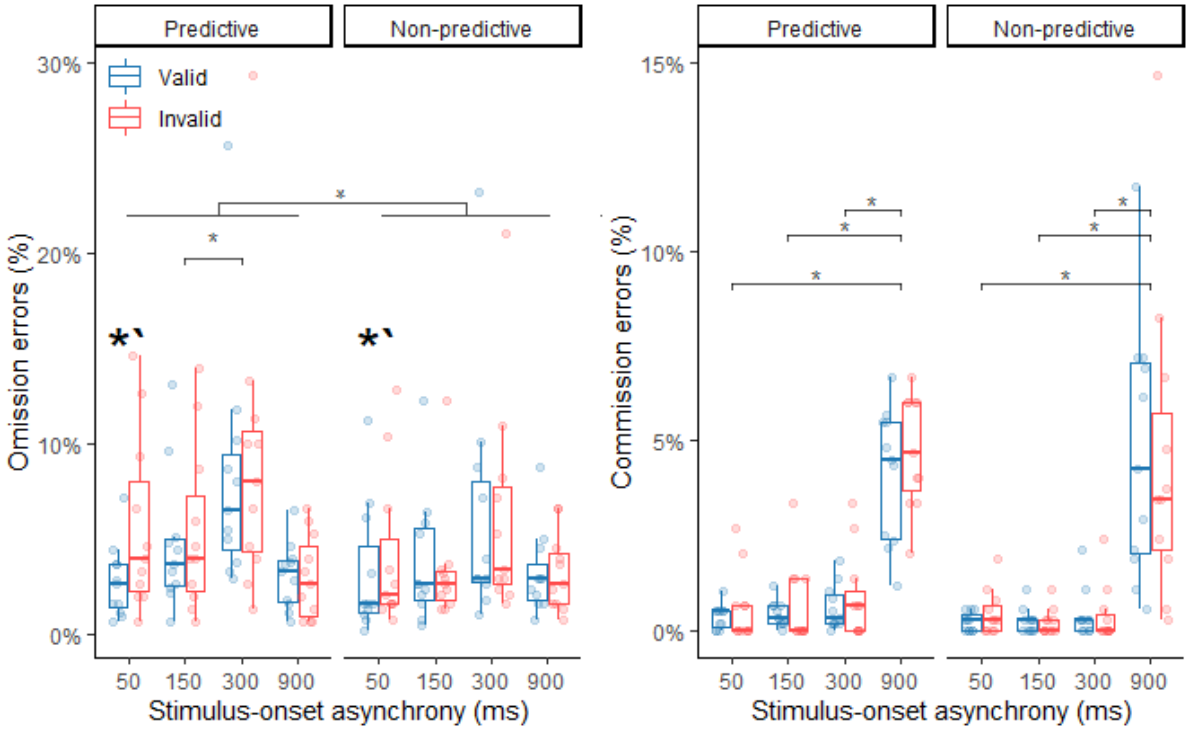
The percentages of omission and commission errors in the final session subset of testing sessions, for P and NP subjects, as a function of SOA, are presented in Figure 38.

Relative to omission errors, statistics revealed no significant Validity effects (Wilcoxon signed rank tests). However, statistics did reveal a marginally significant difference between scores in valid as compared to invalid trials, at the SOA of 50 ms, for both P [v: 2.67 (1.41 – 3.67) %; **i**: 4.00 (2.34 – 8.00) %; p = .065] and NP [**v**: 1.60 (1.20 – 4.67) %, **i**: 2.13 (1.60 – 5.07) %, p = .050] subjects. As Figure 38 shows, the percentage of omission errors were higher in invalid trials, as compared to the corresponding scores of valid trials. These figures are congruent with the marginally significant effects seen for Validity in the analyses of accuracy.

The analysis also showed a significantly lower percentage of omission errors in NP subjects, compared to P subjects [**P**: 3.92 (2.16 – 6.67) %; **NP**: 2.8 (1.60 – 5.34) %; p = .044], similarly to what was seen in the first subset of testing sessions.

Statistical analysis also revealed a significant SOA effect for P subjects ($X^2_F(3)$ = 10.1, p = .018, *Kendall W* = .305). *Post hoc* analyses indicated that the percentage of omission errors at the SOA of 300 ms was both (1) significantly higher as compared to the corresponding scores at the SOA of 150 ms [**150**: 4.00 (2.54 – 5.79); **300**: 7.34 4.17 – 10.1) %; p = .041] and (2) marginally higher as compared to the corresponding scores at the SOAs of 50 and 900 ms [**50**: 3.08 (1.75 – 4.62); **900**: 3.08 (1.41 – 4.00) %; p = .068, for both]. These figures are also congruent with results reported above for omission errors in the initial and intermediate subsets of testing sessions.

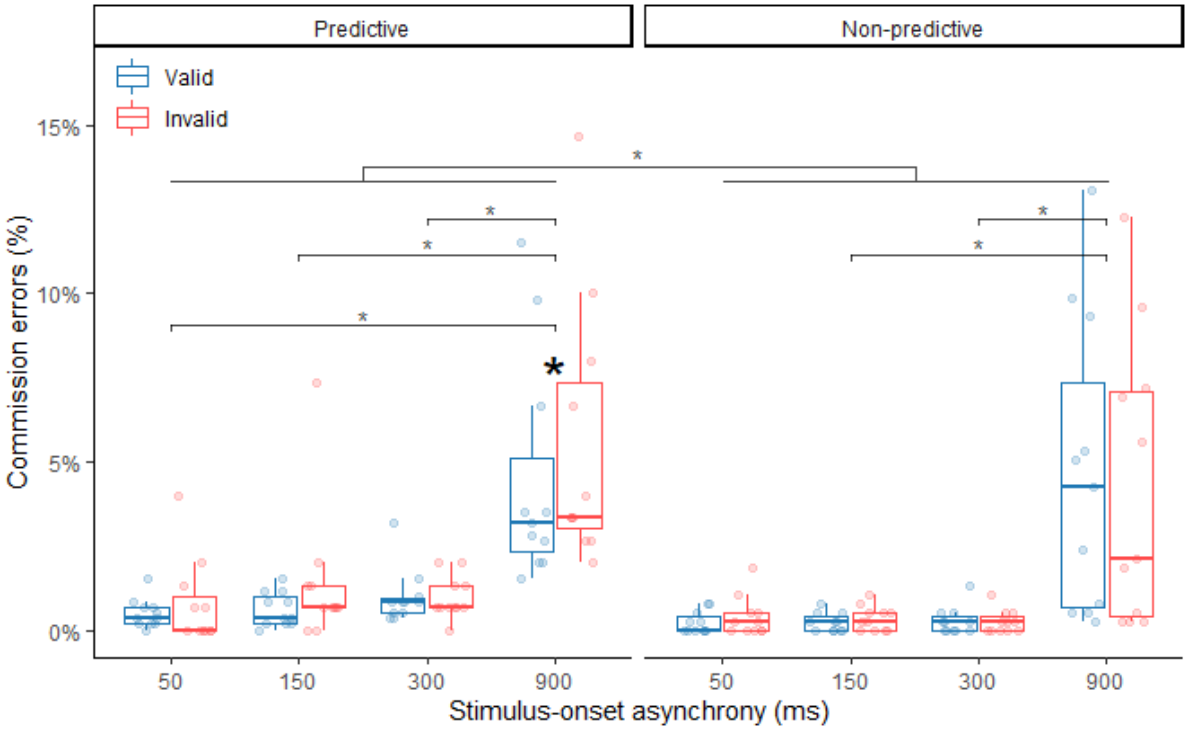
Figure 38 – Percentage of omission (left) and commission (right) errors in the final subset of testing sessions, for P and NP subjects, as a function of SOA, in Experiment 2. All conventions are the same as in Figure 25. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



Relative to commission errors in the initial (Figure 35, bottom right panel) and final (Figure 38, right panel) subsets of testing sessions, statistical analysis revealed lack of significant Validity effect for both P and NP subjects, independently on the SOA (Wilcoxon signed rank tests).

Differently, the analysis of commission errors including data from the intermediate subset of testing sessions did reveal effects involving Validity, as shown in Figure 39. Statistics including data of the P subjects revealed a significant Validity effect at the SOA of 900 ms [**v**: 3.17 (2.34 – 5.08) %; **i**: 3.34 (3.00 – 7.34) %; p = .023] (see Figure 39). The difference, however, seems too small to be considered biologically relevant. In addition, the Validity factor did not produce any significant difference of accuracy at this SOA.

Figure 39 – Percentage of commission errors in the intermediate subset of testing sessions, in valid and invalid trials, as a function of SOA, for P and NP subjects, in Experiment 2. All conventions are the same as in Figure 25. Data analysis involved Friedman, Wilcoxon Signed Rank and Wilcoxon Rank Sum tests.



The statistical analysis also revealed that NP subjects committed less commission errors than P subjects in both initial [**P**: 0.67 (0.16 – 1.34) %; **NP**: 0.26 (0.00 – 0.80) %; p = .010] and intermediate [**P**: 0.83 (0.34 – 2.00) %; **NP**: 0.26 (0.00 – 0.80) %; p < .001], but not in the final subsets of testing sessions [**P**: 0.67 (0.00 – 2.37) %; **NP**: 0.26 (0.00 – 1.07) %; p = .108] (see Figures 35 and 39 for significant statistical comparisons). This is similar to the results for the omission errors and partially mirrors the results of accuracy, especially the analysis including the initial subsets of testing sessions.

Additional data analyses of the percentage of commission errors also revealed significant SOA effects that varied along subsets of testing sessions. That is, in the first subset of testing sessions there was a main SOA effect for P subjects ($X^2_F(3)$ = 20.5, p < .001, *Kendall W* = .623). As can be seen in Figure 35 (bottom right panel), the percentage of commission errors was significantly greater at the SOA of 900 ms (all p ≤ .008). Both P and NP subjects exhibited significant SOA effects at intermediate [**P**: $X^2_F(3)$ = 24.6, p < .001, *Kendall W* = .745; **NP**: $X^2_F(3)$ = 10.5, p = .015, *Kendall W* = .318] (Figure 39) and final [**P**: $X^2_F(3)$ = 22.8, p < .001, *Kendall W* = .692; **NP**: $X^2_F(3)$

= 18.0, $p < .001$, *Kendall W* = .546] (Figure 38, right panel) subsets of testing sessions. Relative to the intermediate and final subset of testing sessions (Figure 39 and the right panel of Figure 38), both P and NP subjects exhibited a pattern of results similar to those seen for P subjects at the initial subset of testing sessions. That is, the percentage of errors is significantly greater at the SOA of 900 ms, relative to the corresponding scores at shorter SOAs ($p \leq .041$ for all comparisons).

5 Discussion

This discussion is organized in four parts. In the first part we discussed the main findings of the present experiments, bringing together all the results we obtained and commenting them in the light of literature in order to extract the main contributions of the present study. In the second part, we discussed findings that, although are not crucial to our main conclusions, point to interesting effects. In the third part, we commented on the possible limitations of the employed approach. Finally, in the last part, we give some closing remarks, suggesting future directions to studies in the field.

5.1 MAIN FINDINGS

Experiment 1 tested whether rats are able to orient visual attention exogenously using peripheral auditory cues in a Posner-like task adapted to rats. The analysis including the first subset of testing sessions showed that valid trials generated a smaller proportion of omission errors, as compared to invalid trials, at a short SOA (150 ms, but not at 50 ms; Figure 10). This effect, accompanied by a marginally higher accuracy in valid trials at this SOA, shows that subjects missed less targets when the auditory cue correctly indicated the target position, as compared to when it indicated the opposite location (invalid trial; Figure 8). This suggests that attention was allocated to the cued location, either facilitating the detection of targets presented at that same location, or impairing detection of targets presented at the opposite side, or both. As the cues were non-predictive about the target's pending location (50 % valid and 50 % invalid) these effects can be explained as an exogenous allocation of attention to the cued side due to the salient sound presented at that location. Note that this effect was only present in the initial five sessions of testing, such that it waned after these few sessions, indicating that repetitive exposure to task stimuli extinguishes it, similarly to what happens with humans (Lupiáñez et al., 2001; Pratt & McAuliffe, 1999; Weaver et al., 1998).

Although the results from Experiment 1 suggest that rats exogenously orient visual attention towards locations where salient peripheral non-predictive sounds are

presented, they lacked an important hallmark of attention orienting, namely a sizeable validity effects for RT and accuracy.

Previous studies using visual cues and visual targets report differences in RTs larger than 30 ms between trials using non-predictive valid and invalid cues (Marote & Xavier, 2011; Phillips et al., 2000; Rosner & Mittleman, 1996; Ward et al., 1998; Ward & Brown, 1996, 1997; Weese et al., 1999). In the present study, using auditory cues and visual targets, RTs were significantly lower in valid trials (1) at the SOA of 50 ms as compared to the corresponding scores in invalid trials, when including all testing sessions (Figures 5 and 6), and (2) at the SOA of 50 ms (Figure 13) as compared to the corresponding scores in invalid trials, when including the intermediate subset of testing sessions. However, the median differences were 1.8 and 0.2 ms respectively, which may seem too small to be biologically relevant. Relative to MT results, scores in invalid trials were significantly higher as compared to scores in valid trials at the SOA of 150 ms when analysis included all sessions pooled (Figure 7) and when the analysis included the initial subset of testing sessions (Figure 15). Similarly to the differences in RTs, these differences in MTs were very small (0.6 and 2.5 ms, respectively) to be considered biologically relevant.

Similar figures were observed relative to accuracy results. That is, in the initial subset of testing sessions, accuracy in valid trials was about 1.7% greater as compared to the corresponding scores in invalid trials at the SOA of 150 ms (Figure 17). This is a much smaller difference when compared to previous studies using visual cues and visual targets for rats (e.g., Marote & Xavier, 2011; Rosner & Mittleman, 1996), even though small accuracy differences have also been reported when using visual cues and visual targets (e.g., Ward et al., 1998; Ward & Brown, 1997).

Human studies involving orienting of attention involving cue and target stimuli of different sensory modalities have shown that the spatial origin of the stimuli is crucial for optimal attentional effects to occur (Spence, 2013; Spence & McDonald, 2004). On admitting that rats could exhibit a similar phenomenon, one raised the hypothesis that the architecture of the operant conditioning chamber employed in Experiment 1 was not optimal for revealing attentional effects when using auditory cues and visual targets, given that cues and targets did not originate from the same location.

Experiment 2 represented an attempt to evaluate this possibility by presenting auditory cues and visual targets originating (approximately) from the same spatial

location (see Figure 21). In addition to changing the location of the sound cues, two independent experimental groups were included, being one non-predictive and another predictive. The general prediction using this novel arrangement was that by presenting stimuli from different modalities at the same spatial location would enhance the integration of cross-modal sensory information and, thus, improve attentional effects.

Therefore, one of the goals of Experiment 2 was to evaluate if auditory cues and visual targets presented in the same spatial location affects exogenous orienting of attention (particularly in the NP group) as compared to the result seen in Experiment 1. One expects results qualitatively similar to those of Experiment 1, but with greater differences when comparing scores in valid and invalid trials.

Another goal of Experiment 2 was to investigate the effect of releasing the auditory cue and the visual target from the same spatial location in a condition that promotes interaction of both endogenous and exogenous orienting of attention, by including subjects exposed to a P condition as compared to subjects exposed to a NP condition. Greater validity effects were expected for P subjects, particularly for longer SOAs, when compared to the NP subjects. Additionally, we expected to observe validity effects larger than those reported by Cruz (2017), when the auditory cue and the visual target were not released exactly from the same location.

Analysis including scores of all sessions of Experiment 2 showed that RTs in valid trials were significantly shorter when compared to the corresponding scores at invalid trials for both P and NP subjects, at all SOAs, except at SOA 300 ms (Figure 22). This result suggests that subjects of P and NP groups oriented attention to the cued side, leading to either faster responses in valid trials or slower responses in invalid trials, or both. Similar shorter RTs in valid trials as compared to invalid trials were observed when analyzing (1) the initial subset of testing sessions, at the SOA of 50 ms for P and NP subjects, and also at the SOA of 150 ms for P subjects (Figures 27 and 28) and (2) the intermediate subset of testing sessions, at the SOA of 50 (significant) and 150 (marginally significant) ms for both P and NP subjects (Figures 29 and 30). However, as in Experiment 1, all differences between RTs in valid and invalid trials were very small (~3 ms), which seems biologically irrelevant.

Relative to the analysis of MTs including data of all testing sessions, statistical test revealed scores 2.8 ms shorter in valid trials as compared to invalid trials at the SOA of 150 ms for subjects of the P group, but not for subjects of the NP group (Figure

23). In line with this finding, MTs including data of the (1) intermediate subset of testing sessions revealed (marginally significant) shorter scores in valid as compared to invalid trials at the SOA of 150 ms for P subjects but not for NP subjects (Figure 33), and (2) final subset of testing sessions revealed (significant) shorter scores in valid as compared to invalid trials at the SOA of 150 ms for P subjects but not for NP subjects (Figure 34).

The percentage of omission errors including scores of all testing sessions showed smaller scores in valid trials as compared to invalid trials both at the SOA of 50 ms for both P and NP subjects, and at the SOA of 150 ms for P subjects (Figure 25). This result is similar to that of Experiment 1 (with the single difference being that in Experiment 1 the validity effect is present only at SOA 150 ms; see Figure 10), and further suggests that the subjects oriented attention at these SOAs. It is interesting to emphasize that in addition to the validity effect observed at the SOA of 50 ms for both P and NP subjects, which indicates the participation of exogenous orienting of attention, there was also a validity effect for P subjects at the SOA of 150 ms, suggesting the engagement of endogenous attention.

The analysis of session subsets showed only marginally significant differences involving accuracy. In the intermediate subjects there were accuracies ~2% higher in valid, compared to than invalid trials, in the P group, whilst in the NP group there was no difference, independently of SOA (Figure 36). The analysis of the final subset, contrarily, showed that valid trials, independently from group and SOA, generated accuracies 0.9% higher than invalid trials, suggesting that both P and NP animals oriented attention (data not shown). In neither of these two last analyses, however, there were significant differences in the percentage of omission or commission errors. Overall, the analysis of accuracy in Experiment 2 only weakly suggests that orienting of attention had an effect in the accuracy of target detection.

A simple comparison of significant and marginally significant validity effects between Experiments 1 and 2 shows that there was a greater number of differences in the second experiment, even after accounting for the introduction of the Predictive condition (Table 1).

Table 1 – Comparison of significant validity effects observed in Experiments 1 and 2. Values in square brackets indicate marginally significant effects. The "-" symbol indicates that the effect was not tested because the experiment lacked that condition.
*** In Experiment 2 we found an interaction between SOA and validity. As it did not involve group, we assumed that both groups showed the indicated difference. Note that these two groups of Experiment 2 are being compared to the subjects of Experiment 1 (which were NP).**
**** These results indicate an IOR-like effect.**
***** These percentages are relative to the total number of trials with targets.**

				Validity effect (Invalid minus Valid)	
Dependent variable	Subset of sessions	Group	SOA (ms)	Experiment 1	Experiment 2
RT	All sessions pooled	*All**	50	1.8 ms	3.1 ms
			150	n.s.	1.8 ms
			900	n.s.	3.5 ms
	Initial subset	Predictive	50	-	-2 ms**
			150	-	2.5 ms
		Non-Predictive	50	n.s.	3.6 ms
	Intermediate subset	Predictive	50	-	4.3 ms
			150	-	[0.2 ms]
		Non-Predictive	50	0.2 ms	4 ms
			150	n.s.	[-0.2 ms]
MT	All sessions pooled	*All**	150	0.6 ms	-
		Predictive	150	-	2.8 ms
	Initial subset	All*	50	2.5 ms	n.s.
	Intermediate subset	Predictive	150	-	[5.2 ms]
	Final subset	Predictive	150	-	2.9 ms
Accuracy	Initial subset	Non-Predictive	150	[-1.7%]	n.s.
			300	2.7%**	n.s.
	Intermediate subset	*Interaction between validity and group*		n.s.	[P: - 1.9%, NP: -0.1%]
	Final subset	*Main effect of validity*		n.s.	[-0.9%]
Omission errors	All sessions pooled	Predictive	50	-	0.6%
			150	-	1%
		Non-Predictive	50	n.s.	0.9%
			150	2.8 trials (0.6%)***	n.s.
	Initial subset	Non-Predictive	150	1.5 trials (0.3%)***	n.s.
	Final subset	Predictive	50	-	[1.3%]
		Non-Predictive	50	n.s.	[0.5%]
Comission errors	Intermediate subset	Predictive	900	n.s.	0.17%

Source: data from the present work.

There were compelling effects observed in Experiment 2, but not in Experiment 1. They include: (1a) the occurrence of validity effects for RT for P and NP subjects at longer SOAs in the analysis involving all testing sessions (150 and 900 ms, in addition to 50 ms also seen in Experiment 1; see Table 1 and Figures 5 and 22) and (1b) for P subjects at the SOAs of 50 and 150 ms when analysis involved the initial subset of

testing sessions, an effect that was restricted to SOA of 50 ms in the NP subjects (Figure 27); and (2a) larger validity effects for RTs in the analysis involving all testing sessions (1.8 *vs* 3.1 at SOA 50, NP group) and (2b) in in the analysis involving the intermediate subset of testing sessions (0.2 *vs* 4.0 ms at SOA 50 ms, NP group). Together, these results may be suggesting that presentation of the auditory cue at a closer location as thar of the visual target did help to render differences of performance involving valid and invalid trials slightly stronger. Note, however, the size differences were not systematically larger in the second experiment and still very distant from the ~30 ms difference in RT found in the rat literature using visual cues and visual targets (Marote & Xavier, 2011; Phillips et al., 2000; Rosner & Mittleman, 1996; Ward et al., 1998; Ward & Brown, 1996, 1997; Weese et al., 1999). There was a significant validity effect for accuracy in Experiment 1, but not in Experiment 2. Therefore, contrary to our predictions, the closer spatial source of auditory cues and visual targets in Experiment 2 did not generate differences between valid and invalid trials similar in magnitude to those seen in similar experiments in rats involving visual cues and visual targets. This suggests that closer spatial proximity of auditory cues and visual targets had little or no effect in orienting of attention in rats.

Alternatively, one could argue that the change in architecture of the cues location was not enough to make auditory and visual stimuli closer enough, such that the task architecture in both experiments were not functionally different. In fact, although we installed speakers as close as possible to the LEDs as we could, this may have not been close enough. In our view, however, this is not the case. As there was a translucent shield between the LED and the subject's eyes, the light "filled" the hole, it seems unlikely that the visual stimuli were perceived as a focal point coming from a different location from the auditory cue.

Although the hypothesis raised when planning Experiment 2 was not fully confirmed, results of this experiment further corroborate the conclusions achieved after Experiment 1. As mentioned in the Preliminary Discussion, in the first experiment we tested whether rats are able to direct attention exogenously to visual targets using peripheral sounds. The validity effects for accuracy (Experiment 1 only) and omission errors (Experiments 1 and 2) obtained in this study using non-predictive cues corroborates that conclusion. Therefore, this study demonstrated that exogenous visuospatial attention may be either captured or engaged, or both, by peripheral

auditory cues. This opens new possibilities to the study of intermodal orienting of spatial attention employing rats as animal model.

Results of the Experiment 2 involving the percentage of omission errors including data of all testing sessions also indicates that in addition to exogenous orienting of visuospatial attention using peripheral non-predictive auditory cues, there is also endogenous orienting of visuospatial attention using peripheral predictive auditory cues. That is, both P and NP subjects exhibited validity effects for omission errors at SOA of 50 ms, and P subjects showed a validity effect at the SOA of 150 ms. This occurrence of validity effects when employing longer SOAs in a P condition is usually ascribed to the effects of endogenous orienting of attention (Chica et al., 2014).

The hypothesis that attentional effects seems to wane along the sessions (see p. 66), which was corroborated by the accuracy results of Experiment 1, was also corroborated by the RT results of Experiment 2. Although the RT differences are slight, we showed validity effects only in the first and intermediate subsets. To our knowledge, this is the first evidence that shows that in rats, as in humans (Pratt & McAuliffe, 1999; Weaver et al., 1998), some attentional effects wane with practice of the task.

5.2 ADDITIONAL FINDINGS

It is worth noting that some of our results showed that the subjects performed faster or more accurately in invalid trials, as compared to valid trials. This type of result is usually ascribed to Inhibition of Return, an effect that generates a detection cost in orienting attention towards places recently attended, supposedly to facilitate detection of relevant stimuli in novel locations (Klein, 2000). This effect seems to transcend sensory modalities, i.e., it seems to be supramodal (Pierce et al., 2018; Spence et al., 2000) such that its occurrence in the present experiment involving auditory cues and visual targets was expected.

Data of Experiment 1 involving the initial subset of testing sessions revealed that accuracy at the SOA of 300 ms is 2.7% higher in invalid trials as compared to valid trials, suggesting that detection of visual targets in locations previously attended is impaired at this SOA. Although the effect was observed exactly at the SOA in which IOR is observed in humans (e.g. Lupiáñez & Milliken, 1999), it was not replicated using similar conditions in Experiment 2. It seems unlikely that presentation of auditory cues

and visual targets in the same location would hinder the effect, since several studies showed that the closer the source of cue and target the greater the IOR effect (Spence et al., 2000). Further, there were no evidence indicating that omission and commission errors explain these higher accuracies in invalid trials and considering that we found an outlier on that analysis, it is possible that the result is due to the outlier's influence. However, taking into account that even without the outlier the effect was marginally significant, we cannot discard the possibility that IOR did occur in Experiment 1. The fact that this phenomenon did not occur in Experiment 2 is puzzling, but reinforces the notion that it is an elusive effect in rats (i.e. difficult to experimentally observe and replicate. See Wagner et al., 2014 for a very clear example of this).

Experiment 2 also revealed shorter RTs in invalid trials as compared to valid trials at the SOA of 50 ms, in the first subset of testing sessions, for P subjects. It seems unlikely that this effect is related to IOR, because the SOA is too short (IOR effects in other animals usually appear at SOAs of 100-150 ms, lasting up to SOAs of 300 ms). In addition, this effect appeared in a group of subjects trained and tested in a Predictive schedule, which, at longer SOAs, usually prevent the IOR effect (Klein, 2000), although there is electrophysiological evidence indicating that IOR occurs even using predictive cues (Chica & Lupiáñez, 2009). A possible explanation for this unexpected result may be related to the use of the median, instead of the mean. By using the mean in this specific comparison, one reveals that the RT is faster in valid trials (162.4 ms) as compared to in invalid trials (165.3 ms). Even though the median seems a more appropriate statistics for this analysis, due to the non-parametric nature of the data.

One of the reasons for employing auditory cues in this study was to explore whether rats exhibit IOR using a cue of a different sensory modality as the target, as reported for humans (Spence et al., 2000). Although the results of the present study did not reveal a consistent IOR when using auditory cues and visual targets, and IOR was reported not to occur for rats when using visual cues and visual targets (Wagner et al., 2014), one cannot discard the possibility that this phenomenon may be revealed in rats using different sensory modalities.

An additional effect seen in Experiment 2 worth noting relates to a drop of accuracy of responses in both P and NP subjects, independently on validity, at the SOA of 300 ms for P subjects and at the SOA of 900 ms for NP subjects. This effect

was consistently observed in the analysis including both all testing sessions pooled (Figure 24) and the initial (Figure 35) and intermediate (Figure 36) subsets of testing sessions, being marginally significant in the last subset (data not shown). These effects seem to be related to the increase in the percentage of omission errors at these specific SOAs (See Figures 25, 35, 37 and 38). This increase in the percentage of omission errors in shorter SOAs of the P subjects as compared to NP subjects is intriguing.

In the present experiments, the auditory cues played a role not only as an indicator of the possible spatial location of the pending target but also as a temporal alerting signal. In this sense, it is not surprising to observe quicker and more accurate responses immediately after the alerting signal (i.e., the auditory cue). Similar patterns of results were reported by Cruz (2017) in a similar experiment using rats and other previous studies in humans showing that auditory signals increase the subjects' alert (Fuentes & Campoy, 2008; Kusnir et al., 2011; Salagovic & Leonard, 2021).

This decline of accuracy at shorter SOAs by P subjects as compared to NP subjects raises speculations related to the predictive nature of the cues. That is, P subjects are actively orienting attention because of the predictive nature of the auditory cue. This could emphasize the auditory cue more as an informative signal that requires cognitive processing, and less as an alerting signal. Thus, it seems likely that the reduction in accuracy at an earlier SOA in P subjects indicates a quicker decline in the effects associated with the alerting signal, as compared to NP subjects, on which the cue is predominantly an alerting signal.

5.3 POSSIBLE LIMITATIONS

The main possible limitation of the results reported in the present experiments relate to the small magnitude of the difference observed between scores in valid and invalid trials.

For instance, even though the statistical analyses did reveal significant validity effects for RTs, as mentioned above, these differences were too small to be considered biologically relevant. In other words, the magnitude of the observed differences does not seem enough for affirming that the animals did benefit from the cues in order to orient attention accordingly and thus to respond faster. The processes

involved in responding in this task involve too many steps, including sensory reception, processing, and generation of a motor response, for affirming that the animals obtained an actual RT advantage by receiving the reward, say, 4.3 ms[9] earlier. The magnitude of the validity effects for RTs found in rats in previous studies was usually much larger than 30 ms, when using visual cues and visual targets (e.g. Rosner & Mittleman, 1996). Even experiments in our laboratory using the same peripheral auditory cues and visual targets found validity effects which magnitude was approximately 10 ms (Cruz, 2017).

However, it is possible to speculate that such differences do indicate attentional effects. Considering that auditory stimuli have been used as a way to detect other lifeforms, thus being crucial for survival, they required the nervous system to work at its utmost limit of processing speed. The small differences in RT observed here, therefore, would be a consequence of the fact that the nervous system is already working near its limit speed, thus not having much “room” to accommodate big RT differences between valid and invalid RTs. This issue remains to be investigated.

Another concern regarding the **magnitude** of differences between scores in valid and invalid trials relates to the percentage of omission errors. Both experiments revealed that there were more errors of omission in **invalid** trials, as compared to valid trials. This increase in omission errors is likely due to orienting of attention towards the side indicated by the auditory cues, which is opposite of the location where the target was presented (note that one is referring to invalid cues). This would lead to a failure in detection of the target appearance and, thus, in the removal of the nose from the central hole. The fact that the effect was present in both experiments in short SOAs (see Table 1) makes it reasonably consistent and further corroborates the aforementioned interpretation.

At a first glance, similarly to the analysis of RTs, the magnitude of the validity effect for accuracy is too small, i.e., of the order of 0.8 %. However, unlike RT results, there have been reports that the percentage of errors[10] in invalid and valid trials differ by about ~2% (Ward et al., 1998; Ward & Brown, 1997), which is, roughly, the same

[9] Difference between valid and invalid RT at SOA 50 of the P group in Experiment 2, which was the largest difference between valid and invalid RT we obtained in our work.

[10] The difference in valid and invalid omission errors in the present experiments is being used as a proxy for the difference between invalid and valid accuracy in other experiments.

order of magnitude found in the present experiments . The present study also found differences of about 1.7% between valid and invalid omission errors, although marginally significant (see Table 1).

It is important to note that these errors decreased the number of rewards that the subjects receive, which seems biologically **relevant** considering that this directly decreases the caloric intake of an animal that was fasting for the last 22 hours.

Another possible limitation refers to the overall accuracy of performance in this task. It was relatively high, particularly in Experiment 2, since subjects consistently exhibited accuracies larger than 85% (Figure 24). In Experiment 1, if one excludes anticipation errors, the mean accuracies per SOA was not less than 90%, ranging from 91.5% at SOA 900 up to 94.9% at SOA 50 ms. This indicates that the subjects learned and performed the task adequately, but may also suggest that the visual target intensities employed were too high, thus interfering with the attentional effects. Note, however, that the target stimulus luminance was calibrated in the absence of attentional orienting such that its threshold for each side of each subject generated accuracies varying between 75% and 85% (data not shown; see the final steps of the conditioning protocols). However, the subjects' accuracies rapidly increased throughout the first few testing sessions (i.e., right after they started to being exposed to the auditory cues), such that, after a couple of sessions, their mean accuracy was much higher than before. In fact, previous studies have shown that humans improve their performance with additional training (Dresp, 1998; Hussain & Bennett, 2020; Solovey et al., 2016) and our results indicate that the same is true for rats. Thus, it seems possible that additional reduction of the visual targets luminance could have favored the appearance of larger validity effects. Since attention plays an important role in perception, particularly of near-threshold stimuli (Chica & Bartolomeo, 2012; Liu et al., 2005; Rimsky-Robert et al., 2019) its effects might be better observed with less intense stimuli.

Future studies could improve calibration of the target stimuli luminance, thus rendering it closer to its detection threshold in order to emphasize the contribution of attentional effects. In the present experiments (1) a between-session staircase procedure (Experiment 1) and (2) a one-session custom staircase procedure based on both the Fixed Step-Size Staircase (García-Pérez, 1998, 2000) and the Unforced Weighted Up–Down (Kaernbach, 2001) procedures (Experiment 2) were employed in

attempts to calibrate the visual target luminance. Another possible approach could be a continuous adjustment of luminance based on subject's performance along testing in order to keep it always close to the threshold level. Another additional possibility would be to employ Detection Theory and use varying target stimuli luminance in order to obtain psychometric curves (Macmillan & Creelman, 2005) for each eye of each subject.

5.4 FUTURE DIRECTIONS

About a dozen of published studies have employed rats in the investigation of spatial attention in this kind of covert attention orienting task (Marote & Xavier, 2011; Phillips et al., 2000; Phillips & Brown, 2000; Rosner & Mittleman, 1996; Wagner et al., 2014; Ward et al., 1998; Ward & Brown, 1996, 1997; Weese et al., 1999). All of the studies used the vision sense. None of these studies investigated the use of cues of one sensory modality and targets of another sensory modality for investigating attentional shifts. Rodents in general use different sense modalities for navigation, including vision (Chen et al., 2013; Tan et al., 2017; Yoder et al., 2019), audition (H. E. Heffner & Heffner, 1985; R. S. Heffner & Heffner, 1992), olfaction (Gire et al., 2016; Osako et al., 2018) and touch (Roohbakhsh et al., 2016). Thus, exploring different sensory modalities in studies of orienting of attention seems important in order to find optimal ways to employ rats as an animal model for the study of attention. In addition, this approach could contribute to our knowledge on how attention is engaged both when using cues of the same sensory modality as that of the target or of a different sensory modality. Presently, most of the attention research has been focused on vision (see also Störmer, 2019). In this regard, future studies should focus both on further exploring senses other than vision and finding more effective ways of presenting them. Here we employed pure tone sound beeps with different frequencies as cues and lights with different intensities as targets, to investigate attention. In our view, it could be productive investigating whether varying other aspects of these stimuli produce more striking attentional effects. Some possibilities include using different sound pressure levels or timbres, or presenting visual objects, or varying contrast and frequency, such as Gabor patches.

An important original feature of the procedures employed in the present study, not used in previous rats studies of attention involving this task, relates to the lack of cues presentation along conditioning. That is, the precedent cues that should promote orienting of attention were introduced after the subjects had been conditioned. This allowed to develop a conditioning protocol that can be used in future studies minimizing the need of repetitive training with these cues, since this repetition reduce attentional effects (Lupiáñez et al., 2001; Pratt & McAuliffe, 1999; Weaver et al., 1998). However, one has to be cautious when using this approach since previous studies of endogenous orienting of attention in rats using predictive peripheral auditory cues and visual targets, revealed validity effects on RT and accuracies at SOAs up to 800 ms (Cruz, 2017) even having introduced auditory cues during the conditioning phase. It seems possible that the lack of bigger differences in scores of valid and invalid trials in Experiment 2 are related to the introduction of the auditory cues after the conditioning phase. This issue remains to be investigated.

Recent studies investigating spatial attention in rodents have been employing mice and, thanks to that, have made some important advancements in understanding the neural underpinnings of attention. The ease of genetic manipulations in this animal model has allowed them to employ optogenetics to influence the activity of subjects during task performance, allowing the investigation of causal relations between certain brain regions and specific components of the animal behavior (e.g. Kim et al., 2016; L. Wang et al., 2020; Zhang et al., 2014). Animal behavior is the ultimate expression of the functioning nervous system. Therefore, efforts to develop and understand behavioral tasks for investigating any function, including attention, are required to understand the whole system. The present study aimed at contributing for improving the behavioral side of this endeavor.

6 Conclusions

The main contribution of this study was the demonstration that rats are able to orient visual attention exogenously when preceding auditory peripheral cues are presented in a Posner-like task adapted to rats.

This intermodal attentional shift does not seem to depend on presentation of the auditory cue and visual target exactly in the same location, since similar effects were observed with and without their spatial superposition.

Attentional effects in rats, similarly to humans, is affected by repetitive training, such that repeated exposure to the task stimuli wanes attentional effects.

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