**UNIVERSIDAD DE MURCIA**
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# Chronotype and time of day as modulators of attention-dependent cognitive processes

# El cronotipo y el momento del día como moduladores de procesos cognitivos que dependen de la atención

**D.ª Lucía Beatriz Palmero Jara**

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UNIVERSIDAD DE
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## DECLARACIÓN DE AUTORÍA Y ORIGINALIDAD DE LA TESIS PRESENTADA EN MODALIDAD DE COMPENDIO O ARTÍCULOS PARA OBTENER EL TÍTULO DE DOCTOR

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EL CRONOTIPO Y EL MOMENTO DEL DÍA COMO MODULADORES DE PROCESOS COGNITIVOS QUE DEPENDEN DE LA ATENCIÓNI

y dirigida por,

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Chronotype and time of day as modulators of attention-dependent cognitive processes

## Abstract

Chronotype as a trait within individual differences together with the time of day has proven to influence the cognitive performance of individuals. In the present doctoral thesis, we delve into the circadian modulation of these variables on attentional subfunctions allowing, on the one hand, to better characterize them and, on the other, to investigate several techniques that may optimize performance when outside the optimal time of day with respect to chronotype. The experimental series of 4 studies we propose is based, first of all, on Michael Posner's theory of attentional networks. In this case, we focus on the study of vigilance in interaction with executive control (Study 1), and on the different components that compose the former (i.e., arousal and executive) also in interaction with the menstrual cycle (Study 2). Secondly, from Schneider and Shiffrin's proposal on automatic and controlled processing, we approach the time course of both processes based on the semantic-priming paradigm (Study 3), and, in a novel way, we use the Self-Attentional Network paradigm in Study 4 to delve into the potential modulating role of circadian rhythms over processes demanding automatic or controlled strategies of response.

## Keywords

Chronotype, time of day, Morning-types, Evening-types, vigilance, sustained attention, automatic processing, controlled processing

# Introduction

Tempus omnia revelat

*Time reveals everything*

Latin proverb in Correa (2010)

The cognitive process of attention is cross-cutting to virtually any mental operation. Nevertheless, explicitly determining what is meant by attention remains a matter of scientific debate.

Among the aspects where there appears to be general agreement at present, two specifically deserve special mention. On the one hand, the fact that attention is more of a system than a unique function has promoted the shift away from the unitary conception of the process and emphasized the study of the different components that are encompassed within it (Hommel et al., 2019). Similarly, it is also widely accepted the tight bond between the cognitive processes of attention and neurophysiology, not only embedding the discipline within the field of cognitive neuroscience (M. I. Posner & Rothbart, 2023; M. Posner & Volpe, 1982; Simon, 1981), but also considering it the nexus between cognition and neuroscience (Beam et al., 2014). In this vein, among the numerous neuroscientific proposals that have emerged to conceptualize attentional functions, two of them are considered central to this work as they constitute the cornerstone on which the experiments presented here are built.

On the one hand, Posner and Petersen's attentional model (M. I. Posner & Petersen, 1990), establishes three attentional networks with distinct functions and underpinning neuroanatomical structures. First, the orienting network is responsible for directing the attentional focus to specific stimulus on the space. Brain structures underlying this network are mainly related to the posterior parietal lobe, the pulvinar nucleus of the thalamus, the superior colliculus, and the frontal eye fields.

The alerting network is implied in reaching and maintaining an optimal level of arousal that enables an efficient response to environmental stimuli. This network likes lateralized in the brain's right hemisphere. Moreover, its functioning is directly dependent on the innervation of the neurotransmitter norepinephrine from the locus coeruleus on right-sided prefrontal and parietal structures.

Finally, the executive control network is responsible for highly sophisticated mental operations related to the control of goal-directed behavior. Therefore, the executive control network would intervene in situations where the inhibition of a response or a conflict resolution is demanded. Brain regions on which this network relies are essentially the anterior cingulate gyrus, the posterior network, mainly related to parietal cortex areas, and the anterior network, where the dorsolateral prefrontal cortex (DLPFC) plays a key role.

In closing, it is noteworthy that the three attentional networks described above can be both directed in a top-down or endogenous manner (i.e., guided by our goals), or bottom-up or exogenous, thus guided by stimuli or signals from the environment (Corbetta & Shulman, 2002).

A further approach to conceptualizing attention-dependent processes is by classification based on the degree of cognitive control they require. This division arises from the observation that conscious information-processing (i.e., controlled processing), and automatic information-processing, understood as non-conscious processing, involve different neural and cognitive mechanisms of functioning. In this line, Schneider and Shiffrin's theory on information processing (Schneider & Shiffrin, 1977; Shiffrin & Schneider, 1977) posits two pathways of information processing whose preponderance is based on both the nature of the incoming stimulation and the individual's previous experience with that stimulation. Hence, automatic processing would be triggered by invariant and well-known information or stimulus conditions where response training has previously occurred. Such a response is considered a sequence of nodes triggered upon a specific input with a particular configuration. Automatic response mode neither demands the involvement of attentional resources nor does it depend on either capacity- or duration-limited memory storages as the short-term memory. The anatomical circuitry underpinning automatic processing is founded on specific regions activated according to the type of incoming information to be encoded (i.e., motor, or sensory areas, for instance). In addition, automatic processing generates the deactivation of the cognitive-control regions mentioned below. It is important to note that all those areas not directly related to controlled processing are considered to serve stimulus coding, thus potentially constituting part of the automatic-processing circuitry (Schneider & Chein, 2003).

In contrast, controlled processing is generally driven by attentional resources, as well as it is closely dependent on short-term memory storage where the information given must be properly represented to provide an adaptive response to a given environment. On this account, and as opposed to automatic processing, control-based responses occur serially rather than in parallel with further operations that require cognitive-resources allocation. As such, the activation of nodes arises under control when faced with variable or unknown information. The given responses tend to develop relatively quickly (i.e., response training is not needed) and are susceptible to easy modification. Regarding the neural circuitry on which controlled processing is based, the structures to be highlighted would be the DLPFC, the anterior cingulate gyrus (ACG), the posterior parietal cortex (PPC), and the thalamus (Schneider & Chein, 2003).

Notwithstanding the seemingly close link between the cognitive process of attention and biology, the study of this relationship has only just begun (M. I. Posner & Rothbart, 2023). Not only is it important to understand the neural mechanisms that underlie attentional processes, but it is also essential to delve into the specific variables that exert significant influences on these circuits in order to move forward. Taking as reference the attentional theories described above, in this dissertation we study in further detail specific biological individual differences that directly influence cognitive performance in terms of attentional processes. In this vein, recent emphasis has been placed on considering participants' baseline in various parameters such as hormonal concentration levels, neurotransmitter balances, or subjective factors such as fatigue or wakefulness. These factors directly bear on the levels of cortical excitation and inhibition, and therefore notably influence cognitive performance and its potential improvement (Colzato et al., 2021; Krause & Cohen Kadosh, 2014). Related to these considerations and comprised within the framework of individual differences is the so-called chronotype, whose influence is evaluated in this thesis through four experimental studies. Also in connection with individual differences, we addressed the menstrual cycle in interaction with chronotype as one of the studies of this work. Specifically, in the first part, we addressed the alerting function of attention by studying both the interaction between alerting and executive control networks and the vigilant attention function through the vigilance components dissociation. On the other hand, in the second part of this dissertation, we go deep into automatic and controlled processing levels using the semantic priming paradigm and the self-attention network.

In the following sections, the reader is introduced more specifically to the central topics of this doctoral thesis, where, explicitly recalling the proverb at the beginning of this section, the variable time becomes of paramount importance.

## Alerting and Executive Control Networks Interaction

Although Posner and Petersen's theory of human attention (M. I. Posner & Petersen, 1990) establishes three distinct networks at the anatomical and cognitive levels, it has been similarly posited that these circuits interact producing multiple influences on each other (Fan et al., 2002; M. I. Posner, 1994). Traditionally, the assessment of the attentional networks proposed by this theory has been conducted through the well-known Attentional Network Test (ANT) (Fan et al., 2002), which objectively quantifies performance in the attentional domains by means of a cueing paradigm and a flanker task. Regarding the executive control network, the target stimuli to which the participant must respond, namely a central arrow, appears flanked by two other arrows pointing either to the same location as the target (i.e., congruent condition), or to the opposite location (incongruent condition). In addition, a neutral condition where instead of arrows straight lines appear on the sides of the target is considered. The efficiency of the executive control network is given by calculating the so-called congruency effect through the subtraction between the incongruent trials (i.e., where the target arrow is different from the rest of the presented stimuli), and the congruent trials, in this case, where the target arrow point to the same location as the rest of flankers.

Prior to the presentation of the target and in order to evaluate the functioning of the alerting network, a double cue condition is considered and presented throughout the trials of the task. In this case, two signals are simultaneously displayed at the two locations where the target is likely to be presented (i.e., above and below the fixation point at the center of the screen). These warning signals do not produce a decrease in attentional diffusion since they do not inform about the location of the target, but they do predispose the individual to respond insofar by predicting the impending appearance of the target. The performance of the alerting network is calculated by the subtraction of RTs or accuracy rates of double-cue condition trials from the RTs or accuracy rates of the no-cue condition trials.

Finally, and even though the orienting network is not of interest in the present work, it is based on a visual cueing paradigm and has three different levels: trials without visual cue, cued trials (i.e., where a cue is presented at the same location as the subsequent target), and uncued trials (i.e., the cue was presented at the opposite location of the subsequent target). Again, the locations where both the cues and the target may appear are either above or below the fixation point that marks the center of the screen. To assess performance concerning the orienting network, the performance indicator (i.e., either reaction times (RTs) or accuracy rates) of the valid and invalid cue-signaled trials must be subtracted.

In order to study the interactions between attentional networks, new versions of the ANT have been designed to compensate for some of its limitations, such as the inability to dissociate the effects of cues related to the alerting network and the orienting network. For instance, Callejas et al., (2004), introduced a novel method to assess the alerting network through the presentation of an auditory tone in 50% of the trials that compose the ANT, thus enabling the study of both the independence and mutual influences of the attentional networks through the Attentional Network Test-Interaction (ANTI).

As for the proposed interaction between the alerting and executive control networks, a negative influence of high levels of alertness on conflict resolution has been documented (Callejas et al., 2004, 2005; Fuentes & Campoy, 2008; M. I. Posner, 1994). In this vein, several theories have attempted to account for the nature of such interaction.

Firstly, M. I. Posner (1994), termed this inhibitory phenomenon as “clearing of consciousness”. Thus, the activation produced by a warning signal or the sustained attention to a source of information from which an infrequent target usually comes (i.e., vigilant attention), produces a sort of mental emptying of thoughts or feelings, thus causing the individual to be more inclined to respond to the subsequent stimulus than to ponder the type of response that should be given (Callejas et al., 2004, 2005). Moreover, this state has appeared to be accompanied by a decrease in the activity of the anterior cingulate cortex, (i.e., the main executive control structure), and an increase in the activation of the neural circuit of alertness, in this case, the right frontal lobe.

Alternatively, Böckler et al., (2011), McConnell & Shore, (2011), and Weinbach & Henik, (2012), focused on the alteration of early attentional processes to explain the negative effect of alertness on executive control. In this sense, this inhibitory effect may be understood as an attentional diffusion toward spatial locations where the forthcoming target is likely to appear. Thus, task-irrelevant stimuli in the visual field are equally processed, leading to an increased influence of the flankers usually resulting in higher congruency effects. On the contrary, attention would remain focused under no warning signal conditions, backing the neglect of the to-be-ignored distracters.

One of the main purposes of the present doctoral thesis, specifically in **Study 1**, is to deepen both the interaction between alerting levels and the executive control and alerting networks from a circadian-rhythms approach.

## Vigilant Attention

Since the consideration of alertness as an attentional network, research on this cognitive process has largely developed, giving rise to several proposals on the various threads that may evolve on the basis of the alertness function. In this connection, it is worth considering the variable time, thus understanding the vigilance function as the ability to maintain the attentional focus over extended temporary periods. Furthermore, the features of the stimulus upon which attention is sustained are emphasized. In this way, it is generally repetitive and non-arousing, leading to a decay of focus over time on task resulting in the so-called vigilance decrement and even distraction by other stimuli inside or outside the context (Davies & Parasuraman, 1982; Robertson et al., 1997; Robertson & O'Connell, 2010).

However, recent research theories have suggested that vigilance is not necessarily a unitary concept (Langner & Eickhoff, 2013; Luna et al., 2018; Martínez-Pérez et al., 2022), but may refer to at least two distinct processes involving separate neuroanatomical structures and behavioral events. Thus, the arousal component of vigilance, also equating to tonic alertness, would solely refer to the maintenance of the attentional focus on a specific source in order to subsequently produce a non-control-based response as quickly as possible, thus excluding the possibility of analyzing and providing alternative responses (Luna et al., 2018). This arousal subfunction of vigilant attention is assumed to be recruited when individuals are faced with usually monotonous, boring, or rather tedious tasks. Some examples of tasks normally used in the laboratory setting to assess this process would be, for instance, the Psychomotor Vigilance Task (PVT) (Lim & Dinges, 2008). During this test, a specific stimulus appears on the screen with an inter-stimulus interval (ISI) of 2 to 10 s. Participants are instructed to respond, whenever the target appears, as fast as possible. The total duration of the test is approximately 10 min.

Besides, the executive component of vigilance is equally accounted for within the dissociation of vigilance components approach. In this vein, the vigilance process would not only be understood as the maintenance of the attentional focus over time but would also be marked by the requirement to involve a cognitive control component responsible for solving a conflict, withholding a response to an infrequent target, or switching between tasks in a flexible manner. To assess the functioning of this system, multiple tasks have been developed, such as the Mackworth Clock Test (Lichstein et al., 2000), or the Sustained Attention to Response Task (SART) (Robertson et al., 1997). This latter is the one that becomes more relevant in the present doctoral thesis. Specifically, this test consists of the random presentation of digits from 0 to 9. Participants are told to respond when presented with any digit other than the number 3, to which no motor response is expected, i.e., a prepotent response based on pressing a button must be withheld in this case. The executive vigilance component unfolds in increasingly difficult tasks, and it is just this variation in task complexity that is essential for a more accurate understanding of the relationship between the brain and behavior (Tkachenko & Dinges, 2018), an aspect that, in addition, has recently given rise to new tasks aimed at measuring the two attentional processes described above, such as the ANTI-Vigilance Executive Attention (ANTI-VEA) (Luna et al., 2018). In addition to a more specific characterization of the vigilance processes, delving into the biological factors producing variations in the functioning of this network is crucial to **Study 2**.

## The Semantic-Priming Paradigm

When it comes to studying attention-dependent processes based on the cognitive control they demand, the semantic-priming paradigm has proven to be a highly efficient tool in the dissociation of automatic- and controlled-processing (Besner & Humphreys, 1991; Neely, 1977; Ortells et al., 2001). In a classical task of this paradigm, participants must give a relatively fast response to a particular stimulus (i.e., the target, which can be a word or pseudoword) and is always preceded by another stimulus (i.e., the prime), that can be either semantically related to the target or unrelated. The semantic priming effect is typically represented through shorter reaction times (RTs) and/or higher accuracy in the related condition (e.g., DOG – cat), than in the unrelatedness condition (e.g., DOG – table) (Meyer & Schvaneveldt, 1971). Faced with this task, Neely (1977) argued the existence of two cognitive processes that might be set in motion. First, the presence and processing of the prime would produce an automatic and unaware activation of all the nodes belonging to the semantic category of the prime caused by over-learned associations within the semantic memory storage, thus generating the possibility of providing faster responses to targets composed of semantically linked words with the prime (Collins & Loftus, 1975).

A version of the semantic priming task consists of using as the prime stimulus the name of a semantic category (e.g., ANIMAL) and as target an exemplar of this category (e.g., tiger). This version of the task is called semantic categorization task, since the participant is usually asked to respond to the semantic category to which the target belongs. The semantic categorization task is not only suitable for assessing semantic activation automatically, especially with rather short prime-target intervals. But it also encourages the use of the prime category name to anticipate the target to be presented next, and thus adapting the subsequent response to it (Langley et al., 2008). However, it is worth noting that the development of conscious, control-based strategies requires time to develop, and thus the time between the prime and target onset (i.e., the stimulus onset asynchrony: SOA) becomes critical (C. A. Becker, 1980). Hence, depending on the length of the SOA, whether the processing takes the form of automatic- or controlled-based will be ascertained (Besner & Humphreys, 1991). At short SOAs (e.g., shorter than 200 ms) (Neely et al., 1989), as conscious strategies would not be able to develop, increased recourse to automatic processing to respond to the target will prevail. Conversely, as the SOA becomes longer, controlled processing may produce the effective development and application of control-based strategies. By using this semantic categorization paradigm, Langley et al. (2008) found evidence of controlled processing in young adults at 200-, 500-, and 800-ms SOA, while automatic processing occurred only at 100-ms SOAs.

Also, for the purpose of either facilitating or hindering the processing of the prime, the use of a mask after its presentation has proven to be relevant. In this sense, it is not only common to use this stimulus in semantic-priming tasks but also to manipulate the interval between the appearance of the prime and the mask. Thus, the immediate presentation of the mask after the prime increases the probability of processing the information automatically, whereas the delayed presentation of the mask after the prime would trigger control-based circuitries (Daza et al., 2002; Merikle & Joordens, 1997).

In closing, another crucial aspect of the semantic-priming approach is the maintained relatedness proportion between the prime and the target throughout the entire experimental task, which yields the best-suited qualitative distinction between automatic and controlled processing with observed patterns of both facilitatory and inhibitory effects in semantic-priming tasks (Merikle & Joordens, 1997).

The facilitatory effect (i.e., positive priming), would refer to the advantage in the processing of stimuli related to the target, and as such, would appear in conditions of high rates of prime-related targets. Conversely, the inhibitory effect (i.e., negative priming) would occur whenever, in most cases, the prime is not directly related to the target to which the participant must respond (i.e., low rates of prime-related targets).

By keeping the proportion of non-prime-related targets fixed at 80%, for instance, so that the implementation of control-based strategies is promoted, the emergence of positive (i.e., facilitatory) priming effects would be depicting a purely automatic-driven way of processing, besides the lack of accurately generated expectations. Conversely, the successful generation of expectations and thus the usage of control-based strategies would be reflected by the emergence of inhibitory priming effects (i.e., shorter RTs to unrelated than to related trials).

Summing up, these two processes can be decoupled and captured in semantic-priming tasks in general, and semantic categorization tasks in particular through the combination of SOA manipulation, the specific presentation of a mask, and the relatedness proportion between the prime and the target.

In **Study 3** of this doctoral thesis, specific combinations of these three variables are carried out in order to clearly distinguish automatic and controlled processes and explore their potential differential modulation by biological factors.

## The Self-Attentional Network

Along the lines of the dissociation of processes that are settled both in an automatic and controlled manner, in **Study 4** we implement, as a novelty, the self-bias paradigm to study such processes and their differential influences by biological factors. In this regard, there exists overwhelmingly consistent evidence of the attentional prioritization on processing stimuli closely linked to oneself in a given environment (Arnell et al., 1999; Dalmaso et al., 2019; Liu et al., 2016; Shapiro et al., 1997; Sui et al., 2009; Sui & Han, 2007), among other cognitive domains. The so-called Self Prioritization Effect (SPE) is supposed to be based on an automatic information processing mode that would be set up in the face of self-related cues, while more remote information from the surroundings would tend to be processed in a fairly controlled manner. Despite the robustness of the SPE, paradigms for studying this effect have been, to a certain extent, methodologically imprecise as they have often been inextricably linked to effects such as familiarity or overlearning. In this vein, (Sui et al., 2012) developed an unbiased approach to addressing the SPE through a perceptual matching task that begins with the learning of an association between three particular geometric figures (i.e., a circle, a square, and triangle, for instance) with three labels related to the self (you), a close person (friend), or an unknown person (stranger). After the learning of associations, participants are presented with both the geometric figures and the labels. In this case, they must judge whether the presented pair is correct or not based on the associations they have trained during the first phase (i.e., matched, or non-matched). The SPE is characteristically reflected through lower RTs and higher accuracy rates for stimuli that have been previously associated with the self (you-label), compared with those linked to the friend and the stranger. RTs normally increase progressively in friend- and stranger- conditions (Dalmaso et al., 2019; Desebrock et al., 2018; Liang et al., 2022; Martínez-Pérez, Campoy, et al., 2020). Moreover, it is noteworthy that the very design of the task shields it from potentially conflicting variables such as the word length, familiarity or concreteness, as well as the frequency of use (Humphreys & Sui, 2015; Sui et al., 2012).

Further evidence, not only for the pervasive existence of the SPE but also for the claim that the processing of the two types of stimuli (i.e., the self-related labels, and others-related labels) are strictly different, stems from studies that have delved into the neural level through the perceptual-matching task designed by Sui and colleagues, (2012).

In this vein, it has been posited that self- and others-processing reside in discrete anatomical structures within the medial prefrontal cortex (MPFC), which is a pivotal region in the mental operation related to oneself- and others-judgments (for a review see a meta-analysis from (Denny et al., 2012)). To this end, the region of the ventromedial prefrontal cortex (VMPFC) would face with self-related stimuli, whereas, in the case of others-related judgments, the DLPFC would be centrally involved. These findings were echoed in a functional magnetic resonance imaging (fMRI) study led by Sui et al., (2013), which additionally highlighted the role of the left posterior superior temporal sulcus (LpSTS), and the intraparietal sulcus (IPS), the former being part of the self-associated stimulus processing circuit together with the VMPFC, and the latter along with the DLPFC, the frontoparietal network responsible for the processing of others-related stimuli. Given the existence of these two distinct systems, Sui and Humphreys (2015) established the concept of the so-called self-attentional network (SAN), made up of an automatically driven ventral attentional network, conducting the processing of oneself-linked stimuli, and an attentional frontoparietal circuit that would be recruited in the face of more cognitive-control demanding tasks (i.e., more complex).

Lastly, it is important to note that both networks would be inversely related, hence as the to-be-performed task requires more control, the activation of the ventral network would be transferred to the frontoparietal circuit and vice versa.

## Chronotype and Time of Day

Out of the manifold influences that are exerted on cognitive performance, it can reasonably be contended that circadian rhythms, understood as fluctuations in 24-hour cycles (i.e., daily) occurring in most organisms to adapt to the environment, are decisive for the underlying behavioral success of a given cognitive activity. Hence, circadian biological clocks not only are responsible for the control of the physiology of an organism through for instance gene expression, but the human most sophisticated activities (e.g., sleep or performance) also operate at the pace of such biological cues (Roenneberg et al., 2003). More than justified along these lines has been the urge to study the life-between-clocks phenomenon, mainstreamed in the field of neuroscience but largely and historically overlooked in both clinical and experimental settings (Schmidt et al., 2007), despite the early evidence of its influence in the cognitive domain yet outlined by Ebbinghaus, (1913) who alleged that the learning of nonsense syllables improved during the morning hours, as opposed to later times during the day. The rationale for this absence of interest may be mainly related to other cognitive processes that have been drawing the most emphasis in conjunction with circadian rhythms, in this case, basic attentional domains primarily linked to simple vigilance processes (i.e., arousal vigilance). The focus on this field has paralleled the detriment of higher-order cognitive operations study, the relevance of which resides in the potential consequences their failure or merely a deficit in their optimal functioning may entail. Increasing the knowledge on this latter issue is one of the central points of this thesis, and as such, it is reflected in all the studies that comprise it (**Studies 1, 2, 3, and 4**).

Delving into the link between circadian rhythms and cognition, it is relevant to highlight that the variations produced under the strict command of these biological rhythms envisage variations at the interindividual level (Levandovski et al., 2013; Schmidt et al., 2007), an aspect that directly connects with one of the paradigms of study of the circadian influences on cognitive processes, and transversal in the present work: the so-called chronotype. The chronotype can be defined as the preference that individuals develop for carrying out their basic activities of daily living and their rest hours at one time of the day or another. Thus, this trait would be considered a sort of continuum where all individuals would be located, ranging from extreme morningness (i.e., people who have an earlier profile when planning their activities, working, and also when resting), to extreme eveningness, where people preferring later hours both for resting and for conducting any type of daytime activity would be found (Taillard et al., 2002). The continuum would also include moderate-types of both morning- and evening-profiles and intermediate-types or neither-types (i.e., those who do not possess a specific pattern of time of day for planning their sleep/wake cycles). The different time-of-day fluctuations covered within a chronotype profile specifically include variations in their neurobiological functioning, such as the melatonin and cortisol secretions (Duffy et al., 2001; Oginska et al., 2010), or body temperature rhythms and peaks (Kerkhof & Van Dongen, 1996; Sarabia et al., 2008), as well as peak times of day in which their arousal vigilance levels are at their maximum (Valdez et al., 2012), among others. The chronotype trait is sustained in a permanent gene-environment dialogue, factors which continuously feeds back to. Thus, at the endogenous level, the chronotype is mainly regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus, which integrates both endogenous (i.e., cortisol secretion) and exogenous (i.e., artificial light) inputs. Moreover, this trait is derived from specific genes called clock genes, which interact to develop particular circadian patterns (Montaruli et al., 2017). This disposition, in turn, would be influenced by the behavior of the individual, who can voluntarily modify their rhythms through activities that have been shown to entrain the circadian rhythms. For instance, physical exercise, food intake, or general daytime habits, act as the so-called zeitgebers, leading the efficiency of the trait to be based on the interaction between physiology and behavior (Bonaconsa et al., 2014). Assessing the chronotype as a factor that explains cognitive performance and using it as a tool on one's behalf to improve it at certain junctures constitute one of the fundamental purposes of current research. In this regard, what becomes truly intriguing in the experimental setting when it comes to delving into chronotype is not only the determination of the trait, which is addressed below, but also the conjunction of this time preference with the time of day when participants are asked or evaluated on their performance. One of the most common methods of assessing the chronotype trait is through self-report questionnaires. Among them, we highlight the (Horne & Ostberg, 1976) Morningness-Eveningness Questionnaire (MEQ), which classifies participants into Extreme-Morning-type, Moderate-Morning-type, Intermediate- or Neither-types, Moderate-Evening-type, and Extreme-Evening-type. A reduced version of the MEQ, namely rMEQ, was also validated and adapted to the Spanish population by Adan & Almirall, (1991). This reduced scale has shown to have excellent psychometric properties for the assessment of chronotype. The resulting classification is based on the same categories as in its original 30-item version. Once participants have been selected on the basis of their scores, the paradigm sets the assessment of extreme chronotypes in two radically opposite time slots (i.e., 8 AM and 8:30 PM), coinciding with the optimal and non-optimal time of day, and vice versa, for each of the extreme-chronotypes considered. This factorial crossover enables the evaluation of the so-called synchrony effect (May & Hasher, 1998), consisting of an increase or improvement in performance at the optimal times according to the participants' chronotype, and a significant worsening outside their preferred time range (i.e., non-optimal times).

As mentioned above, this effect has traditionally been observed in arousal-type vigilance tasks such as the PVT (Correa et al., 2014; Lara et al., 2014; Mongrain et al., 2008).

The aim of the present dissertation with respect to chronotype is not only to study the synchrony effect in relation to higher-level cognitive tasks (**Study 1, Study 2, Study 3, and Study 4**), but also to investigate the potential restoration of off-optimal performance through the consideration of other potentially influential factors in this interaction (**Studies 1 and 2**).

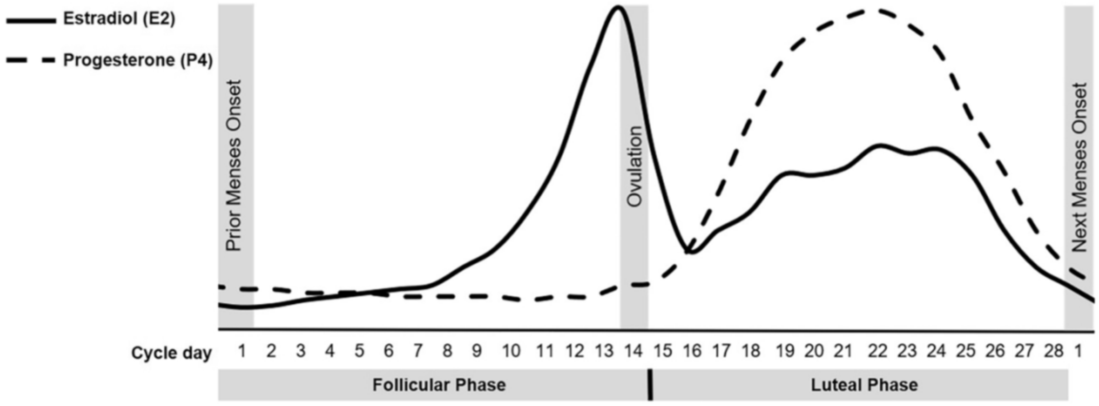
## Human Menstrual Cycle

Another biological variable that has been traditionally linked to cognitive performance in women has been the menstrual cycle. The female menstrual cycle results from the interaction between the structures that make up the hypothalamic-pituitary-gonadal axis and can be understood as the variation in sexual hormonal levels over periods of 28 to 30 days, whose final aim is the liberation of an ovocyte that may later be fertilized, or eliminated through bleeding, thus triggering a subsequent menstrual cycle. Within that period, specific variations of two fundamental hormones occur: estradiol and progesterone (Yen et al., 1999). Specifically, understanding the menstrual cycle as the division of the 28-30 days into two major phases, the follicular and the luteal, the first one would comprise the initial 14 days of the cycle and would end by giving rise to the ovulation around day 14-15 of the cycle, when the maximum estradiol peak would be detected. On the other side, the luteal phase would be characterized by the peak in progesterone around day 21 of the cycle, while maintaining estradiol levels at intermediate concentrations (Jaffe, 1981).

Figure 1 depicts the variations in hormonal concentrations occurring during a regular menstrual cycle. It is important to note that, while the standard menstrual cycle length varies between 28-30 days, natural cycles in fertile, healthy women can last between 21 and 37 days (Long, 1990).

**Figure 1**

*Ovarian progesterone (P4) and estradiol (E2) fluctuations in an idealized 28-day cycle fragmented into two main phases: Follicular and Luteal. Adapted from Schmalenberger et al., (2021).*



Female sex hormone concentrations have been demonstrated to modulate various cognitive processes such as global-local information processing and visual perception (Álvarez-San Millán et al., 2021; Marful et al., 2021; Pletzer, 2014), sustained attention (Solís-Ortiz & Corsi-Cabrera, 2008), vigilance (Vidafar et al., 2018), or performance in the aforementioned attentional networks  (Cohen et al., 2019), among others. The rationale behind this modulation in directly related to the vast of hormonal receptors held by specific brain regions such as the frontal cortex, amygdala, hypothalamus, thalamus, and hippocampus (Guerra-Araiza et al., 2000, 2003; Kato et al., 1994), where it is assumed that these substances would arrive and act by modifying the functioning of the specific anatomical network and its underlying function. Although it has been common to link certain cognitive processes with the menstrual cycle, the linkage thereof with attention has produced scant clear and reliable outcomes (Pletzer B et al., 2017). Besides, yet a further aspect that must be considered when addressing the process of attention, as mentioned elsewhere in previous sections, is the myriad of functions involved therein so that unravelling them and studying each in depth in relation to the menstrual cycle is still a major task to be accomplished.

Otherwise, still orbiting -and to a great extent detrimental- to research on the menstrual cycle is the fact that there remains a lack of consensus on the definition and operationalization of menstrual cycle phases in order to carry out precise evaluations in the experimental setting that can be linked to specific hormonal patterns (Pletzer B et al., 2017; Sundström Poromaa & Gingnell, 2014). In this regard, an extraordinary work has recently been carried out by the team of Schmalenberger and colleagues, (2021), who thoroughly discuss the gold-standard guidelines to be adopted by any research that intends to delve into the field of the menstrual cycle. Several recommendations become essential and are listed below:

- The menstrual cycle is fundamentally a within-person process and therefore should be treated as such in the clinical and experimental setting. This point directly prompts the repeated-measures design as the reference model.
- Concerning the determination of the menstrual-cycle phase, a biological marker beyond the simple counting of days or recording of menstrual cycles is widely recommended, (see also J. B. Becker et al., 2005). Methods such as the measurement of luteinizing hormone (LH) in urine through ovulation tests or the confirmation of hormonal levels through blood or saliva analysis are recommended in this respect.

Taking these issues together, in **Study 2** of the present doctoral thesis, we address the study of certain menstrual-cycle phases in connection with attentional domains that are of relevance to the present work.

## Objectives and Hypothesis

The overarching objective of the present doctoral thesis is to study how certain individual differences in biology-related variables (i.e., the chronotype trait transversally, and menstrual cycle specifically highlighted in **Study 2**) modulate attention-dependent cognitive processes. To conceptualize attentional processes, we focused on two main theoretical models. First, based on Michael Posner's proposal of attentional networks, in **Study 1**, we intend to further explore the interaction between the alerting network and the executive control network through the chronotype paradigm. This paradigm also enables the identification of specific operation patterns in both attentional networks as a function of the interaction between chronotype traits and the time of day (i.e., synchrony effect). We set out four specific objectives in this work. First, we studied how the synchrony effect operates in arousal-type vigilance tasks (PVT), and in more challenging tasks such as ANTI. In general, we expected that performance would consistently improve at the optimal times according to the chronotype of individuals. On the other hand, we examined certain compensatory mechanisms for improving performance at non-optimal times of the day such as the novelty effect. Specifically, we raised the possibility that the first experience with the task may increase endogenous alertness levels and thus compensate the drop in performance observed in non-optimal times provided that the first session was at off-peak times. Furthermore, we address the potential countervailing relationship between phasic and tonic alertness (i.e., exogenous, and endogenous alertness) on performance in tasks demanding executive control. In this vein, it is possible that warning signals compensate for the low level of endogenous alertness when participants perform tasks outside their optimal time. Finally, we examine which type of alertness is responsible for the negative effect typically observed on the executive control network. Based on two different accounts of this negative interaction, we propose, from the perspective of the phenomenon of clearing of consciousness, the possibility that the higher levels of endogenous alertness inherent to the optimal time of day compensate for the negative effect of phasic alertness on executive control. On the other hand, considering the theory of diffusion of attentional focus, we propose that such negative effect is observed irrespective of the time of day. **Study 2** expands on specific aspects of the alerting function of attention, based on the recently described dissociation of vigilance components (Luna et al., 2018), thus considering the arousal vigilance type and the executive vigilance task. In addition to studying the synchrony effect on both types of vigilance (i.e., the PVT to study arousal-type vigilance, and the SART to address the executive component of vigilance), in **Study 2**, we tested the ability of female sex hormones related to the menstrual cycle to produce performance-enhancing benefits in the two attentional processes we assessed. Specifically, we delved into the interaction between progesterone during the mid-luteal phase and participants' cortisol peak during the morning hours to boost performance at non-optimal times according to their chronotype. Thus, we hypothesize that progesterone will act as a trigger of alertness levels, likely compensating for the participants' worsened performance at their non-optimal times notably in the mid-luteal phase compared to follicular. On the other hand, we also consider the possibility that this effect is mediated by the specific chronotype of the participants, which determines the timing of cortisol secretion. Depending on previous cortisol levels, progesterone exerts different activation effects, which may directly affect the cognitive performance of participants.

Besides, from the theoretical proposal of differentiating cognitive processes depending on the demanded degree of cognitive control (Schneider & Shiffrin, 1977; Shiffrin & Schneider, 1977), we investigate the influence of chronotype and time of day through two different paradigms that allow us to delve into various aspects of these functions. First, in **Study 3**, we used a semantic categorization task to dissociate automatic and controlled processing. We do not only aim to tease out whether chronotype and time of day exert different influences on each type of processing (i.e., the synchrony effect is produced unevenly), but also address the time course from the unfolding of automaticity-based responses to the required application of sustained control in order to provide a correct response. Hence, we first hypothesize the development of positive (i.e., facilitatory) priming effects on shorter SOAs (i.e., 100 ms), as well as inhibitory priming effects at longer SOAs. However, we also estimate that the time course of both processes will be variable depending on the chronotype and the specific time of day at which participants perform the task. In this connection, we dig deeper into the role that the chronotype and the time of day play in the change of response strategy promoted by the nature of the task itself. Finally, in **Study 4**, we press on with the dissociation of automatic and controlled attentional-based processes, in this case, through a perceptual matching task used to assess the SPE. We sight to determine whether the synchrony effect occurs in an unequal fashion in the processing of stimuli related to oneself (i.e., self-related labels; automatic processing), and those related to others, which require controlled processing. In this sense, we attempt to extend previous results regarding the circadian modulation of automatic and controlled processing by a novel approach to dissociating the two cognitive processes. Drawing on theories of circadian modulation of cognitive processes, we hypothesize that performance on self-related labels will not vary as a function of the time of day, while those related to others (i.e., friend-, and stranger-related labels) might be sensitive to the observation of synchrony effects.

# Experimental section:

**Study 1:** *The role of chronotype in the interaction between the alerting and the executive control networks.*

**Study 2:** *Mid-luteal phase progesterone effects on vigilance tasks are modulated by women's chronotype.*

**Study 3:** *Circadian Modulation of the Time Course of Automatic and Controlled Semantic Processing.*

**Study 4:** *Testing the Modulation of Self-related Automatic and Others-related Controlled Processing by Chronotype and Time-of-day.*

## Study 1:

**Reference**: Martínez-Pérez, V., **Palmero, L. B***., Campoy, G., & Fuentes, L. J. (2020). The role of chronotype in the interaction between the alerting and the executive control networks. *Scientific Reports*, *10*(1), 11901. https://doi.org/10.1038/s41598-020-68755-z. Affiliation: All authors (Universidad de Murcia).

**Abstract**:

Chronotype refers to the time of day preferred by individuals to perform daily activities according to their circadian rhythm. We asked whether synchrony effects, that is, the difference in performance between the optimal and non-optimal time of day as a function of chronotype, are observed in two tasks that differently involve the endogenous component of the alerting network, the psychomotor visual task (PVT) and the flanker task. From an initial sample of 132 students that filled in the Morningness–Eveningness Questionnaire (MEQ), 18 were classified as Morning-types and 16 as Evening-types. Evening-types showed synchrony effects in both tasks, whereas Morning-types failed to show synchrony effects in the flanker task and when the PVT was first performed at the nonoptimal time of day. thus, Morning-types might have seen increased their vigilant attention at their non-optimal time of day due to the cognitive demands of the flanker task and to the novelty with the PVT. Phasic alerting generated by alerting tones increased conflict score in the flanker task, but time of day did not modulate the congruence effect. Chronotype determines vigilant attention more decisively in evening-types than in Morning-types individuals. Also, exogenous but not endogenous alerting exerts a deleterious effect on conflict resolution.

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# The role of chronotype in the interaction between the alerting and the executive control networks

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**Chronotype refers to the time of day preferred by individuals to perform daily activities according to their circadian rhythm. We asked whether synchrony effects, that is, the difference in performance between the optimal and non-optimal time of day as a function of chronotype, are observed in two tasks that differently involve the endogenous component of the alerting network, the psychomotor visual task (PVT) and the flanker task. From an initial sample of 132 students that filled in the Morningness–Eveningness Questionnaire (MEQ), 18 were classified as Morning-types and 16 as Evening-types. Evening-types showed synchrony effects in both tasks, whereas Morning-types failed to show synchrony effects in the flanker task and when the PVT was first performed at the non-optimal time of day. Thus, Morning-types might have seen increased their vigilant attention at their non-optimal time of day due to the cognitive demands of the flanker task and to the novelty with the PVT. Phasic alerting generated by alerting tones increased conflict score in the flanker task, but time of day did not modulate the congruence effect. Chronotype determines vigilant attention more decisively in Evening-types than in Morning-types individuals. Also, exogenous but not endogenous alerting exerts a deleterious effect on conflict resolution.**

In dealing with daily activities, organisms need to reach an appropriate level of arousal (activation) to perform efficiently, which varies according to the famous Yerkes–Dodson's inverted-U shaped law[1]. According to this law, the optimal level of arousal very much depends on task difficulty, so that performance on rather easy tasks profits from high levels of arousal whereas low levels of arousal are beneficial for rather difficult tasks. In other words, depending on its difficulty, each task requires an optimal level of arousal, with important decrements in performance when such level deviates, below or beyond, from the optimal one.

In Posner and Petersen's[2] theory, such arousal state is linked to the alerting network of the attention system, which is involved both in achieving and maintaining an optimal level of activation, and in preparing the person to perceive and/or respond to a forthcoming target. The alerting network involves both cortical and subcortical areas, which share norepinephrine modulation rooted in the locus coeruleus[2,3]. The alertness state can be reached either exogenously (phasic alertness) or endogenously (tonic alertness). The former refers to a rather transient and nonspecific activation state that increases response readiness and that is triggered automatically by the presence of external warning signals (e.g., a white noise). This phasic alerting component depends on ascending thalamic projections to the right parietal lobe, which are also involved in orienting visual attention[4]. Tonic alertness develops slower and refers to the ability to maintain attention for rather long periods of time-on-task, sometimes at the scale of hours. This endogenous component is activated when the task is rather monotonous and tedious, usually because it does not require strong perceptual, cognitive, or motoric demands to be performed (examples of laboratory tasks with these characteristics are the psychomotor visual test, PVT; the continuous performance test, CPT; and the sustained attention to response task, SART). During this kind of tedious tasks, a vigilant attention network is recruited to maintain the attentional state endogenously in a top-down manner[5]. Vigilant attention involves a right lateralized cortical network including the anterior cingulate cortex (ACC) and the right dorsolateral prefrontal cortex (DLPFC), two brain areas that are also involved in executive control[6], as well as the right inferior parietal lobe[7,8].

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Beside time-on-task, vigilant attention seems to be also affected by circadian rhythm, our endogenous biological clock that determines our physiological and behavioral processes, usually in synchrony with external time. Vigilance levels stabilize along daytime when the circadian rhythm system offsets the sleep-regulation homeostatic system, which accumulates pression to sleep as a function of time spent awake[9]. However, some people undergo shifts in circadian phase. As a consequence, individuals may differ in neurobiological functioning, such as in their peak times of day (periods of high alertness[10,11] or vigilance[9,12]), in their sleep times, bedtime, wake up time and daytime sleepiness[13], in their melatonin levels[14], cortisol levels[15], and in their body temperature rhythm[16] and peaks[17]. All these variations are based on one's innate circadian rhythm. In fact, people display preferences for activity at certain time of day, leading to a circadian phenotype that may be classified with the concept of chronotype. Thus, chronotype can be defined as the time of day preferred by individuals to perform daily activities or to sleep[9,18].

Chronotype is derived from genetic factors since it depends on clock genes that interact among them determining specific circadian patterns[19]. The central coordination of circadian rhythms at an endogenous level takes place through the suprachiasmatic nucleus (SCN) of the hypothalamus, which in turn integrates external inputs such as natural and artificial light[20,21]. Despite endogenous regulation, it is important to note that humans can voluntarily modify circadian rhythms through some activities such as physical exercise, food intake or time spent resting and being awake. Such practices become inputs that modulate the circadian pattern[19]. In this sense, the efficiency of the circadian system always depends on the conjunction of physiology and behavior of the organism[20].

These preferences for performing daily activities and resting are often determined through the use of questionnaires[22], but also thorough some physiological indices such as body temperature or melatonin secretion, both deemed as the best markers of circadian rhythm[19]. Accordingly, three different chronotypes can be described: Morning-types (referred to as "larks"), characterized by circadian rhythmicity that occurs earlier with their optimal functioning early in the morning, Evening-types (referred to as "owls"), characterized by circadian rhythmicity that occurs later with their optimal functioning late in the evening, the two representing the two extreme typologies. The delay of Evening-types with respect to Morning-types oscillates between 2 and 4 h in the circadian rhythmicity of all variables described above[23]. Finally, a third chronotype has been referred to as the Neither-types (or intermediate), that is, those without a pronounced circadian preference[9]. Synchrony effects are frequently reported in a variety of tasks[24], so it is possible to reach the best performance during the optimal time of day (in the morning for Morning-types, in the evening for Evening-types) compared to the non-optimal time of day (in the morning for Evening-types, in the evening for Morning-types), following the circadian typology.

As circadian variability differentially affects endogenous alertness at different times of day, it is expected that performance in tasks that require vigilant attention differs in people with extreme chronotypes depending on time of testing. Previous studies have found chronotype and time of day interactions (synchrony effect) in tasks requiring vigilant attention (e.g., the PVT)[23,25]. Besides vigilant attention tasks, synchrony effects are also expected in cognitive demanding tasks that require sustained attention to maintain an accurate level of performance, such as those involving attentional control (e.g., Stroop or flanker interference tasks), or inhibitory control (e.g., some versions of the SART[25]).

Important interactions between the two components of the alerting system have also been widely documented. For instance, top-down vigilant attention seems to compensate for the lack of sufficient arousal that monotonous and tedious tasks convey. On the other hand, the phasic component of alerting compensates for the decrement on vigilant attention as time-on-task increases. For instance, when clonidine is administered to inhibit the release of norepinephrine from the locus coeruleus, lapses of vigilant attention are observed, but they are attenuated when white noise, activating the exogenous component of the alerting network (phasic alert), is supplied while performing the task[8,26].

The objective of the present study is threefold. As a first objective, we aimed to determine whether the high level of endogenous alert, that we assume to occur in the optimal time of day according to the individual's chronotype, improves performance in both a rather monotonous task that is supposed to activate the vigilant attentional network (the PVT), and in a conflict demanding task that requires sustained attention throughout the task (the flanker task). Additionally, as attention is thought to be biased to novel stimulus and/or locations[27,28], we assessed whether synchrony effects in the two aforementioned tasks can be modulated by task-related novelty, that is, first-experience-with-task occurring in the first session. It might be that first-experience-with-task increases alerting levels, compensating the low endogenous alertness when participants complete the tasks for the first time at their non-optimal time. If that were the case, we expect synchrony effects in this situation to be reduced in comparison with when the first session takes place at the optimal time of day and the second at the non-optimal time of day.

As a second objective, we aimed to determine whether the exogenous component of the alerting system modulates also people's performance according to their chronotypes. Given the interactive relationship between the exogenous and endogenous components of alerting, we expect that warning auditory signals that are usually used to assess the effect of phasic alerting (e.g., in the interactive version of the Attention Network Test, ANT-I[29]), compensates for the low level of endogenous alertness when people perform the task at their non-optimal time of day.

Finally, as a third objective, we aimed to assess the impact of the two different components of the alerting system on executive control as a function of chronotype. Phasic alerting seems to have a negative impact in some measures of cognitive control[30–34]. For instance, conflict based on stimulus–response interference, like in the Simon task[35] is increased when targets are preceded by an auditory signal[36], but conflict based on stimulus-stimulus interference, like in the Stroop task is not[34]. These results suggest that alerting improves the translation of the visual code into the correspondent motor code[36], rather than the alerting tone producing a general state of readiness to respond.

However, of greatest relevance for the present study is the negative effect of phasic alerting on flanker interference. With this task, two main accounts have been proposed. The deactivation account[29,31,37] suggests that the alerting tone would inhibit the attentional control network involved in conflict resolution[38], producing a subjective feeling of clearing of consciousness, and rendering the individual more prone to react to forthcoming external stimuli than to current thoughts or internal states[39,40]. Alternatively, phasic alerting might prioritize spatial processing of stimuli in the visual field, being them task-relevant or task-irrelevant, and consequently enhancing the processing of to-be-ignored distracters when they are presented separated from the target, as it happens in the flanker task[34]. Thus, the increased conflict effect with alerting warning tones would be the indirect consequence of higher accessibility of spatially presented flankers.

All these results suggest that the interaction between phasic alerting and conflict may depend on the type of interference involved. Thus, when interference is based on stimulus–response mapping, alerting may facilitate translation between visual and motor codes, whereas when interference is based on spatial processing of target and distracters, like in the present study, alerting may either inhibit the executive network or facilitate spatial processing of task-irrelevant flankers.

To elucidate which is the best explanation for the alerting and conflict network interaction is beyond the aim of the present study. However, data from synchrony effects on the conflict effect can help assess the pertinence of the two aforementioned accounts in the flanker task. According to the former explanation (clearing of consciousness), conducting the flanker task in the optimal time of day could compensate for the reduced activity of the executive control network produced by the alerting tone, as the vigilant attention network and the executive attention network share common brain areas, such as the DLPFC and the ACC[8]. Therefore, such increment in the conflict effect with the alerting tone should not be observed when participants perform the task in the optimal time of day. However, if the alerting tone increases spatial attention to distracters in the flanker task, such increment in conflict effect should be observed irrespective of the moment of day. Importantly, tonic alertness has been found to enhance spatial processing in orienting tasks[41–44], like the phasic component does[37,45]. Therefore, according to the spatial processing account, the endogenous component of the alerting network should have similar effects on conflict effects as the alerting tone has been found to have. If that were the case, we should observe increased conflict effects when testing occurs in the optimal time of day compared with when testing occurs in the non-optimal time of day with the alerting tone absent. However, previous findings have reported no differences in flanker interference due to the endogenous component of the alerting network either when tonic alerting is promoted throughout the whole task[46] or as a function of chronotype and time of testing[47,48]. These results suggest that the endogenous component of the alerting network has influence in spatial orientation but not in how people deal with cognitive conflict in the flanker task.

## Method

Morning-types and Evening-types participants were tested in two different sessions separated by 1 week. One session took place in the morning and the other in the evening. In each session, all participants completed two computer-based reaction-time tasks. First, the PVT, a rather monotonous task that measures vigilant attention[49]. Second, a flanker task, preceded or not by alerting tones, in which target and distracters were located in the center of the screen to avoid uncertainty about target location.

**Participants.** Details of the study were announced through the distribution list existing in the Faculty of Psychology (University of Murcia) to recruit participants in exchange of course credit. Thirty-four undergraduate students (27 females; M age = 21.0 years, SD age = 2.3) were selected from a total of 132 students who agreed to participate and complete a reduced Spanish version of the Horne and Östberg's Morningness–Eveningness Questionnaire (MEQ)[22]. On the basis of MEQ scores, we selected 16 students (14 females; M age = 21.6, SD age = 3.0) for the Morning-types group, and 18 students (13 females; M age = 20.4, SD age = 1.5) for the Evening-types group. We excluded participants with intermediate chronotype to maximize differences in vigilant attention between extreme chronotypes. We did not explore the influence of sex because only 7 participants were males. All participants reported normal or corrected-to-normal vision and no chronic medical conditions.

**Procedure.** Participants first signed the written informed consent, and then completed the reduced Spanish version of the reduced Morningness–Eveningness Questionnaire (rMEQ) develop by Adan and Almirall[50]. The rMEQ consisted of five items with scores ranging from 4 (definitively Evening-types) to 25 (definitively Morning-types). Participants who scored between 17 and 25 (M = 19.4) formed the Morning-types group, and participants who scored between 4 and 11 (M = 8.6) formed the Evening-types group.

Participants were tested individually in sound-attenuated booths. All tasks were programmed in E-Prime 3 (Psychology Software Tools)[51]. Visual stimuli were presented on a 22″ TFT monitor with a screen resolution of 1920 by 1,080 pixels. A Chronos device (Psychology Software Tools) with five buttons was used to collect responses and present auditory stimuli (via headphones).

All participants came to the laboratory twice, with an interval of seven days between the two sessions. One of the sessions was scheduled to begin at 8:00 AM (the morning session), and the other at 20:30 PM (the evening session), both lasting by 30 min of duration, approximately. This allowed us to evaluate participants in both their optimal and their non-optimal time of day according to their chronotype (when we expected their vigilant attention to be maximum and minimum, respectively). Testing was carried out during the week days, and the order of the sessions was counterbalanced across participants within each chronotype group, so that half of the participants from each group completed the first session in their optimal time and the second session in their non-optimal, with the reverse order for the other half.

Both sessions had the same structure. We began with a 5-min interview by asking participants whether they had consumed coffee during the previous two hours or other stimulants during the previous 24 h (all reported no consumption) and whether they had slept at least 5 h the night before (all reported having slept between 7 and 9 h). Then, participants performed a ten-min version of the PVT. Each PVT trial began with a random interval ranging from 2 to 10 s in which the computer screen remained black. Then, a red circle (50 pixels in diameter) appeared in the center of the screen and participants had to press, as quickly as possible, the central bottom of the response box with the index finger of their dominant hand. When the response was made, the screen went blank and a new trial began.

Immediately after the PVT, participants completed the flanker task for 15 min, approximately. The task consisted of five arrows (pointing left or right) as stimuli and alerting tones preceding half of the trials in each congruency condition. Each trial began with a fixation point (a plus sign) presented in the center of the screen for 2,500 ms. Then, a row of five arrows appeared in the center of the screen and participants indicated, as quickly and accurately as possible, whether the arrow in the middle (the target) pointed left or right by pressing the leftmost or rightmost button of the response box, respectively. The five arrows remained visible until a response was made or for 3,000 ms. In half of the trials, the four flanking arrows pointed in the opposite direction as the target (incongruent condition), whereas, in the other half, flankers and target pointed to the same direction (congruent condition). In each congruence condition, an alerting tone (a 50 ms beep of 2000 Hz) was presented prior to the target in half the trials, with a tone-target interval of 500 ms (from onset to onset). Participants completed three blocks of 72 trials with a short break between blocks. Experimental trials were preceded by 16 practice trials.

**Ethical approval.** This study was approved by the Ethics Committee of the University of Murcia and was conducted conformed with the ethical standards laid down in the 1964 Declaration of Helsinki.

## Results

Data were preprocessed with R[52], and analyzed by analysis of variance (ANOVA) with JASP 0.9.2[53]. We adopted a significance level of 0.05 for all analyses.

**Psychomotor vigilance task (PVT).** The first trial of each session was considered as practice and discarded. Besides, we considered extreme outliers and discarded reaction times (RTs) shorter than 150 ms or longer than 1,200 ms (0,11% of the data), in addition to those separated by more than six interquartile ranges from the median value of each participant in each session (an additional 0.22% of the data). We employed this lenient trimming procedure because, in order to exploit the additional information that emerges from RT distributions, and taking advantage of the relatively large number of trials per condition, we planned to perform a bin-means analysis[54,55]. Note that, with this kind of analysis, the presence of moderate outliers (as those that could result from fluctuations in alertness during a session) turns out to provide valuable information, rather than compromise data analysis and interpretation.

RTs for each participant in each session were rank ordered and divided into ten bins as equally sized as possible (the number of data per bin ranged from 8 to 11; M = 9.4). Mean RTs were calculated for each bin and submitted to a mixed analysis of variance (ANOVA) with the within-participants factors test time (optimal, non-optimal) and RT bin (1–10), and the between-participants factors chronotype (Morning-types, Evening-types) and order (optimal session first, optimal session second). Main statistical results are presented in Table 1 (rightmost column, bottom row). Neither the main effect of chronotype nor the main effect of order reached statistical significance (both *F*s < 1). However, there was a main effect of test time (the synchrony effect), revealing shorter RTs at the optimal time (M = 300 ms) than at the non-optimal time (M = 319 ms; synchrony effect = 19 ms). There was also an interaction between test time and RT bin, showing that the synchrony effect varied across bins. As illustrated in Fig. 1, the effect increased towards the slower end of the distribution (for bins 1 to 10, synchrony effect = 10, 14, 13, 14, 16, 17, 18, 21, 28, and 41 ms). Finally, there was an interaction between test time, RT bin, and chronotype; and also, between test time, RT bin, and order. To disentangle these interactions and further understand the pattern of results, we performed separate analyses for each chronotype and for each order (see Table 1). Evening-types participants showed synchrony effects that increased at the slower end of the RT distribution and that did not significantly differ as a function of session order (when the optimal session was completed first, synchrony effect for bins 1 to 10 = 8, 13, 12, 14, 19, 23, 25, 33, 50, and 73 ms; M = 27 ms; when the optimal session took place second, synchrony effect = 18, 18, 18, 17, 18, 19, 21, 22, 27, and 42 ms; M = 22 ms). This is the same pattern that was observed for Morning-types participants when the optimal session was completed first (synchrony effect = 9, 19, 20, 22, 23, 24, 26, 34, 40, 68 ms; M = 29 ms). However, the synchrony effect was completely abolished for Morning-types participants when the non-optimal session took place first (synchrony effect = 6, 4, 3, 3, 2, 0, − 1, − 6, − 7, − 24 ms; M = − 2 ms).

The PVT allowed us to evaluate the tonic alert that resulted from the synchrony between participants' chronotype and the time of day in which the task was performed. In addition to this form of tonic alert, the flanker task allowed us to also evaluate the phasic alert generated by the presentation of alerting tones. We also evaluated the interaction between tonic and phasic alert and the modulation of the congruency effect by these two kinds of alerting.

**Flanker task.** We excluded from the RT analysis trials with incorrect responses (2.78% of the data) and trials with RTs separated by more than three interquartile ranges from the median value of each participant in each condition (1.95% of the data). Mean RTs were submitted to a mixed ANOVA with test time (optimal, non-optimal), alerting tone (tone, no tone), and congruency (congruent, incongruent) as within-participant factors;

| Order | Effect | Morning-types | Evening-types | Both groups |
|---|---|---|---|---|
| Optimal session first | Test time | $F(1, 7) = 7.615$<br>$p = 0.028, \eta^2 = 0.521$ | $F(1, 8) = 5.667$<br>$p = 0.045, \eta^2 = 0.415$ | $F(1, 15) = 12.843$<br>$p = 0.003, \eta^2 = 0.461$ |
| | Test time × RT bin | $F(9, 63) = 6.666$<br>$p < 0.001, \eta^2 = 0.488$ | $F(9,72) = 7.194$<br>$p < 0.001, \eta^2 = 0.473$ | $F(9, 135) = 13.220$<br>$p < 0.001, \eta^2 = 0.463$ |
| | Test time × chronotype | – | – | $F < 1$ |
| | Test Time × bin × chronotype | – | – | $F < 1$ |
| Optimal session second | Test time | $F < 1$ | $F(1, 8) = 4.462$<br>$p = 0.06, \eta^2 = 0.358$ | $F(1, 15) = 2.119$<br>$p = 0.166, \eta^2 = 0.105$ |
| | Test time × RT bin | $F(9, 63) = 2.022$<br>$p = 0.051, \eta^2 = 0.224$ | $F(9,72) = 2.221$<br>$p = 0.03, \eta^2 = 0.217$ | $F < 1$ |
| | Test time × chronotype | – | – | $F(1,15) = 3.088$<br>$p = 0.099, \eta^2 = 0.153$ |
| | Test time × bin × chronotype | – | – | $F(9,135) = 4.198$<br>$p < 0.001, \eta^2 = 0.218$ |
| Both orders | Test time | $F(1, 14) = 3.912$<br>$p = 0.068, \eta^2 = 0.169$ | $F(1, 16) = 10.128$<br>$p = 0.006, \eta^2 = 0.386$ | $F(1, 30) = 13.352$<br>$p < 0.001, \eta^2 = 0.272$ |
| | Test time × RT bin | $F < 1$ | $F(9, 144) = 9.171$<br>$p < 0.001, \eta^2 = 0.337$ | $F( 9, 270) = 7.675$<br>$p < 0.001, \eta^2 = 0.154$ |
| | Test time × Order | $F(1, 14) = 5.218$<br>$p = 0.038, \eta^2 = 0.226$ | $F < 1$ | $F(1, 30) = 3.008$<br>$p = 0.093, \eta^2 = 0.061$ |
| | Test time × bin × order | $F(9, 126) = 8.064$<br>$p < 0.001, \eta^2 = 0.353$ | $F(9, 144) = 2.041$<br>$p = 0.039, \eta^2 = 0.075$ | $F(9, 270) = 8.600$<br>$p < 0.001, \eta^2 = 0.172$ |
| | Test time × bin × chronotype | – | – | $F(9, 270) = 2.591$<br>$p = 0.007, \eta^2 = 0.052$ |

**Table 1.** Results of ANOVA tests on the mean reaction time (RT) for the psychomotor vigilance task (PVT).

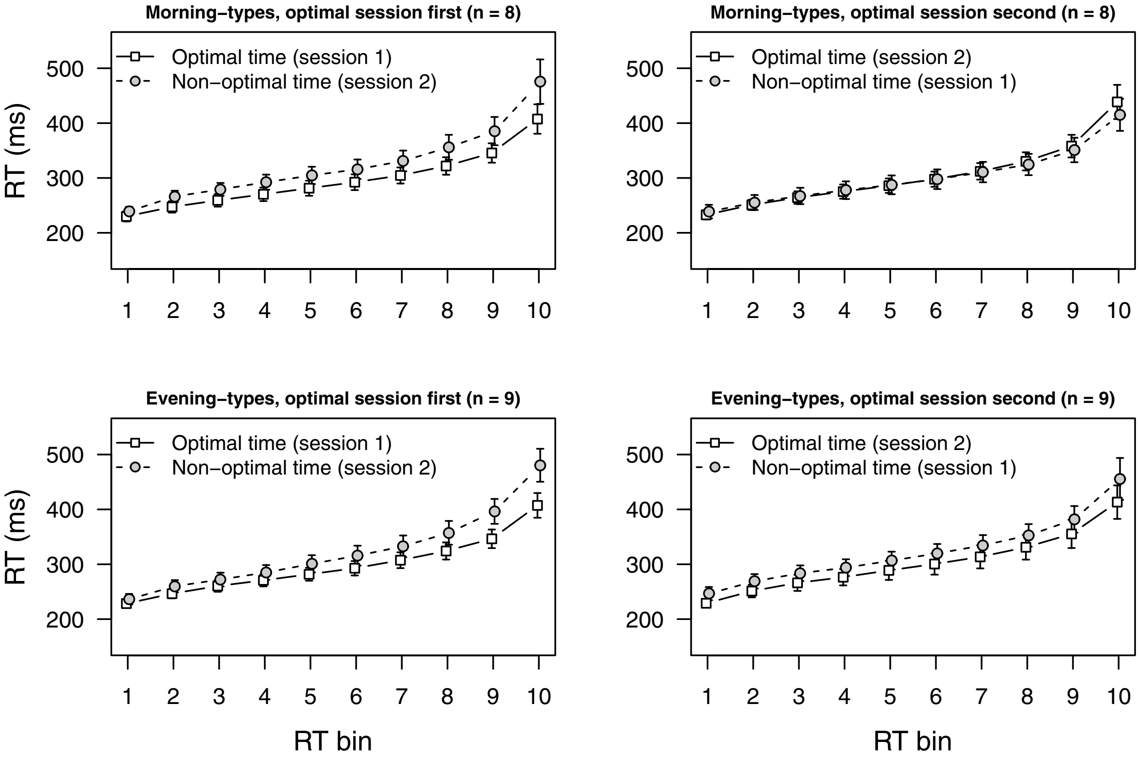

**Figure 1.** Mean reaction time (RT) in the psychomotor vigilance task, PVT (error bars represent standard error of the mean).

and chronotype (Morning-types, Evening-types) and order (optimal session first, optimal session second) as between-participants factors.

There was a main effect of test time, $F(1, 30) = 8.212$, $p = 0.008$, $\eta^2 = 0.169$, revealing that RTs were shorter at the optimal time (M = 394 ms) than at the non-optimal time (M = 412; synchrony effect = 18 ms). However, this effect was not equivalent for Morning-types and Evening-types participants, as revealed by the interaction

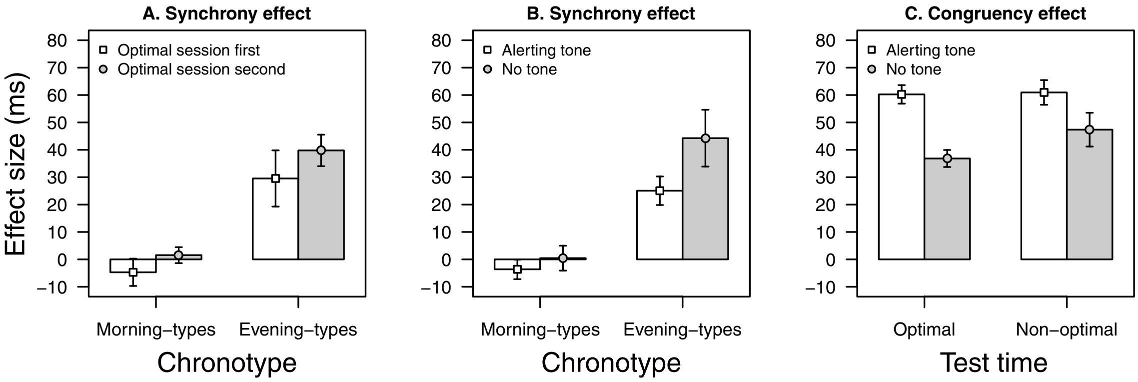

**Figure 2.** (**A**) Synchrony effect (RT at the non-optimal time minus RT at the optimal time) as a function of chronotype and order. (**B**) Synchrony effect as a function of chronotype and alerting tone. (**C**) Congruency effect (RT in the incongruent condition minus RT in the congruent condition) as a function of alerting tone and test time.

between test time and chronotype, $F(1, 30) = 9.864$, $p = 0.004$, $\eta^2 = 0.203$. In fact, only the Evening-types participants showed a significant effect of test time (synchrony effect = 35 ms), whereas this effect was completely abolished in the Morning-types group (synchrony effect = − 1.6 ms). As illustrated in Fig. 2A, the synchrony effect was numerically greater when the optimal session took place second than when the optimal session was completed first, in contrast to what was observed with the PVT. However, this tendency was not statistically significant (for both the test time × order interaction and the test time × chronotype × order interaction, $F < 1$).

In addition, we found a statistically significant interaction between test time and alerting tone, $F(1, 30) = 4.653$, $p = 0.039$, $\eta^2 = 0.119$, revealing greater synchrony effect in the no-tone condition than in the tone condition (synchrony effect = 24 and 12 ms, respectively). An inspection of Fig. 2B suggests that this interaction was mainly consequence of Evening-types participants' performance. In fact, for Morning-types participants just a main effect of alerting tone was observed, $F(1, 14) = 31.399$, $p < 0.001$, $\eta^2 = 0.684$, that is, RTs were shorter with the tone than when the tone was not presented. However, for Evening-types participants we also observed a main effect of alerting tone, $F(1, 16) = 23.093$, $p < 0.001$, $\eta^2 = 0.586$ but test of time and alerting tone interacted in this group of participants. Although admittedly the interaction was only marginally significant, $F(1, 16) = 4.111$, $p = 0.060$, $\eta^2 = 0.195$ it is worth noting that the alerting tone reduced the differences between the optimal and non-optimal time of day by almost half (synchrony effect = 25 ms) in comparison to when the alerting tone was absent (synchrony effect = 44 ms).

Finally, there was statistically significant main effects of congruency, $F(1, 30) = 233.828$, $p < 0.001$, $\eta^2 = 0.884$ and alerting tone, $F(1, 30) = 49.415$, $p < 0.001$, $\eta^2 = 0.614$. RTs were shorter in the congruent condition (M = 377 ms) than in the incongruent condition (M = 418 ms; congruency effect = 51 ms); and RTs were also shorter in trials with alerting tone (M = 387 ms) than in trials with no tone (M = 418; alerting tone effect = 31 ms). However, the congruency × alerting tone interaction was statistically significant, $F(1, 30) = 33.400$, $p < 0.001$, $\eta^2 = 0.515$ because the congruency effect was greater in the tone condition (congruency effect = 61 ms) than in the no tone condition (congruency effect = 42 ms). Importantly, however, the congruency × test time interaction did not reach statistical significance, $F(1, 30) = 1.934$, $p = 0.175$, $\eta^2 = 0.055$ suggesting equivalent congruency effect at the optimal time (congruency effect = 49 ms) than at the non-optimal time (congruency effect = 54 ms; Fig. 2C).

## Discussion

Three main objectives were addressed in the present study regarding the role of chronotype in attention-related tasks. We first asked whether synchrony effects, that is, the difference in performance between the optimal and non-optimal time of day as a function of chronotype, are observed in two tasks that differently involve the endogenous component of the alerting network, the PVT and the flanker tasks. The second objective was to explore the role of phasic alerting as a function of chronotype and time of testing when participants performed the flanker task. In the final objective we assessed the role of the two components of the alerting network in executive attention-dependent conflict resolution, and whether chronotype and time of testing had any modulatory effect.

**The role of chronotype in attention tasks.** The first task, the PVT, requires vigilant attention that is activated when rather monotonous tasks are performed during long periods of time. Our results showed that when participants performed the PVT at the non-optimal time of day reaction times to targets were longer than when participants performed the task at the optimal time of day[25,56]. This synchrony effect increased towards the slower end of the RT distribution, which suggests that the effect largely emerged from more frequent or extreme fluctuations of attention at the non-optimal time of day. Besides, the synchrony effect was modulated by the first-experience-with-task, but very especially for the Morning-types participants. Specifically, whereas Evening-types participants showed synchrony effects irrespective of whether the task was performed first at either the non-optimal or optimal time of day, Morning-types participants showed synchrony effects only when they performed the task first at the optimal time of day. When they performed the task first at the non-optimal time of day, the synchrony effect vanished away, being performance at their non-optimal time of day similar to that observed at their optimal time of day. This novel result suggests that novelty of the task produced an increment in the level of alerting that overcame the deleterious effect of poor vigilant attention characteristic of the non-optimal time of day, but that this novelty effect only occurred or was much more pronounced for Morning-types chronotype. Consequently, the novelty effect fully compensated the fact of being at the non-optimal time of day for Morning-types participants, but not for Evening-types participants. These results extend what is usually observed when novel objects or locations are presented along task performance[27] to the task as a whole when it is presented for the first time under low vigilant attention conditions.

As in the PVT, Evening-types participants showed reliable synchrony effects in the flanker task irrespective of whether first-experience-with-task took place at the non-optimal or at the optimal time of day. Morning-types participants, however, did not show any synchrony effect in the two session, and first-experience-with-task did not have any modulation effect like that observed with the PVT. This unexpected pattern of results showed by Morning-types participants across tasks deserves further discussion. One possible explanation for these results is that Morning-types individuals are, in general, more sensitive than Evening-types individuals to certain factors potentially capable of increasing alerting levels at their non-optimal time of day. The observed difference between the PVT and the flanker tasks may emerge from the fact that the flanker task itself fosters sustained attention due to the high level of cognitive demands of dealing with conflicting information (the flankers), having to choose between two possible responses, and monitoring errors, whereas the PVT does not contain strong perceptual, cognitive, or motoric features that promote high level of attention. In this situation, Morning-types participants could saw their low vigilance level at the non-optimal time of day compensated by task-novelty in the PVT. In the flanker tasks, however, they reached appropriate levels of sustained attention because the high cognitive demands of the task, regardless of the session in which the task is performed.

It is not clear though, why only Morning-types participants benefited from task novelty (PVT) and cognitive demands (flanker task) to increase their alerting levels at the non-optimal time of day.

### Interaction between the two components of alerting in the flanker task.

With the flanker task we also asked about the role of the exogenous component of alerting in performance as a function of chronotype and time of testing. The reduction in RTs when the alerting tone was present compared with when it was absent was observed at both the optimal and non-optimal times of day, and for both Morning- and Evening-types participants. However, in line with the previous contention, it was expected that phasic alerting compensates for the deleterious effect of reduced vigilance in the non-optimal time of day, mainly in Evening-types participants. Whereas Morning-types participants showed faster responses with warning tone present (phasic alerting effect) irrespective of time of testing, Evening-types participants showed more effect of phasic alerting when testing occurred in the non-optimal than in the optimal time of day.

These results comply with the suggestion that the phasic component of alerting, when activated, reduces the demands on the endogenous component. Let us illustrate that contention with some examples. In a fMRI study, O'Connor et al.[57] used the SART, a task that activates the right fronto-parietal network as well as the thalamus. When participants performed the SART with auditory alerting tones being randomly presented, activation in the right DLPFC was absent. Thus, the activation of the alerting network by exogenously presented alerting tones seems to reduce the need of top-down modulation of the endogenous alerting component mediated by the right DLPFC. Also, the compensatory role of phasic alerting illustrated here, may have relevant consequences in pathology. For instance, ascending thalamic projections to the parietal lobe characteristic of phasic alerting can ameliorate parietal lobe-dependent orienting deficits shown by left-side neglect[45,46]. Lewy Bodies dementia patients showed a deficit in orienting attention, which was regulated by the presence of an alerting tone[58]. Mild cognitive impairment patients showed their conflict effect restored up to the level showed by healthy control participants when the alerting tone was present, compensating for the deficit of such patients in keeping an adequate level of tonic alertness thorough the task[59]. Finally, the fact that RTs were faster even when participants performed the task in the optimal time of day, when vigilant attention levels are high, leads us to conclude that phasic alert plays a role beyond the mere compensatory effect observed in conditions of low vigilance. Phasic alerting is thought to activate initial phases of response initiation[60], accelerating responses sometimes at expenses of accuracy[61].

### Effects of alerting on conflict resolution: the role of chronotype and time of testing.

A final goal was to determine the role of the two components of the alerting network in executive attention-dependent conflict resolution, and whether chronotype and time of testing have any modulatory effect. As previously found, the endogenous component of alerting did not influence the congruency effect at all[46]. Tonic alertness, sustained throughout the performance of a cognitive demanding task, was sufficient to achieve an appropriate functioning of the executive attention network involved in conflict monitoring and resolution[6], so that any further increment in vigilance, as expected in the optimal time of day, did not add any efficiency in such network functioning. Regarding the exogenous component of alerting, our results replicate those of previous studies that found an increment in the congruency effect with the alerting tone present. If phasic alerting had produced a deactivation of some components of executive attention, the increment of vigilance level usually observed in the optimal time of day, should have compensated for such reduced activity in conflict-based brain areas also involved in the vigilant attention network (e.g., ACC and right DLPFC)[8]. The fact that such deleterious effect of phasic alerting on conflict resolution was observed irrespective of participants' chronotype and time of testing goes against a negative relationship between the alerting and the executive control networks. These results fit better with an account based on phasic alerting prioritizing spatial attention in the visual field[34]. The alerting tone might have expanded the focus of attention, enhancing processing of flankers and therefore fostering response competition with the target. As a result, the conflict effect increased. This account agrees also with the alerting tone enhancing attention orientation triggered by peripheral cues in healthy younger adults[37].

Briefly, the exogenous and endogenous components of the alerting network interact with each other, so that phasic alerting compensates for reduced activation of the endogenous component due either to low vigilance level dependent on circadian phase, or to pathology affecting the vigilant attention network. However, executive attention-dependent conflict resolution in cognitive demanding tasks, is not affected by the endogenous component of alertness, but it is seriously compromised when the exogenous component of alerting is transiently activated, irrespective of participants' chronotype and time of testing.

**Limitations and future directions.** In line with previous related research, here we have observed that the decline in performance at sub-optimal times impacts more in Evening-types individuals than in Morning-types ones[25], maintaining the latter more stable performance over longer periods of time at any time of day[62]. We still lack a convincing account for why Morning-types participants are more sensitive than Evening-types participants to some characteristics of the attentional tasks (novelty, cognitive demands) that compensate for their low alerting level at the non-optimal time of day. Although the number of participants in the present study is well within the range of previous related studies or even larger (see[25]) we acknowledge that insufficient power might have mainly affected our analysis of novelty effects, for which very small samples of participants in each first-experience-with-task condition were tested. Future research should address such an important issue with larger samples. Other issue is whether the pattern of results observed with extreme chronotypes generalizes to individuals of the Neither-types, which represent the 60% of the adult population[24] or to individuals of different sex and age. For instance, during adulthood, females tend to be more morning-oriented than men[63]. Regarding age, it is well known that preadolescents and old people tend to be more morning-oriented than adolescents and young people, who are more evening-oriented[64]. Young people tend to compensate during the weekends for their sleep debt accumulated during the schooldays, producing a rather irregular sleep–wake cycle pattern, which might lead them to show misalignment between their biological and social time (social jet-lag)[65]. We suggest that the irregular circadian rhythm shown by Evening-types students attending morning classes, might affect their sleep quality, and consequently a disadvantage when testing happens in their non-optimal time of day. In contrast, Morning-types participants may show more alignment between their internal clock (circadian rhythm) and academic activities, usually starting early in the morning, rendering them more efficient in their cognitive and academic performance. Accordingly, we hypothesize that in the present study, even when Evening-types participants did not show any difference regarding sleep hours in comparison with Morning-types participants, they usually report some difficulty in waking up early to attend morning classes, which might affect sleep quality[66], compromising academic achievement[67–69]. Future studies should be conducted to determine whether the effects of the alerting system on cognitive control as a function of chronotype generalize to individuals with either extreme or intermediate chronotypes that differ in sex and age.

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## Author contributions

V.M.P.: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original draft preparation. L.B.P.: Conceptualization, Investigation, Writing- Original draft preparation. GC: Conceptualization, Methodology, Formal analysis, Writing-Original draft preparation, Writing-Reviewing and Editing. L.J.F.: Conceptualization, Methodology, Writing-Original draft preparation, Writing-Reviewing and Editing, Funding acquisition.

## Competing interests

The authors declare no competing interests.

## Additional information

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## Study 2:

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**Abstract**:

**Background:** In this study we assessed the effects of progesterone on vigilance tasks that require sustained attention. In contrast to previous research, we differentiated two components of vigilance: the exogenous component, involved in monotonous and tedious tasks such as the Psychomotor Vigilance Task (PVT); and the endogenous component, involved in tasks that require cognitive control such as the Sustained Attention to Response Task (SART).

**Methods:** A sample of 32 female participants differing in extreme chronotypes were tested at their optimal and non-optimal time-of-day, as secretion of sex hormones follows biological rhythms. Ovulation tests that measure the presence of luteinizing hormone (LH) in urine were used to minimize methodological errors. Women of Morning-type or Evening-type chronotypes completed 4 experimental sessions of the two attentional tasks when they were in their follicular (low progesterone level) and mid-luteal (high progesterone level) phases, both in the morning (8:00 AM) and the evening (8:30 PM).

**Results:** Compared with the follicular phase, performance in the mid-luteal phase improved in the Morning-type participants and worsened in the Evening-type

participants. This pattern of results was observed only when testing occurred at the optimal time-of-day and with both the PVT and the SART tasks.

**Conclusion:** These results suggest that the simultaneous presence of both progesterone and cortisol at 8:00 AM may explain the benefit observed in Morning-type females. In contrast, the low concentration of cortisol along with the reduced benefit of mid-luteal phase progesterone in the evening may account for the worsening in performance observed in Evening-type females.

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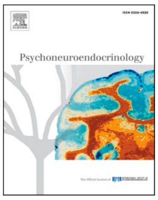

# Mid-luteal phase progesterone effects on vigilance tasks are modulated by women’s chronotype

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A R T I C L E  I N F O

*Keywords:*
Vigilance tasks
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A B S T R A C T

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## 1. Introduction

Cognitive performance involves many different processes associated to specific psychological functions, and the efficiency of such processes may depend on variations in the pre-existing state of the organisms (see Colzato et al., 2020). Here we will focus on how the level of some sexual hormones usually observed along the menstrual cycle, concretely progesterone, influence women's performance when they perform some vigilance tasks that require sustained attention.

Progesterone is crucial in the implantation of the fertilized ovocyte and its receptors are distributed among areas such as amygdala, hippocampus, hypothalamus, thalamus and frontal cortex (Kato et al., 1994; Guerra-Araiza et al., 2000, 2002, 2003). In these regions, progesterone binds to receptor membrane component-1 (PGRMC1) (Intlekofer and Petersen, 2011). Once bound to its receptor, it induces rapid non-genomic changes. Metabolization of progesterone produces neuroactive steroids such as pregnanolone and allopregnanolone, which in turn stimulate GABA receptors, related to the excitation/inhibition balance of brain regions (Inghilleri et al., 2004; Smith et al., 2002) and thus to the modulation of cognitive function (Sundström Poromaa and Gingnell, 2014). However, the influence of sexual hormones on attentional tasks is particularly sparse (Pletzer et al., 2017). Moreover, it must be taken into account that attention is not a unitary concept and several attentional functions have already been dissociated at both the behavioral and neural levels (Posner and Petersen, 1990).

Importantly, most attentional functions seem to be fostered in the mid-luteal phase, when the progesterone level is at its peak and cortical inhibition is at its maximum. For example, enhancement has been observed in tasks that require spatial attention (Brötzner et al., 2015), decision-making (Solis-Ortiz et al., 2004), inhibition (Lord and Taylor,

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1991), vigilance (Vidafar et al., 2018), and conflict resolution (Cohen et al., 2019). All of these attention-based processes require the ability to maintain attention on task over time, an ability that has been termed sustained attention or vigilance (Davies and Parasuraman, 1982). From a neuroanatomical point of view, vigilant attention comprises a network of right-lateralized cortical areas involving the anterior cingulate cortex (ACC), the dorsolateral prefrontal cortex (DLPFC), and the right inferior parietal lobe (Sturm and Willmes, 2001; see Robertson and O'Connell, 2010, for a review). There is some consensus regarding the effects of progesterone on sustained attention tasks, suggesting a possible facilitatory effect on any task that directly involves the prefrontal cortex (Solis-Ortiz et al., 2004), where, as previously mentioned, there are many progesterone receptors that modulate activity of that particular brain region. Some researchers have shown an improvement in performance when the tasks were performed during the luteal phase (*e.g.*, Solís-Ortiz and Corsi-Cabrera, 2008), while others showed such improvement in the follicular phase, when the level of progesterone is low (*e.g.*, Matthews and Ryan, 1994; Pletzer et al., 2017). Both theoretical views on vigilant attention and procedural differences between studies may underlie these discrepancies.

Regarding the concept of vigilance, there are two mechanisms that could be involved when people have to maintain attention on the task over time. The activation of these mechanisms depends on the cognitive demands required by the task at hand. When the cognitive demands are low and the task is rather monotonous and tedious, an *arousal vigilance* state would be activated to maintain attention for the duration of the task. In contrast, when the nature of the task is more difficult and involves high-level cognitive resources, a type of *executive vigilance* is then activated (Luna et al., 2018; Martínez-Pérez et al., 2020, 2021). To our knowledge, this distinction regarding the components of vigilance has not been taken into account when researchers have investigated the effects of the menstrual cycle on attentional tasks.

Regarding procedural differences in determining the phase throughout the menstrual cycle, there is broad agreement that cycle phases are determined as a function of some hormones of interest. However, there is no specific agreed protocol for assessing menstrual cycle influences on cognition and, therefore, any comparison between the results of different studies is rather problematic (Pletzer et al., 2017; Sundström Poromaa and Gingnell, 2014). In this regard, the way these hormones are measured has to be distinguished. For example, the amount of hormones can be determined directly by capturing the peak of luteinizing hormone (LH) through ovulation testing, or indirectly as when estimating the early follicular phase, characterized by low sex hormone levels, without necessarily measuring them. The use of a marker beyond the menstrual cycle recording itself is highly recommended (*e.g.*, Becker et al., 2005).

Finally, there are other important variables that modulate women's performance in relation to the menstrual cycle that are often not taken into account. It is well known that hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes interact. For example, a stress-induced increase in glucocorticoids secretion leads to hypothalamic suppression of GnRH-secreting neurons, which supresses reproduction (Kirby et al., 2009). Likewise, the interplay between the axes has been used to explain concepts of social dominance or competitiveness (DHH: dual hormone hypothesis) in relation to both testosterone (Mehta and Josephs, 2010) and estradiol (Tackett et al., 2015). Importantly, progesterone exerts hyperactivation effects on the HPA axis (Henderson, 2018), culminating in cortisol secretion (Goldstein et al., 2005; Keller-Wood, 1998; Roca et al., 2003). Thus, an interaction between the day of the menstrual cycle when the level of progesterone increases (*i.e.*, the mid-luteal phase) can directly impact cortisol levels, which also increase in the early morning. The interaction between these two substances would also influence participants' performance. Another variable relates to the time-of-day when hormone assessment is performed (Cohen et al., 2019). The secretion of female sex hormones follows biological rhythms (Becker et al., 2005) and links the menstrual cycle to circadian rhythmicity. Circadian rhythms include a set of variables that vary according to a 24-hour daily cycle. This endogenous biological clock works in synchrony with external signals to determine individuals' time preferences for daily activities and sleep. These preferences allow individuals to be classified into Morning-types, Evening-types, or Neither-types, which is the definition of chronotype (Levandovski et al., 2013; Schmidt et al., 2007). The chronotype paradigm is frequently used for studying the influence of circadian rhythms on higher order cognitive processes (Schmidt et al., 2007). Given the interaction between the HPA and HPG axes, the use of the chronotype paradigm could be useful to study the modulation of the menstrual cycle by circadian rhythms. In addition, it also takes into account the time-of-day when the assessment is performed. Previous research on the cognitive domain has found differences in performance depending on the time of testing according to the chronotype of the participants. Thus, Morning-types perform more efficiently during the early hours of the day, while their performance decreases in the afternoon. On the contrary, Evening-types have their optimal time in the afternoon, while their worst performance occurs in the morning. Neither-types refer to individuals who do not develop any marked time preference for either performance or rest (Schmidt et al., 2007). The interaction between chronotype and time of testing determines the synchrony effect (May and Hasher, 1998). The synchrony effect has previously been reported in tasks that require sustained attention, and Morning-types tend to be less affected by the time of testing than Evening-types (Mongrain et al., 2008; Adan et al., 2012).

In the present study, a sample of female participants performed two sustained attention tasks that differed in the vigilance component involved. The tests took place at their optimal and non-optimal time-of-day according to their chronotype. Recent studies (*e.g.*, Lara et al., 2014; Martínez-Pérez et al., 2020, 2021, 2022) have found the Psychomotor Vigilance Task (PVT; Dinges and Powell, 1985) to be an adequate test of arousal vigilance, whereas the Sustained Attention to Response Task (SART; Robertson et al., 1997) is an adequate test of executive vigilance. The main aim of the present study was to determine whether performance in these vigilance tasks was differently affected by progesterone level across different phases of the menstrual cycle, as a function of women's chronotype and time of testing. Two indices were used to estimate phase: an indirect index (menstruation) for the follicular phase; and a direct index, luteinizing hormone (LH) using ovulation tests for the mid-luteal phase. Given that progesterone has been shown to increase attentional capacity (Brötzner et al., 2015; Cohen et al., 2019; Lord and Taylor, 1991; Solis-Ortiz et al., 2004; Solís-Ortiz and Corsi-Cabrera, 2008; Vidafar et al., 2018), we hypothesize that it will act as an alertness activator. Thus, performance is expected to be optimized in the mid-luteal phase compared to the follicular phase. However, it is possible that this performance-enhancing effect may be modulated by chronotype and time of testing, since as we mentioned above progesterone and cortisol secretion, as well as how they interact to affect cognitive performance, will depend on the timing of the test, differentially affecting Morning-type and Evening-type participants.

## 2. Methods

### 2.1. Participants

Thirty-two female undergraduate students from the University of Murcia (M age = 19.75, SD age = 1.57) participated in our experiment for course credit. Recruitment of participants was as follows. First, we made a selection of participants from an available database of previous chronotype studies. From this database we selected 83 potential female participants with extreme chronotypes (42 Morning-types; 41 Evening-types) classified according to the reduced Spanish version of the Horne and Östberg's Morningness-Eveningness Questionnaire (rMEQ) developed by Adan and Almirall (1990) and whose scores ranged from 4 (definitely Evening-types) to 25 (definitely Morning-types). Participants who scored between 17 and 25 (M = 18,4) formed the Morning-type group, and those who scored between 4 and 11 (M = 8,6) formed the Evening-type group.

Fifty-six potential participants who attended the meeting, where the requirements for participation were explained, filled out a questionnaire that included data on their last three menstrual periods (dates, regularity and total duration) and contraceptive use. All those on contraceptive treatment were excluded and all those with natural menstrual cycles between 28 and 32 days and with a certain regularity were invited to participate. Based on these criteria, only 27 women were selected. Subsequently, we made a second call for participants following the same procedure described above and selected a total of eight additional participants, four Morning-types and four Evening-types. The COVID-19 pandemic forced us to interrupt the experimental sessions, so only 6 participants completed the experiment before lockdown. The study continued after the confinement and we interviewed another 46 potential participants. Based on their scores on the rMEQ questionnaire and menstrual cycle regularity, the sample consisted of 32 women, 16 Morning-types and 16 Evening-types. However, data from one of the Morning-type participants at optimal and non-optimal time-of-day just during the mid-luteal phase could not be collected because she tested positive for coronavirus and dropped out of the study. Consequently, the final sample consisted of 31 participants.

A post-hoc power (1- β) analysis was performed using G*Power to detect a medium effect size of $f = .25$ at $\alpha = .05$, with a final sample of 31 participants, two groups, four assessments per participant, and given a repeated measures correlation of $r = .5$. The resulting statistical power was .91.

All participants reported the absence of mental or physical illness, as well as being under psychological or pharmacological treatment at the time of testing. They also declared having normal or corrected-to-normal vision and not suffering from any chronic disease. Written informed consent was obtained from all participants.

### 2.2. Tasks

Participants were tested individually in sound-attenuated booths. The two tasks were programmed in E-Prime 3 (Psychology Software Tools; Schneider et al., 2012). The visual stimuli were presented on a 22” TFT monitor with a screen resolution of 1920 × 1080 pixels. A Chronos® device with five buttons was used to collect responses. In the PVT, each trial began with a random interval of between 2 and 10 s in which the computer screen remained black. Then, a red circle (50 pixels in diameter) appeared in the center of the screen and participants had to press, as quickly as possible, the center button of the response box with the index finger of their dominant hand. Once the response was made, the screen went blank and a new trial began. All participants were instructed to respond as quickly as possible in all conditions. In the SART, a go/no-go paradigm, individuals' ability to retain a response to an infrequent target digit is assessed. Digits from 1 to 9 were presented and participants were required to respond by pressing the center button of the response box with the index finger of their dominant hand, except when the target digit “3” appeared (Robertson et al., 1997). Each of the 9 digits was displayed 25 times for 250 ms, so that the total number of stimuli presented was 225. After the presentation of each digit, a mask appeared for 900 ms. The mask consisted of a circle with a diagonal cross in the center. Both the digits and the mask appeared in the center of the screen in white on a black background. In addition, the digits were presented in 5 different fonts: 48, 72, 94, 100 and 120 points. The interval between the digits was 1150 ms. Participants were asked to respond as quickly as possible trying not to make mistakes.

### 2.3. Procedure

Participants completed a total of 4 experimental sessions. Regarding the menstrual cycle, they came to the laboratory during the early follicular phase (1–3 days of the cycle) and during the mid-luteal phase (approximately on the 21st day of the cycle). In addition, the experimental sessions for both phases were scheduled at 8:00 AM and 8:30 PM. Accordingly, each participant was examined 4 times, during the early follicular phase in the morning; in the early follicular phase in the afternoon; in the mid-luteal phase in the morning; and in the mid-luteal phase in the afternoon. During the experimental sessions, participants completed the two attentional tasks. They first performed the PVT, and then the SART. The order in which participants performed the attentional tasks was counterbalanced for menstrual cycle phase (follicular, mid-luteal) and the time-of-day (morning, evening), resulting in 4 experimental conditions: (1) follicular phase/morning first – mid-luteal phase/afternoon after; (2) follicular phase/afternoon first – mid-luteal phase/morning after; (3) mid-luteal phase/morning first – follicular phase/afternoon after; (4) mid-luteal phase/afternoon first – follicular phase/morning after. Eight participants from both chronotypes were initially randomly assigned to each experimental condition.

Participants contacted the experimenter by e-mail on the same day as the onset of menstruation. The tests then proceeded as follows. For the follicular phase, participants came to the laboratory between days 1 and 3 of the cycle and completed the first two experimental sessions. Based on the onset and duration of menstruation, the probable day of ovulation was estimated and corroborated with DIAGNOS Ovulation (LH) Test Strips (Manufacturer: Cuckool, ref.: 74t5486gg-jj 197) which have a measurement accuracy of 99%. The main advantage of this test it that it detects the presence of LH in urine and thus the presence of ovulation. It allows researchers to more accurately define the phase of the woman's cycle and thus to determine the days on which cognitive assessment should be performed.

Ovulation tests were carried out between 12:00 and 13:00 h on the scheduled day, following the manufacturer's instructions. Once in the laboratory, participants were given a bottle to collect urine for analysis. A positive result meant that ovulation should occur within 24–48 h. The mid-luteal phase was expected to occur about 6–7 days after the time interval when ovulation was supposed to have occurred, so participants were invited to come to the laboratory on those days. It is known that the mid-luteal phase is stable and that its duration is approximately 14 days (Lenton et al., 1984). Thus, it is possible to confirm that the participant was in that phase once the exact day on which she started menstruating again was known. Accordingly, we asked participants to inform us about the onset of their next menstruation to confirm that the evaluation had occurred in their mid-luteal phase. In case the ovulation test was negative, the test was continued to be administered during the following days until a positive result was obtained. As Sundström Poromaa and Gingnell (2014) point out, a single administration of the test is not sufficient to determine the phase of a woman's menstrual cycle.

Once the participant was summoned, she had to come to the laboratory either in the morning and then in the afternoon, or in the afternoon and then in morning of the following day, depending on the experimental condition to which she had been assigned. This procedure allowed us to assess participants at their optimal and non-optimal time-of-day according to their chronotype. Participants were asked not to drink coffee or other stimulants at least 2 h before the start of the experimental tasks. They were also asked to try to get between 6 and 9 h of sleep the night before. All participants complied with these requirements.

### 2.4. Statistical analysis

Data were pre-processed with R software, (R Core Team, 2017) and analyzed with JASP .9.2 (JASP Team, 2019). Also, outliers were considered to be all reaction times (RTs) that after logarithmic transformation were separated by more than four semi-interquartile ranges from the median value. We adopted a statistical significance level of $\alpha = .05$ for all statistical analyses. Data were entered into three-ways mixed 2 (chronotype) × 2 (phase) × 2 (time-of-day) ANOVAs, separate for each task. In the PVT, transformed means of RTs were considered the dependent variable. In the SART, the dependent variables were transformed means of RTs and accuracy on go trials, and percentage of non-responses on no-go trials. When an interaction probed statistically significant, further simple main effects were carried out through either paired (between-participants factor) or unpaired (within-participants factor) *Student's t* tests, because none of such analyses required the comparison of more than two groups/conditions.

## 3. Results

The results of the two attentional tasks in the different experimental conditions are shown in Table 1.

### 3.1. Menstrual cycle statistics

All participants reported having regular natural menstrual cycles ($M = 28.02$, $SD = 1.29$). In addition, the duration of their three menstrual cycles prior to the experiment was recorded ($M = 28.68$, $SD = 2$). The difference between the reported and recorded duration was not statistically significant, $t(29) = 1.36$, $p = .19$ The correlation between the two scores was statistically significant ($r = .58$, $p < .01$. As mentioned above, women were examined in two phases of their menstrual cycle: early follicular phase and mid-luteal phase. The mean cycle days for data collection in the early follicular phase was 3.06 ($SD = 1.32$). The mean cycle days for data collection in the mid-luteal phase was 20.43 ($SD = 2.80$). The date predicted by a positive ovulation test result was, on average, around day 13.47 ($SD = 2.85$) of the cycle. The mean number of days elapsed between the mid-luteal phase and the next menstruation was 7.83 days on average ($SD = 1.83$), confirming that the tests were performed at the appropriate time.

### 3.2. Psychomotor Vigilance Task (PVT)

The first trial of each session was considered as practice and subsequently discarded. Transformed means of RTs were calculated and subjected to a mixed analysis of variance (ANOVA) with phase (follicular, mid-luteal) and time-of-day (optimal, non-optimal) as within-participants factors, and chronotype (Morning-types, Evening-types) as the between-participants factor. The main effect of time-of-day was statistically significant, $F(1, 29) = 11.05$, $p < .002$, $\eta_p{}^2 = .28$, indicating faster RTs at the optimal time ($M = 283$ ms) than at the non-optimal time ($M = 292$ ms) for all participants. The synchrony effect was modulated by the significant phase × time-of-day × chronotype interaction, $F(1, 29) = 4.85$, $p = .036$, $\eta_p{}^2 = .14$. We further analyzed the interaction separately for each chronotype (see Fig. 1). For the Morning-type participants, the synchrony effect was observed only in the mid-luteal phase, $t(14) = 2.06$, $p = .059$, but not in the follicular phase, $t(14) = .56$, $p = .58$. For the Evening-type participants, the synchrony effect was observed only in the follicular phase, $t(15) = 3.68$, $p = .002$, but not in the mid-luteal phase, $t(15) = 1.19$, $p = .25$. The three-way interaction also revealed an interesting pattern of opposite results when we compared performance between the two phases at the optimal time-of-day as a function of participants' chronotype. Compared to performance in the follicular phase, the mid-luteal phase tended to produce shorter RTs in the Morning-types, although the difference was not statistically significant, $t(14) = 1.15$, $p = .27$), but longer RTs in the Evening-types, the difference being statistically significant, $t(15) = 2.65$, $p = .018$.

### 3.3. Sustained Attention to Response Task (SART)

Trials in experimental blocks in which participants did not respond to a go trial or emitted a response on no-go trials when the target digit was presented were excluded from statistical analyses. Transformed means of RTs and accuracy on go trials, and percentage of non-responses on no-go trials were subjected to a mixed analysis of variance (ANOVA) with phase (follicular, mid-luteal) and time-of-day (optimal, non-optimal) as within-participants factors, and chronotype (Morning-types, Evening-types) as the between-participants factor.

For the go trials, in the RTs analysis we found only a marginally significant main effect of chronotype, $F(1, 29) = 3.90$, $p = .058$, $\eta_p{}^2 = .12$. Morning-types tended to show longer RTs (292 ms) than Evening-types (227 ms). In the analysis of accuracy we found only a main effect of time-of-day, $F(1, 29) = 4.85$, $p = .036$, $\eta_p{}^2 = .14$. Participants showed higher accuracy at the optimal time (98%) than at the non-optimal time (97%). For no-go trials, the nonresponse percentages are illustrated in Fig. 2. The chronotype × time-of-day interaction reached statistical significance, $F(1, 29) = 8.34$, $p = .007$, $\eta_p{}^2 = .22$. No other main effect or interaction was significant. The interaction was further analysed for each chronotype separately. For the Morning-types, the synchrony effect was observed, *i.e.*, higher accuracy at the optimal time (70%) than at the non-optimal time (64%), $F(1, 14) = 4.61$, $p = .05$, $\eta_p{}^2 = .25$. Although phase did not interact with time-of-day, it is noteworthy that the synchrony effect was significant only in the mid-luteal phase (8.4% of increment; $t(14) = 2.98$, $p = .01$, but not in the follicular phase (3.5% of increment), $t(14) = .81$, $p = .43$ For the Evening-types we observed no significant effects (all $ps > .05$). An inspection at the optimal time-of-day (see Fig. 2) reveals that, as with the PVT, an opposite pattern of results is also observed when comparing the two phases. Compared to the follicular phase, mid-luteal phase accuracy increased in the Morning-types, $t(14) = 1.89$, $p = .08$, but showed a slight decrease in the Evening-types, although not statistically significant, $t(15) = .91$, $p = .38$

Finally, a correlation between participants' speed on go trials and non-response accuracy on no-go trials showed that Morning-types were slower but more accurate in retaining responses to the target digit, $r = .90$, $p < .001$ whereas Evening-types showed the opposite pattern, $r = .92$, $p < .001$

## 4. Discussion

We conducted the present study to determine whether progesterone, a sex hormone that varies according to the phase of the menstrual cycle in women, has an effect on vigilance tasks that require sustained

**Table 1**
Mean RTs (in ms) in the Psychomotor Vigilance Task; mean RTs (in ms) and accuracy (in percentages) on go trials, and accuracy in retaining responses (in percentages) on no-go trials in the Sustained Attention to Response Task (SART), as a function of menstrual cycle phase (follicular, mid-luteal) and time-of-day (optimal, non-optimal) for both Morning-type and Evening-type chronotypes. Standard deviations are shown in parentheses.

| Menstrual cycle phase and Time-of-day | Morning-types | | | | Evening-types | | | |
|---|---|---|---|---|---|---|---|---|
| | PVT | SART | | | PVT | SART | | |
| | Mean RTs | Mean RTs | Accuracy go trials | Accuracy no-go trials | Mean RTs | Mean RTs | Accuracy go trials | Accuracy no-go trials |
| **Follicular** Optimal | 294(29) | 293(99) | 99(1) | 67(24) | 270(19) | 224(91) | 97(4) | 55(23) |
| Non-optimal | 297(33) | 272(103) | 96(7) | 63(29) | 284(22) | 230(92) | 98(2) | 56(22) |
| **Mid-luteal** Optimal | 289(32) | 302(108) | 99(1) | 73(21) | 281(29) | 221(86) | 95(6) | 52(22) |
| Non-optimal | 300(42) | 302(109) | 99(1) | 65(25) | 287(24) | 232(85) | 97(7) | 57(21) |

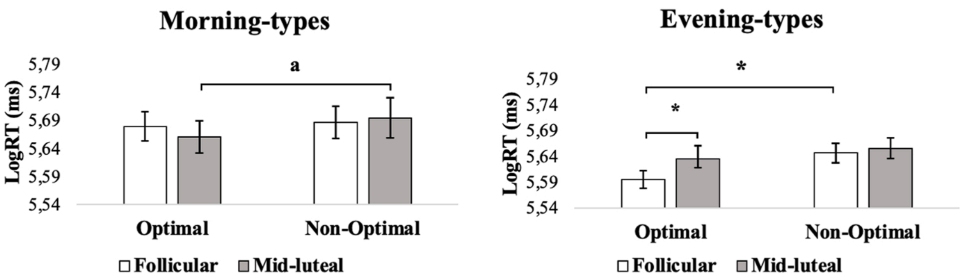

**Fig. 1.** Results with the PVT. Transformed mean reaction times (RTs) for each chronotype (Morning-types, Evening-types) as a function of time-of-day (optimal, non-optimal) and menstrual cycle phase (follicular, mid-luteal). Error bars represent the standard error from the mean. $^{a}p < .06$, $^{*}p < .05$.

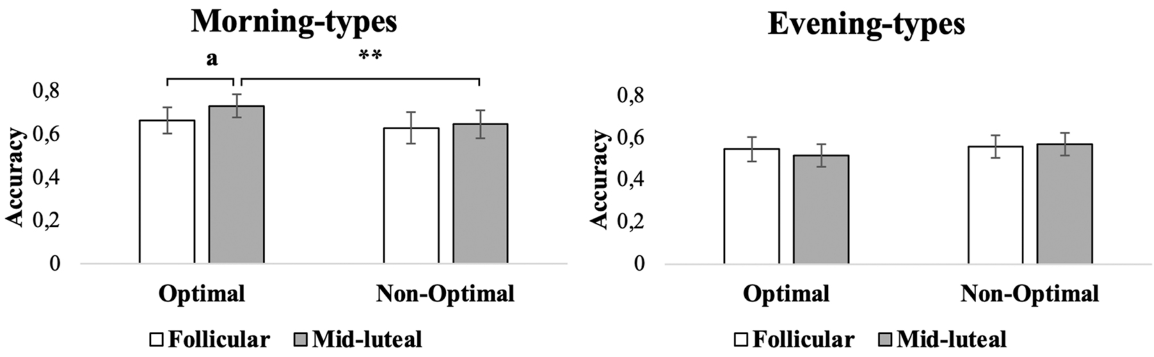

**Fig. 2.** Results with the SART. Percentage of retained responses to the target digit for each chronotype (Morning-types, Evening-types) as a function of time-of-day (optimal, non-optimal) and menstrual cycle phase (follicular, mid-luteal). Error bars represent the standard error from the mean. $^{a}p = .08$, $^{**}p = .01$.

attention. We used the ovulation test, and thus measurement of the amount of LH, for prediction of the mid-luteal phase, which proved to be an accurate method. This method is in line with previous research in which it has been suggested that the ovulation test is the most suitable test to be applied in menstrual cycle studies (Becker et al., 2005). Interestingly, there is a large interindividual variability in progesterone hormone secretions (Sundström Poromaa and Gingnell, 2014). Therefore, we estimated a margin of error of approximately ± 2 days around the day on which the progesterone peak was expected to be observed, *i.e.*, day 21st. This ensured that testing took place in the range of days of the mid-luteal phase (Pletzer et al., 2017; Scheuringer and Pletzer, 2016).

Here we went further to investigate some relevant factors that might have been neglected in previous research. First, the involvement of different components of vigilance depending on the attentional task being performed. Second, the interaction between participants' preferences in performing their daily activities according to their chronotype and time of testing. It should be noted that this last factor may have important implications for how progesterone modulates certain types of vigilance, as progesterone secretion has been found to be linked to biological rhythms. It is important to note that the time-of-day variable has been treated here in terms of optimality. The optimal time-of-day for each participant depends directly on her chronotype. That is, the optimal time for Morning-types is the morning (08:00 AM), while for Evening-types it is the evening (08:30 PM). This difference in optimality with respect to morning and evening is of crucial importance in explaining the present results. Third, progesterone has been found to affect the secretion of cortisol, a hormone that has been thought to affect cognitive performance (DiMenichi et al., 2018; Dolcos, 2014; Lupien et al., 2009). The results showed that the effects of different phases of the menstrual cycle affected performance as a function of both chronotype and time-of-day interaction (the synchrony effect).

The standard synchrony effect was observed with the PVT, the arousal component of vigilance. Participants of both chronotypes showed differences in performance between the optimal and non-optimal time-of-day, although the synchrony effect was smaller in the Morning-types, a result that has also been observed previously (Lara et al., 2014; Martínez-Pérez et al., 2020; Molina et al., 2013). In contrast, with the SART, the executive component of vigilance, the synchrony effect was observed in Morning-types, but only when they performed the task in the mid-luteal phase, not in the follicular phase. Evening-types showed no synchrony effect with this task. These results suggest that the cognitive control demands of the SART may have produced an increase in alertness that counteracted the low-level arousal usually observed during the non-optimal time-of-day, affecting participants of both chronotypes (Martínez-Pérez et al., 2020). Importantly, Morning-types were more conservative than Evening-types when performing the SART, leading the former to be more effective in retaining responses to the target digit at the expense of slowing responses to digits on go trials. Taken together, these results suggest that Morning-types, compared with Evening-types, are characterized by greater adjustment, flexibility and efficiency, as well as greater synchrony between endogenous biological rhythms and social demands.

Of particular relevance is the observation that the presence of high levels of progesterone in the mid-luteal phase further enhanced performance in the Morning-types when the task was carried out at their optimal time-of-day, *i.e.*, in the morning. This improvement in performance was clearly expected in the SART, as that task requires a high-level of cognitive control involving brain regions such as the prefrontal cortex, influenced by progesterone (Guerra-Araiza et al., 2000, 2002, 2003; Kato et al., 1994). Importantly, the fact that a similar result is also observed with a task such as the PVT, which does not require a high level of cognitive control, suggests that progesterone has a rather general nonspecific impact on the performance of tasks that simply require maintaining attention over a fairly long period of time. At the physiological level, progesterone exerts modulatory effects in brain regions involved in attentional processes such as the prefrontal cortex. Specifically, there is consensus that progesterone induces the activation of GABAergic receptors through its main metabolites: pregnanolone and allopregnanolone. GABA is the main inhibitory substance in the brain.

For this reason, the mid-luteal phase is considered to have the highest rates of cortical inhibition (Epperson et al., 2002; Inghilleri et al., 2004; Smith et al., 2002). This pattern would explain the negative results of progesterone on attentional functions observed here in the Evening-type participants.

However, we observed an opposite pattern of results in the two chronotypes just when they performed the vigilance tasks at the optimal time-of-day. Compared to the follicular phase (baseline), the presence of high levels of progesterone in the mid-luteal phase tended to improve performance in the Morning-types (more evident in the SART) and worsen performance in the Evening-types (more evident in the PVT). This differential effect of progesterone on the two chronotypes, when testing was performed at the optimal time-of-day, may be accounted for on the basis of the interaction between the hypothalamic-pituitary-adrenal (HPA) and the hypothalamic-pituitary-gonadal (HPG) hormonal axes. There is evidence that both axes produce bidirectional effects on each other. It is well known that ovulation is regulated by regions that drive circadian rhythms, such as the suprachiasmatic nucleus (Chappell, 2005; De la Iglesia and Schwartz, 2006), as well as the fact that the ovary is a peripheral regulator of circadian rhythm, as there is a rhythmic expression of clock genes controlled by LH in this organ (Fahrenkrug et al., 2006; Karman and Tischkau, 2006). More specifically, it has been observed that progesterone induces a hyperactivation of the HPA axis in healthy women (Roca et al., 2003), although attending to the physiological aspect, it may depend on the basal activation of the HPA axis, which culminates in the secretion of glucocorticoids among which cortisol stands out. Thus, we argue that if there are high concentrations of cortisol, progesterone would act by inhibiting the inhibition of the production of glucocorticoid, it would act as a cortisol agonist. Progesterone can facilitate that cortisol remains longer in circulation and that its secretion does not decrease. Conversely, if endogenous secretion or exogenous administration of progesterone occurs in the absence of cortisol, or if cortisol is present in low concentrations, progesterone may act by inhibiting the release of ACTH and CRH by the pituitary and hypothalamus, respectively (Keller-Wood, 1998; Goldstein et al., 2005; Roca et al., 2003). This hypothesis may also explain the elevated cortisol levels detected in pregnant women whose progesterone levels are normally elevated. Consequently, in Morning-types the cortisol peak occurs in the morning hours (Oginska et al., 2010), roughly coinciding with the participant's test at her optimal time-of-day (8:00 AM). Therefore, it is likely that the performance-enhancing effect observed in the Morning-type participants at their optimal time-of-day, during the mid-luteal phase, is due to the interaction between progesterone and cortisol being present in the body simultaneously. The beneficial effect of progesterone in the mid-luteal phase disappears in the evening, coinciding with the optimal time-of-day for Evening-type participants. It should be noted that cortisol peak is delayed in Evening-types by about 2–4 h compared to Morning-types (Mongrain et al., 2008), so cortisol concentration levels are expected to be lower in the evening, coinciding with the optimal time-of-day for those participants. Consequently, task performance worsened in the mid-luteal phase compared to the follicular phase in the Evening-types participants.

Although in the present study we focused on progesterone levels throughout the menstrual cycle, it should be noted that mid-luteal phase is also associated with elevated estradiol levels, but unlike progesterone, this hormone does not peak in that phase (Jaffé, 1982). Nonetheless, we must also consider some other potential explanations for the effects linking the HPA axis with estradiol. There is evidence that elevated plasma cortisol levels impair the positive effect of estradiol on some cognitive processes. This effect has been observed both in studies under stress conditions (Liston et al., 2009) and in experiments with exogenous cortisol administration (Baker et al., 2012). However, in those studies, plasma cortisol levels were quite elevated, which *a priori* is not the case in our study. Moreover, under continuous exogenous and pulsatile cortisol administration, high or low estradiol levels (as occurs in the mid-luteal phase) inhibit the synthesis of ACTH, a hypothalamic hormone involved in the final glucocorticoid secretion (Sharma et al., 2014). Given this fact, estradiol would act as a regulator of cortisol synthesis, which at elevated levels would impair cognitive performance. It is possible that this physiological phenomenon occurred in Morning-type participants, who have plasma cortisol in the morning hours, which increases their state of activation and cognitive ability. In contrast, in the Evening-type participants, the absence of cortisol in the morning hours would preclude any influence of estradiol on the performance of these participants.

Several limitations of the present study should be kept in mind. One important limitation concerns the lack of direct measurement of menstrual cycle hormones and consequently, the results should be taken with caution. Future studies should include the measurement of estradiol and progesterone, as well as cortisol concentrations, to corroborate the possible explanations we have put forward in order to establish a causal relationship between these hormones and the cognitive processes involved in tasks that require sustained attention or vigilance. While here we have focused only on progesterone levels throughout the menstrual cycle, the mid-luteal phase is also associated with elevated levels of estradiol, although as we stated above, unlike progesterone this hormone does not peak in that phase. Therefore, further studies that include a periovulatory phase, when estradiol is at its highest concentration, are also needed. It is also important to note that our participants show extreme chronotypes, but it is also very important to evaluate the effects of progesterone in Neither-type participants, who represent by 60% of the population (Adan et al., 2012). Finally, due to the COVID-19 pandemic, the number of participants was dramatically reduced, which meant that some of the tests might lack sufficient statistical power to declare some of the observed differences as statistically significant.

## 5. Conclusions

The present results highlight the relevance of individual differences in baseline vigilance levels, explored here through differences in women's chronotype, in assessing the effects of sex hormones on sustained attention tasks. Importantly, our results reveal that the beneficial effects of progesterone, which peaks in the mid-luteal phase, may depend on whether or not that sex hormone promotes high concentrations of cortisol, a hormone that has been observed to affect cognitive performance. The interaction of the two hormones appears to be crucial in improving (Morning-types) or worsening (Evening-types) task performance when testing is conducted at the participants' optimal time-of-day.

## Ethical standards

The research protocol was approved by the Ethics Committee and the Biosafety in Experimentation Committee of the University of Murcia and was carried out in accordance with the Declaration of Helsinki.

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## Authors contribution

**LBP:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **VMP:** Conceptualization, Investigation, Writing – original draft. **MT:** Conceptualization, Investigation, Writing – original draft. **GC:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **LJF:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition.

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### Declaration of Competing Interest

None of the authors have any conflict of interest to declare.

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## Study 3:

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**Abstract**:

We investigated whether chronotype and time-of-day modulate the time course of automatic and controlled semantic processing. Participants performed a category semantic priming task at either the optimal or non-optimal time-of-day. We varied the prime-target onset asynchrony (100-, 450-, 650-, and 850-ms SOAs) and kept the percentage of unrelated targets constant at 80%. Automatic processing was expected with the short SOA, and controlled processing with longer SOAs. Intermediate-types (Experiment 1) verified that our task was sensitive to capturing both types of processes and served as reference to assess both types of processes in extreme chronotypes. Morning-type and evening-type participants (Experiment 2) differed in the influence of time of testing on priming effects. Morning-types applied control in all conditions, and no modulation of performance by time-of-day was observed. In contrast, evening-types were only able to suppress automatic processing when the task was performed at their optimal time of day. Also, they were considerably slower in the implementation of controlled processing as inhibitory priming occurred in the longest 850-ms SOA only. These results suggest that extreme chronotypes may be associated with different styles of cognitive control: while Morning-types would be driven by a proactive control style, a reactive control style might be applied by Evening-types.

# Circadian Modulation of the Time Course of Automatic and Controlled Semantic Processing

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Keywords: chronotype, automatic processing, controlled processing, category semantic priming task, facilitatory priming, inhibitory priming.

## Introduction

Human circadian rhythms are physiologically driven by a central pacemaker that is the suprachiasmatic nuclei (SCN) (J. D. Miller et al., 1996) and connects the organism with the 24-h period of a day on Earth. Nevertheless, circadian functioning may vary from one individual to another. Thus, we refer to the existence of different chronotypes, that is, the preferences that individuals develop for performing their daily life activities and resting earlier or later in the day (Levandovski et al., 2013; Schmidt et al., 2007). The assessment of chronotype allows individuals to be classified into different circadian profiles that are distributed along a Gaussian curve at the population level. Intermediate-types (i.e., those who do not develop any specific circadian preference), would be located around the centre, while morning-types (i.e., those who prefer to engage in their routines in the early morning hours) and evening-types (i.e., those with a more later-day profile) occupy the extremes of the distribution (Roenneberg et al., 2007).

The study of chronotype has become interesting since the continuous development of society has forced individuals not to rely on a 24-h diurnal cycle. For instance, when a doctor must operate at 03:00h in an emergency, a student must plan her or his study schedule for exams, or an air traffic controller must stand guard at her or his post from 05:00h, the consideration of chronotypes and how they operate can predict success. Recently, this trait has been linked to aspects of mental health (Walsh et al., 2022), effects of shift work (Cheng et al., 2021), and school performance (Goldin et al., 2020).

Delving into the cognitive level, processes involving the prefrontal cortex have been shown to be directly related to chronotype and time-of-day, usually showing an enhancement of performance at the time of day that matches the individual’s preference (optimal time). This effect is known as the *synchrony effect* (May & Hasher, 1998), and has been reflected in a variety of cognitive tasks (for a review, see Adan et al., (2012)). Nevertheless, the synchrony effect is often not the same for morning- and evening-types. In contrast to evening-types, morning-types have shown better adjustment and flexibility at non-optimal times (Lara et al., 2014; Martínez-Pérez et al., 2020; Palmero et al., 2022). It has been argued that personality traits such as impulsivity (Di Milia et al., 2011) and even hormonal secretions during the menstrual cycle in women (Palmero et al., 2022) are linked to these chronotype-based differences. Furthermore, morning- and evening-types have shown general differences in brain connectivity patterns (Facer-Childs, Campos, et al., 2019), and in neuro-cognitive functioning reflected in alpha and theta oscillations recorded via electroencephalography (EEG) (Venkat et al., 2020) and fMRI (Orban et al., 2020). More specifically, they have also shown variations in the neural networks activated in response to certain cognitive demands such as conflict resolution or cognitive control (Schmidt et al., 2012). These results, together with findings that have revealed the development of specific biological patterns in each of the chronotypes, such as the delay in the secretion of activation and relaxation substances, (i.e., cortisol and melatonin), (Duffy et al., 2001; Oginska et al., 2010), or peaks and rhythms in body temperature (Kerkhof & Van Dongen, 1996; Sarabia et al., 2008), have prompted researchers to propose that morning- and evening-types are not only the two extremes of a continuum, but also they are independent entities whose functioning, associated conditions and even specific needs, are particular within each group. This aspect is explicitly reflected in the existence of specific research on each of the chronotypes (Facer-Childs, Middleton, et al., 2019; Martínez-Pérez et al., 2022), and in the fact that the results show differential patterns in both groups (Correa et al., 2014; Martínez-Pérez et al., 2020; Palmero et al., 2022; Venkat et al., 2020).

Following with the circadian influences on high-order cognitive operations, results obtained both at the behavioral and physiological levels related to the prefrontal cortex and the time-of-day in general have led researchers to argue that, in general, all mental operations that require control are more vulnerable to the time of testing than those that are more automatic in nature (Lara et al., 2014; Manly, 2002; May et al., 2005). Automatic and controlled processes refer to the differential modes of processing information of our environment that result in an appropriate response (18). It has commonly been argued that controlled processing is the more adaptive since it prevents responses that may have important negative consequences (E. K. Miller & Cohen, 2001), although the need for automatic processing and the importance of not overcontrolling in certain circumstances have also been highlighted (Bocanegra & Hommel, 2014).

In relation to people’s preferences for when to perform daily life activities, it is controlled rather than automatic processing that seems to fluctuate throughout the day. For instance, Fisk & Schneider (Fisk & Schneider, 1981), showed that the decrease in performance typically observed in vigilance tasks occurred specifically when controlled processing was necessary, while decrements in vigilance performance were not observed when automatic processing was involved. Thus, using the Sustained Attention to Response Task (SART), Manly et al. (Manly, 2002), found differential effects due to the time of testing only in no-go trials, which required cognitive control and therefore controlled processing. In addition, Lara et al. (Lara et al., 2014), manipulated two response strategies while participants performed the SART: *speed* (automatic responding set) vs. *accuracy* (controlled responding set). Selective synchrony effects were found only when focused on accuracy. Finally, regarding memory, May et al. (May et al., 2005), and Yang et al. (Yang et al., 2007) found a pattern of increased explicit recall (controlled retrieval) at the optimal time of day for both the morning- and evening-types. However, although automatic and controlled processes have previously been linked to chronotype and time-of-day, to our knowledge, studies that have addressed the emergence and time course of both types of processing in relation to circadian rhythms in a single task are rather scarce.

In the current study, we approached this issue using a paradigm commonly used to dissociate the involvement of automatic and controlled processing: the semantic priming paradigm. In this paradigm, a prime stimulus (e.g., a word) is briefly presented and followed by a target stimulus that requires a quick response by the participant. The target can be a word or a pseudoword if a lexical decision is needed. The semantic priming effect refers to the advantage in responding (shorter reaction times, RTs, and better accuracy) in related prime-target trials (e.g., DOCTOR – nurse) compared to unrelated prime-target trials (e.g., DOCTOR – bread) (Meyer & Schvaneveldt, 1971). Although a discussion of the different models that account for the semantic priming effect is beyond the scope of the present study, semantic priming has been thought to involve the rapid activation of semantic information conveyed by the prime, which spreads to other associated words among which would be the associated target (Collins & Loftus, 1975). Once the prime has been processed, participants may also consciously generate expectancies about candidate targets in a controlled manner; however, these expectancies require time to build up, and therefore they may only occur if there is enough time between the prime and the target onsets (Becker, 1980). Thus, the prime-target onset asynchrony (usually referred to as prime-target SOA) is crucial for determining whether semantic processing is mainly due to automatic or controlled processing (Besner & Humphreys, 1991), with the former being better captured with short prime-target SOAs and the latter with long prime-target SOAs (e.g., longer than

200 ms) (Neely et al., 1989). Semantic priming studies usually reveal facilitation in target responses when only the prime-target SOA is manipulated to engage either automatic or controlled semantic processing. Additionally, in relation to prime processing, the use of a mask after its presentation has proven to influence automatic and controlled processes in semantic priming tasks. The immediate appearance of a mask fosters processing of the prime in a strategy-free automatic way, whereas the delayed appearance of the mask allows the use of strategies after processing the prime and guides the individual to act in a more controlled manner (Daza et al., 2002; Merikle & Joordens, 1997).

However, a more appropriate way of dissociating the involvement of automatic and controlled processing in a semantic priming task is to find qualitatively different patterns of results associated with each type of process (Merikle & Joordens, 1997). Whereas the use of associative links between the prime and the target usually produces facilitatory priming effects, both facilitatory and inhibitory effects can be found with category semantic priming tasks when both the relatedness proportion and the prime-target SOA are manipulated (Besner & Humphreys, 1991; McNamara, 2005). Facilitatory priming refers to the advantage in performance with related prime-target trials than with unrelated prime-target trials, and the other way around for inhibitory priming. In the category semantic priming task, the prime is the name of a category (e.g., ANIMAL), and the target can be either an exemplar of the prime category (e.g., *cat*) in the related condition or an exemplar of a different category (e.g., *table*) in the unrelated condition. Participants will create expectations about the forthcoming target based on the frequency with which a related target follows a prime category and on the interval between the prime and the target (Neely, 1977). With a low rate of related targets, expectations based on the prime category will favour unrelated targets. Thus, the observation of shorter RTs with unrelated compared to related targets (inhibitory priming) will confirm that an expectation has been successfully generated. In contrast, any facilitatory effect despite the low rate of related targets will confirm that expectations were not developed; therefore, facilitatory priming would simply reflect the intervention of automatic processing. By using this paradigm, Ortells et al. (Ortells et al., 2003), showed facilitatory priming effects with 200- and 300-ms prime-target SOAs, whereas the effect became inhibitory with a 500-ms SOA. Using a similar design with young participants, Langley et al. (Langley et al., 2008), found a facilitatory priming effect only with a 100-ms SOA and inhibitory priming effects with 200- 500- and 800-ms SOAs, effects being larger as the SOA value increased.

In the current study, we employed a category semantic priming task in which the name of a semantic category served as the prime stimulus and was followed by either a related or an unrelated target exemplar. A low rate of related targets (20%) was used to promote expectancy-based priming effects, and a short prime-target SOA and long prime-target SOAs were used in different blocks of trials to assess the emergence and, as a novelty, the time course of facilitatory (automatic) priming and inhibitory (controlled) priming effects, respectively. Thus, we were not only interested in the contrast between automatic and controlled processes, but also in the process by which individuals were able to reverse an automatic process in a context where control had to be recruited to give a correct response.

Experiment 1 involved intermediate chronotype participants, who have been consistently neglected in chronotype-related studies (May & Hasher, 2017) despite representing 60% of the population (Roenneberg et al., 2007). This experiment allowed us to test the suitability of this category semantic priming task to dissociate the two types of processes. In Experiment 2, morning- and evening-type participants, who combined represent the 40% of the population and are referred to as extreme chronotypes, performed the task to assess whether chronotype and time of testing are crucial factors that modulate the earlier appearance of automatic processing and the later emergence of controlled processing. Moreover, in view of the above-mentioned differences between the two chronotypes, our main interest was to observe how this cognitive strategy (i.e., the shift from automatic to controlled processing) may follow a different time course in each chronotype. Based on previous results, we hypothesize facilitatory priming with the short SOA involving automatic processing. However, it is likely that the facilitatory effect turns into inhibitory at different moments for each chronotype. Therefore, and based on the results obtained in previous pilot studies with intermediate-types, the time course of automatic processing was addressed based on the division of a 100-ms SOA block in three consecutive subblocks. That division has not been usually done in previous related studies, however, is of special relevance here because participants performed the task in optimal (also non-optimal) arousal conditions according to their chronotype, which may promote the anticipated use of control-based strategies even in the short prime-target SOA block. Note that the younger adult group in Langley's et al. study (Langley et al., 2008) showed inhibitory effects in the rather short 200-ms SOA. On the other hand, to assess the effects of time of testing on controlled processing, we explored both the emergence and time course of inhibitory priming with longer prime-target SOAs (450, 650, and 850 ms). In this case, based on the differences between the two extreme chronotypes, we also hypothesized that inhibitory priming may exhibit a different time course in each chronotype.

We also tested the existence of synchrony effects in each chronotype and expected them to generally affect controlled but not automatic processing, but we went further and investigated how the time course of such effects may change as a function of both chronotype and time of testing.

Finally, to ensure the appropriate selection of our sample of extreme chronotypes, we used the psychomotor vigilance task (PVT). This task has been shown to be very sensitive to circadian-related performance (Blatter & Cajochen, 2007) and has been widely used in cognitive research, mainly related to vigilance and sustained attention (Lara et al., 2014; Martínez-Pérez et al., 2020, 2022; Molina et al., 2019).

## Materials and Methods

### ***Participants***

Sixty-four undergraduates ($M_{age}$= 21.14 years; $SD_{age}$= 5.45) completed the experiment for course credit. The sample was recruited based on the scores obtained in the reduced version of the Horne and Östberg's Morningness-Eveningness Questionnaire (rMEQ) standardized by (Adan & Almirall, 1991) for the Spanish population. This brief questionnaire classifies individuals depending on their circadian preference into definite morning- or evening-types and neither or intermediate-types. In this case, we considered the chronotype as a continuum, with the morning- and evening-chronotype groups consisting of moderate and extreme individuals, following previous studies (Lara et al., 2014; Martínez-Pérez et al., 2020; Palmero et al., 2022; Salehinejad et al., 2021). We had three chronotype-based groups. Twenty-five students with scores ranging from 12 to 16 were assigned to the intermediate-type group (M= 13.60; SD= 1.66) and participated in Experiment 1.Twenty students with scores between 17 and 25 (corresponding to moderate-to-extreme morning-types) were assigned to the morning-type group (M= 18.50; SD= 1.60), and 20 students with scores between 4 and 11 (corresponding to moderate-to-extreme evening-types) were assigned to the evening-type group (M= 9.25; SD= 1.68). An a posteriori sensitivity analysis using G*Power was performed to corroborate the effect size that our study was able to detect given that our main interest was in testing the synchrony effect. Given 2 groups (morning and evening), and 2 measures for each (optimal and non-optimal times), with an alpha level of .05, a statistical power of at least .80, a sample size of n = 40 participants, a correlation between repeated measures of $r = .5$ and a non-sphericity correction of 1, the effect size $f$ that our design was able to detect was .22. Thus, the sample size that comprised our study warranted the detection of small-to-medium effect sizes. Both morning- and evening-type participants participated in Experiment 2. All participants reported normal or corrected-to-normal vision and the absence of chronic medical conditions. Note that although both groups were composed of moderate and extreme participants, throughout the manuscript we refer to morning- and evening-types as extreme chronotypes to differentiate them from intermediate-types.

### ***Tasks and design***

Two experimental tasks programmed with E-Prime 3 (Psychology Software Tools, Pittsburgh, PA, 2016; Schneider, W et al., 2012) were used. Stimuli were presented on a 22-inch computer screen with a resolution of 1920 × 1080 pixels. Responses were recorded by using a Chronos® device (Psychology Software Tools, Pittsburgh, PA, 2016).

The PVT assessed the morning-types and evening-types arousal vigilance state by presenting a red circle (50 pixels in diameter) in the centre of the screen to which the participants had to respond as quickly as possible. In each trial, the red circle was presented after a random interval between 2 and 10 s in which the computer screen remained black. The participants were instructed to press the central button of the response box with the index finger of their dominant hand when the stimulus appeared. Once the trial was completed, a new trial began.

On the other hand, the category semantic priming task assessed performance under automatic or controlled processes. The stimuli were all presented on the centre of the screen in black against a white background. The categories used as primes were the Spanish words ANIMAL (animal) and MUEBLE (furniture) (on average: 3.72° in width; 0.71° in height) and were always presented in uppercase letters. The target words were familiar examples of each of the two categories. For animals, *burro* (donkey), *gato* (cat), *tigre* (tiger), and *foca* (seal) were used. For furniture, *mesa* (table), *silla* (chair), *percha* (hanger), and *cama* (bed) were used. The stimuli subtended 2.90° width and 0.57° height on average. All stimuli were presented in Spanish and in lowercase letters.

For all participants, on 80% of the trials, the prime and the target stimuli were unrelated, so the target (e.g., seal) did not belong to the category of the prime (e.g., FURNITURE), and in the remaining 20% of trials, the prime (e.g., ANIMAL) preceded a target of the same category (e.g., cat). The time between the onset of the prime stimulus and the onset of the target (i.e., SOA) was manipulated to foster the development of automatic and controlled processing. Four different SOAs were considered. First, to promote automatic processing, a short 100-ms SOA was chosen on the basis of previous studies (Langley et al., 2008). To assess controlled processing and the time course of strategy acquisition, we used 3 long SOAs of 450, 650 and 850 ms.

For the presentation of the different SOAs, a blocked design was used. The short-SOA block had 125 trials in total, from which the first 10 were considered practice. Thus, the automatic-processing experimental block consisted of 115 trials. Subsequently, all long SOAs were randomly presented in a different block comprised of 300 trials. The first 30 trials were also considered practice (approximately 10 trials per

SOA). Each long SOA was presented in 90 different trials, bringing the total number of trials to 270. Finally, the task comprised 425 trials, and the design was similar to that used in other previous studies (Langley et al., 2008; Ortells et al., 2003). The order of the blocks was the same for all participants: first, they completed the short-SOA block (reflecting automatic processing) and then the long-SOA block (reflecting controlled processing). The rationale for not using a single block with the four SOAs randomly presented, nor counterbalancing the order of the short and long SOA blocks was to avoid applying the control elicited by the long SOA trials to the automatic processing evaluated through the short SOA, as previous studies have shown that the two types of processes can interact during a semantic priming experiment (Balota et al., 1992; Lerner et al., 2014; Neely et al., 2010).

Moreover, to maximize the development of automatic processing and controlled strategies, a random letter stimulus "XDGTKSN" (4.30° width; 0.72° height) was used as a mask between the prime and the target. Although it was present in all blocks, the delay between the presentation of the prime and the mask varied depending on the SOA. In the short-SOA block, the mask was presented immediately after the prime and lasted 50 ms. In the long-SOA block, the mask lasted 50 ms but was presented with an adapted delay after the prime according to the SOA of each of the trials (i.e., 350, 550 and 750 ms, respectively). It is important to note that the use of the mask in the short-prime task is meant to promote automatic processing, but by no means it prevents the participants from being aware of the prime stimulus, and hence it is not a design to promote unconscious priming effects.

The participants were instructed to press two different buttons on the response box (the leftmost and rightmost) to classify the target words. The order of the buttons was counterbalanced between participants. Thus, half of them responded by pressing button 1 to the exemplars of the category ANIMAL and button 5 to the category FURNITURE, and the button assignment was reversed for the other half of participants. The time to respond to the target was unlimited. Once a response was made, a new trial began. An example of each type of trial can be seen in Figure 1.

Before the task started, the participants were informed of the relatedness of the prime-target proportion. That information was given to foster the use of expectations based on the prime stimulus from the very beginning of the experimental session. Also, to prioritize an accuracy strategy, we instructed the participants to give a correct response rather than a quick response.

Insert here Figure 1

Figure 1. Sequence of trials in the category semantic priming task with the short 100-ms SOA (left) and the long 450-, 650- and 850-ms SOAs (right).

***General procedure***

After being selected based on their scores on the rMEQ, the participants were invited to complete the experimental sessions.

In Experiment 1, the participants came to only one experimental session scheduled from 10:00h to 16:00h. The experimental session consisted of completing only the category semantic priming task, which lasted approximately 40 min.

In Experiment 2, each participant came to the laboratory twice, with an interval of approximately 7 d between sessions. The sessions were scheduled at 08:00h (morning session) and at 20:30h (evening session). This procedure is commonly implemented in studies using the extreme-chronotypes paradigm (Blatter & Cajochen, 2007; Schmidt et al., 2007) and allows us to obtain an assessment of the participants’ performance at their optimal and non-optimal time-of-day according to their chronotype. The order of the sessions was counterbalanced across participants within each chronotype group so that half of the sample started the experiment at 08:00h and the other half at 20:30h. All sessions had the same structure and duration (~1 h). In all cases, the participants in Experiment 2 started the session by performing the PVT and subsequently performed the category semantic priming task. Instructions were given in all sessions in the same way.

Participants were interrogated about the total number of hours they had slept the night before the experimental session, as well as about intake of stimulants, as caffeine intake has been shown to be related to prefrontal cortex-dependent cognitive performance (Zhang et al., 2020). All participants were asked not to take any stimulant substances, such as coffee or tea, in the 2h prior to the start of the session.

## Results

Data were preprocessed with R software (R Core Team, 2022) and analysed with JASP 0.9.2 (JASP Team, 2022). Regarding the PVT, the first trial, trials with RTs shorter than 150 ms, and trials with RTs separated by more than 3.5 semi-interquartile ranges from the median value of each participant in each session were considered outliers and discarded (1.77% of trials). In relation to the category semantic priming task, we applied two different filters according to the graphical distribution of the RT raw data. Considering the graphical representation, we first established a long-time interval where most of the participants' responses were concentrated: 100 ms to 4000 ms. Specifically, only 3 participants made some responses above 4000 ms, and no one made responses below 100 ms. Second, we considered outliers all RTs that were separated by more than 3.5 semi-interquartile ranges from the median value of each participant in each condition. The percentage of discarded trials did not exceed 1.10% in any experimental condition. Mean RTs were calculated considering only RTs associated with correct responses and after discarding practice trials.

For the accuracy data the statistical analysis was conducted with the proportion of correct responses. Accuracy rates and mean RTs from all participants in the study are shown in Table 1S in the supplementary material (see category_semantic_priming_data.xlsx).

We adopted a statistical significance level of $\boldsymbol{\alpha} = .05$ for all statistical analyses. Moreover, we present effect size values for each of our contrasts through the partial eta squared ($\eta_p^2$) for ANOVA and Cohen's d (*d*) for Student's *t* tests.

### ***Demographic data***

The analysis of the scores on the rMEQ used to classify individuals according to their chronotype showed a main effect of chronotype, $F(2, 62) = 157.40$; $p < .001$; $\eta_p^2 = .84$. Post-hoc tests with Bonferroni correction showed significant differences between the three groups: morning-types vs intermediate-types, $t(63) = 9.90$, $p < .001$, $d = 2.99$; evening-types vs intermediate-types, $t(63) = 8.79$, $p < .001$, $d = 2.60$; and morning-types vs evening-types, $t(63) = 17.73$, $p < .001$, $d = 5.62$. The analysis of age showed no statistically significant differences between the groups ($p = .99$). The participants reported 5h or more of sleep the night before the experiment. As instructed, none of the participants reported caffeine consumption in the 2h prior to the experiment.

### ***Experiment 1: Intermediate-type chronotype***

*Category semantic priming task*

The analyses were conducted separately for the 100-ms SOA and the long SOAs. For the 100-ms SOA, data were subjected to 2 × 3 repeated-measures ANOVAs with relatedness (related and unrelated) and subblock (first, second and third) as within-participants factors. For the long SOAs, data were subjected to 2 × 3 repeated-measures ANOVAs with relatedness (related and unrelated) and prime-target SOA (450, 650 and 850 ms) as within-participants factors.

*Accuracy analysis*. The results are shown in Figure 2a. For the 100-ms SOA, the main effect of relatedness was not significant, $F < 1$. Also, priming effects did not vary across the three subblocks of trials (all $p$s > .24). For the long SOAs, we observed a significant main effect of relatedness, $F(1, 25) = 6.75$, $p = .01$, $\eta_p^2 = .21$, indicating higher proportion of correct responses in unrelated (M = .94) than in related trials (M = .90), that is an inhibitory priming effect. No other effects or interactions were significant (all $p$s > .61).

*RTs analysis.* The results are shown in Figure 2b (short SOA) and Figure 1c (long SOAs). For the 100-ms SOA, the main effect of subblock was significant, $F(2, 46) = 3.54$, $p = .04$, $\eta_p^2 = .07$, showing that RTs were longer in the first subblock (M = 784 ms) than in the second and third subblocks (M = 734 and 746 ms, respectively). However, neither the main effect of relatedness nor the relatedness × subblock interaction were significant, (all $p$s > .15). However, an inspection to Figure 1b reveals that the lack of priming may be due to the facilitatory effect observed in the first subblock of trials, which proved statistically significant (M priming = 53 ms), $t(23) =$

2.14, $p < .04$, $d = .44$, being counteracted by the lack of effect in the second subblock (M priming = 2 ms) ($p = .95$), and the non-significant trend for an inhibitory effect in the third subblock (M priming = -7 ms) ($p = .74$). For the long SOAs, the main effects of relatedness and SOA were significant, $F(1, 23) = 10.22$, $p = .004$, $\eta_p^2 = .31$ and $F(2, 46) = 10.50$, $p < .001$, $\eta_p^2 = .31$, respectively. The relatedness effect showed that, in general, the participants were faster in unrelated (M = 656 ms) than in related trials (M = 695 ms) and the effect of SOA reflected that RTs decreased as SOA increased (M = 691, 669, and 667 ms, for the 450-, 650- and 850-ms SOAs, respectively), In addition, a significant relatedness × SOA interaction was also found, $F(2, 46) = 4.24$, $p = .02$, $\eta_p^2 = .16$, which reflected a change in the priming effect across the SOAs. Further analyses of the interaction showed the progressive development of an inhibitory priming effect. With a SOA of 450 ms, priming (M = -9 ms) was not yet significant, $t(23) =.71$, $p = .48$, $d = .14$, but priming was statistically significant with SOAs of 650 ms (M = -35 ms), $t(23) = 2.25$, $p = .034$, $d = .46$, and 850 ms (-75 ms), $t(23) = 3.85$, $p < .001$, $d = .78$.

An inspection to Figure 2b and Figure 2c suggests that intermediate-type participants showed automatic processing at the very beginning of the experimental session, being apparent only in the first subblock of the short prime-target SOA block of trials. The lack of priming effects in both the two later subblocks of the short SOA block and the 450-ms SOA of the long SOA block, suggest that automatic and controlled processes cancelled out each other during that interval of time. With longer prime-target SOAs, automatic processing may have already dissipated and therefore the results show the development of controlled processing, which makes stronger with time. This interplay between automatic and controlled processing as a function of prime-target SOA suggests that the pattern of priming effects showed by intermediate-type participants constitutes an appropriate referent to assess the performance of extreme chronotype participants when testing occurs in their optimal and non-optimal time-of-day.

Insert Figure 2 here

Figure 2. Intermediate-type participants. In (a), priming effects with accuracy data are shown as the difference in the proportion of correct responses between unrelated and related trials as a function of SOA. Priming effects with RTs (RTunrelated – RTrelated) as a function of (b) subblocks of trials in the short 100-ms SOA, and (c) long 450-, 650- and 850-ms SOAs. Note that contrary to RTs, inhibitory priming in the accuracy analysis is expressed with positive scores (higher proportion of correct responses in unrelated than related trials).

### ***Experiment 2: Extreme chronotypes***

#### *Psychomotor Vigilance Task (PVT)*

Mean RTs were subjected to a mixed ANOVA with time-of-day (optimal and non-optimal) as the within-participants factor and chronotype (morning-types and evening-types) as the between-participants factor. The main effect of time-of-day was statistically significant, $F(1, 38) = 5.61$; $p = .02$; $\eta_p^2 = .13$, which indicated that, in general, performance at the optimal time produced shorter RTs (M = 319 ms) than at the non-optimal time (M = 341 ms). The difference in performance between the optimal and non-optimal time-of-day according to the different chronotypes is referred to as the synchrony effect and the current results replicate those obtained in other experiments using the same task (Correa et al., 2014; Lara et al., 2014; Martínez-Pérez et al., 2022). Accordingly, the current synchrony effect confirms the appropriate selection of our sample of extreme chronotypes. No other effects or interactions were significant (all *p*s > .31).

*Category Semantic Priming Task*

As with intermediate-types, statistical analyses were conducted separately for the 100-ms SOA and the long SOAs. For the 100-ms SOA, data were subjected to 2 × 2 × 3 × 2 mixed ANOVAs with relatedness (related and unrelated), time-of-day (optimal and non-optimal), and subblock (first, second and third) as within-participants factors, and chronotype (morning- and evening-types) as the between-participants factor. For the long SOAs, data were subjected to 2 × 2 × 3 × 2 mixed ANOVAs with relatedness (related and unrelated), time-of-day (optimal and non-optimal) and prime-target SOA (450, 650, and 850 ms) as within-participants factors, and chronotype (morning- and evening-types) as the between-participants factor.

*Accuracy analysis*. For the 100-ms SOA, only the relatedness × chronotype interaction reached statistical significance, $F(1, 38) = 5.58$, $p = .023$, $\eta_p^2 = .13$. The interaction analysis showed that evening-types showed facilitatory priming (M = .016) and morning-types inhibitory priming (M = -.005). For the long SOAs, the main effect of relatedness was significant, $F(1, 38) = 14.78$, $p < .001$, $\eta_p^2 = .28$, the relatedness × SOA interaction was also significant, $F(2, 76) = 4.12$, $p = .02$, $\eta_p^2 = .10$, and the time-of-day × SOA × chronotype interaction was marginally significant, $F(2, 76) = 2.78$, $p = .068$, $\eta_p^2 = .07$. However, these effects were qualified by the significant relatedness × time-of-day × SOA × chronotype interaction, $F(2, 76) = 3.13$, $p = .05$, $\eta_p^2 = .08$. This four-way interaction means that in morning-types the differences in inhibitory priming effects between the optimal and non-optimal time of day was only observed with 800-ms SOA (M synchrony effect = .042), while in evening-types the pattern of inhibitory priming was completely different. Priming effects were observed in all SOA values at the optimal time (M = .043 in 450-, .056 in 650-, and .072 in 850-ms SOAs), and only in 850-ms SOA at the non-optimal time (M = .061).

*RTs analysis*. For the 100-ms SOA, none of the main effects nor the interactions were significant (all $p$s > .05). For the long SOAs, we observed significant main effects of relatedness, $F(1, 38) = 15.30$, $p < .001$, $\eta_p^2 = .29$, and SOA, $F(2, 76) = 22.19$, $p <.001$, $\eta_p^2 = .37$. RTs with unrelated trials (M = 660 ms) were shorter than those with related trials (M = 690 ms), meaning an inhibitory priming effect. Also, RTs reflected a general decrease as SOA increased (M = 694, 672, and 658 ms, for the 450-, 650- and 850-ms SOAs, respectively). The main effects of time-of-day and chronotype and the relatedness × time-of-day interaction were not significant, (all $p$s > .10). Importantly, we observed a significant relatedness × time-of-day × SOA interaction, $F(2, 76) = 5.62$, $p = .005$, $\eta_p^2 = .13$. This three-way interaction means that the priming effects were affected by the time of testing (synchrony effect) and varied as a function of SOA. However, contrary to the accuracy analysis, the interaction involving relatedness, time-of-day, SOA and chronotype was not significant, $F < 1$.

The lack of a significant four-way interaction in the RTs analysis deserves further comments. Regarding priming, the differences between the two chronotypes were expected to be subtle rather than extreme, i.e., mainly in the time where the effects emerge. Therefore, given the hypothesized patterns of results in the performance of the two chronotypes, we consider the expected differences to be more qualitative than quantitative. Importantly, previous studies have shown that the differences in performance between the optimal and non-optimal time-of-day are only pronounced for evening-types, while a greater stabilization of performance throughout the day is only found in morning-types (Lara et al., 2014; Martínez-Pérez et al., 2020; Palmero et al., 2022). Thus, it should be expected differences in the emergence and time course of controlled priming effects between the two chronotypes to be mainly observed at the non-optimal time-of-day. This is further supported by the significant relatedness x SOA x chronotype interaction when performance was assessed only at the non-optimal time-of-day, $F(2, 76) = 3.40$, $p = .039$, $\eta_p^2 = .082$. Thus, we consider that there is a strong case for separate analyses for each chronotype to assess both the emergence and time course of automatic and controlled processing in both accuracy and RTs data.

For the 100-ms SOA, given that subblock did not show any effect neither as main effect nor in the interaction with the other factors, that factor was omitted in the accuracy and RTs analyses for each chronotype. Thus, accuracy and RTs data were subjected to 2 × 2 repeated measures ANOVAs with relatedness (related and unrelated) and time-of-day (optimal and non-optimal) as within-participants factors. For the long SOAs, data were subjected to 2 × 2 × 3 repeated-measures ANOVAs with relatedness (related and unrelated), time-of-day (optimal and non-optimal) and SOA (450, 650, and 850 ms) as within-participants factors.

### *Automatic and controlled processes in morning-type chronotype*

*Accuracy analysis*. The results are shown in Figure 3a. For the 100-ms SOA, none of the main effects nor the interactions reached statistical significance (all $p$s > .30). For the long SOAs, we observed a significant main effect of relatedness, $F(1, 19) = 6.90$; $p = .02$; $\eta_p^2 = .27$, that is the proportion of correct responses was higher in unrelated ( M =

.98) than in related trials (M = .94). Moreover, the relatedness × time-of-day × SOA interaction was statistically significant, $F(2, 38) = 4.67$; $p = .01$; $\eta_p^2 = .20$. The interaction was due to a significant synchrony effect (the difference in priming between the optimal and non-optimal time-of-day) only with the 850-ms SOA, $t(19) = 2.79$, $p = .01$, $d = .62$.

*RTs analysis*. The results are shown in Figure 3b. For the 100-ms SOA, neither the main effects nor the interactions reached statistical significance (all ps > 20). For the long SOAs, the main effects of relatedness and SOA were statistically significant, $F(1, 19) = 8.32$; $p = .009$; $\eta_p^2 = .30$ and $F(2, 38) = 12.38$; $p < .001$; $\eta_p^2 = .39$, respectively. RTs were longer for related (M = 750 ms) than for unrelated trials (M = 711 ms), reflecting an inhibitory priming effect. Also, RTs reflected a general decrease as SOA increased (M = 733, 707, and 694 ms, for the 450-, 650- and 850-ms SOAs, respectively). However, the relatedness × time-of-day × SOA interaction did not reach statistical significance, $F(2, 38) = 2.74$; $p > .05$; $\eta_p^2 = .13$. Thus, we did not find any modulation of priming due to time of testing for the morning-type participants in the RTs analysis.

Insert Figure 3 here

Figure 3. Morning-type participants. Priming effects (unrelated – related) at the optimal and non-optimal time-of-day across prime-target SOAs for accuracy (proportion of correct responses) (a) and RTs (b). Priming effects did not vary for the 3 subblocks of the 100-ms SOA and consequently they are not shown. Note that contrary to RTs, facilitatory priming in the accuracy analysis is expressed with negative scores and inhibitory priming with positive scores.

### *Automatic and controlled processes in evening-type chronotype*

*Accuracy analysis*. The results are shown in Figure 4a. For the 100-ms SOA, we observed a main effect of relatedness, $F(1, 19) = 7.23$; $p = .015$; $\eta_p^2 = .28$, indicating higher proportion of correct responses in related (M = .97) than in unrelated trials (M = .95), reflecting automatic facilitatory priming. There were no other statistically significant main effects nor interactions (all *p*s > .40). For the long SOAs, we also observed a main effect of relatedness, $F(1, 19) = 7.91$; $p = .01$; $\eta_p^2 = .29$, indicating higher proportion of correct responses in unrelated (M = .96) than in related trials (M = .91). There were no other statistically significant main effects nor interactions (all *p*s > .14).

*RTs analysis*. The results are shown in Figure 4b. For the 100-ms SOA, only the relatedness × time-of-day interaction was marginally significant, $F(1, 19) = 3.89$; $p = .06$; $\eta_p^2 = .17$. The analysis of the interaction showed a trend for facilitatory priming (M = 16 ms) only at the non-optimal time of day, although the effect was not statistically significant, $t(19) = 1.05$, $p = .31$, $d = .23$, and a clear inhibitory priming effect (M = -29 ms) at the optimal time of day, $t(19) = 2.00$, $p = .06$, $d = .45$. For the long SOAs, we observed significant main effects of relatedness, $F(1, 19) = 7.53$; $p = .013$; $\eta_p^2 = .28$, and SOA, $F(2, 38) = 9.99$; $p < .001$; $\eta_p^2 = .34$. RTs were larger for related (M = 651 ms) than for unrelated trials (M = 628 ms), and decreased as SOA value increased (M = 656, 639, and 623 ms, for the 450- 650- and 850-ms SOAs, respectively). Importantly, the relatedness × time-of-day × SOA interaction reached statistical significance, $F(2, 38) = 5.46$; $p = .008$; $\eta_p^2 = .22$. The analysis of the three-way interaction showed significant inhibitory priming at the 600-ms SOA, $t(19) = 4.74$, $p < .001$, $d = 1.06$, and 850-ms

SOA, $t(19) = 2.01$, $p = .05$, $d = .45$, only when testing occurred at the optimal time-of-day, but no priming effects were observed when testing occurred at the non-optimal time-of-day (all *p*s > .08).

Insert Figure 4 here

Figure 4. Evening-type participants. Priming effects (unrelated – related) at the optimal and non-optimal time-of-day across prime-target SOAs for accuracy (proportion of correct responses) (a) and RTs (b). Priming effects did not vary for the 3 subblocks of the 100-ms SOA and consequently they are not shown. Note that contrary to RTs, facilitatory priming in the accuracy analysis is expressed with negative scores and inhibitory priming with positive scores.

## Discussion

It is well established that automatic processes occur without the need to invest cognitive resources, whereas controlled processes require cognitive control. A key question is whether both types of processes can be modulated by the time of testing in extreme chronotypes, that is, whether the development of attention-based strategies can be different by the fact that people perform cognitive tasks at the optimal or non-optimal time-of-day according to their chronotypes. Although the influence of chronotype and time-of-day on automatic and controlled processing has been addressed separately in most studies, it is important to note that both processes coexist in most of our daily activities, and the interaction between them gives us the ability to deal with relatively complex demands. Thus, in the present study, we used a single-category semantic priming task in which a combination of a high proportion of unrelated targets, which promoted strategic processes, and varying intervals between the prime and target, allowed us to assess with finer detail the chronotype influence on the emergence and time course of automatic and controlled processes.

In the first experiment, the results obtained with intermediate-chronotypes, who represent the largest part of the population (Roenneberg et al., 2007), allowed us to verified that our task was sensitive to capturing both types of processes. When the short SOA was used, the prime stimulus activated the semantic category in an automatic way, and the participants showed facilitatory priming. However, an automatic influence was apparent with low levels of experience with the task because the facilitatory effect was cancelled out as the task progressed, suggesting that the intermediate-type participants were able to engage the inhibitory strategy and implement it relatively quickly despite the very short SOA. The early emergence and dissipation of automatic processing may have been promoted by the instructions given to the participants at the beginning of the experimental session about the low rate of related trials as well as the emphasis in accuracy which surely fostered the development of controlled processes. With longer prime-target SOAs, intermediate-type participants were able to progressively apply inhibitory control as the SOA value increased. Inhibitory priming effects increased as a function of prime-target SOA, indicating effective controlled processing. Thus, the results of Experiment 1 with intermediate-types confirm that our task was capable of capturing both processing styles, replicating those observed in some previous studies (Langley et al., 2008; Ortells et al., 2003).

In addition to the capability to dissociate automatic and controlled processes, the category semantic priming task used here has proven to be sensitive to the effects of time-of-day, according to the results obtained with the participants showing extreme chronotypes, in line with the results of other related studies that used conflict tasks such as the Stroop task (Schmidt et al., 2012). Based on the important differences that distinguish the two chronotypes regarding physiological and cognitive measures, and how the two are differentially affected by the time of testing, we discuss the results observed in each group separately.

Concerning morning-type participants, their performance at the short SOA was marked by the development of attention-based strategies throughout almost the entire block of trials, as we observed a lack of priming effect, but with a clear trend to be inhibitory in both accuracy and RTs (see Figure 2). The lack of priming effect may be due to both automatic and controlled processes cancelling out each other (see a similar finding in Langley et al. (Langley et al., 2008) with the older adult participants). Thus, we can conclude that these participants did not show any variation in performance as a function of time-on-task, thereby suggesting that morning-types very efficiently suppressed the automatic activation of the prime category even at the very start of the task. Moreover, their performance was not modulated as a function of the time of testing, which means that their ability to reverse automatic activation was robust and unaffected by diurnal variations. Regarding the time course of controlled processing, we did not find any modulation by either the SOA value or the time of testing, as morning-type participants showed significant inhibitory priming effects in all conditions (although accuracy data showed a synchrony effect just at the longest SOA). These results suggest that morning-type performance seems to be constant and invariable throughout the day.

The lack of time-of-day modulation of the performance of morning-type participants deserves further explanation. It has been suggested that circadian modulation of performance depends on the difficulty of the task (Lara et al., 2014; Manly, 2002; May et al., 2005; Yang et al., 2007) such that synchrony effects usually appear in those tasks that demand high levels of cognitive resources. Therefore, notwithstanding the evident efficiency in controlled processing shown by morning-types it could be argued that the category semantic priming task used here was not truly challenging for this group of participants, so that the implementation of attention-based strategies would have been relatively easy and remained constant at both the optimal and non-optimal time-of-day. This approach is directly supported by the lack of facilitatory priming with the short SOA and the large inhibitory priming effects observed with the long SOAs.

Concerning evening-types, despite the small facilitatory priming effects usually observed in this type of task, a time-of-day modulation of the time course of automatic processing occurred in these participants in the RTs analysis. Evening-types showed facilitatory priming at the non-optimal time-of-day and were only able to suppress the automatic activation of the prime category when the task was performed at their optimal time-of-day. This result is particularly striking because it marks a substantial qualitative difference from both the intermediate-types, who despite starting with an automatic processing mode, could quickly cancelled it out by the second subblock of trials, and the morning-types, whose performance was always guided by the effective application of the controlled strategy. As far as we know, this is the first evidence of such modulation by chronotype and time-of-day.

Concerning controlled processing, we observed that evening-types were considerably slower in the implementation of the controlled strategies. Note that at the optimal time-of-day, inhibitory priming appeared with the 650-ms SOA onwards like intermediate-types, whereas at the non-optimal time priming was no longer significant. These results are consistent with the suggestion of difficulty-based modulation of cognitive processes by time-of-day, such that the evening-type participants were not only affected by time of testing for applying control, but their inhibitory priming effects (reflecting controlled processing) were rather small.

The results obtained in the present study reveal a dissociation between the magnitude of the effect based on controlled processes (i.e., inhibitory priming effects) and the difficulty of the task. Theories on which this study was based have pointed out that time-of-day modulation occurs most explicitly in tasks that require the involvement of controlled processing. However, we found that the strongest evidence of controlled processing (which occurs in morning-type individuals throughout almost the whole task) was paralleled by the absence of time-of-day modulation. Nevertheless, the evening-types, whose performance reflected less controlled processing, were severely influenced by time-of-day in both automatic and controlled processing. This pattern of results suggests that the difficulty of the task varied for the two extreme chronotypes being harder for the evening-types than for the morning-types.

In this vein, it is also important to address the qualitative differences found in performance of the category semantic priming task between the two extreme chronotypes. Our results are in line with studies suggesting better adaptation by morning-types at non-optimal times (Lara et al., 2014; Martínez-Pérez et al., 2020; Palmero et al., 2022); however, we go further by suggesting that it is plausible that their cognitive-control styles may be substantially different. Thus, following Braver's (Braver, 2012a) theory on the dual-mechanism framework of cognitive control, the proactive control style refers to the maintenance of active response strategies so that the individual can anticipate the occurrence of a conflict or a cognitively demanding event and resolve it without producing noticeable negative effects. On the other hand, the reactive control mode is related to a "late correction", which suggests that the resolution of the conflict takes place once it has occurred and has been detected. Both styles are variable at the intraindividual level, but interestingly, it has been suggested that there are differences at the interindividual level that place cognitive control as an individual trait driving the way different people deal with and resolve tasks that demand cognitive control. In this sense, the response style observed in the evening-types could be associated with a greater degree with the reactive style, while the consistent application of the attention-based strategy from the very beginning of the task by the morning-types would be evidence of a proactive mode of response in these individuals. In addition, the development of reactive control strategies has been linked with anxiety traits (Fales et al., 2008), to which evening-types are selectively associated (Antypa et al., 2016). The study of cognitive control styles in morning- and evening-types constitutes a line of future research that deserves further attention.

### *Limitations and future directions*

The present study has certain limitations that need to be mentioned. The main conclusions regarding the different pattern of results shown by the two extreme chronotypes should be taken with caution, as when entering chronotype as a between-participants factor in the overall ANOVAs, the four-way interaction was statistically significant only with the accuracy data but not with the RTs. However, further analysis showed that the interaction was significant when performance was assessed only at the non-optimal time-of-day, probably because morning-types showed greater stabilization of performance throughout the day compared to evening-types.

A second limitation is that our task was not able to capture large automatic semantic priming effects (see (Langley et al., 2008)), which could have made it difficult to observe a clearer circadian modulation of automatic processing. Instructions about the low rate of related trials and the emphasis on accuracy might have promoted the activation of controlled processing earlier than in previous studies.

A third limitation is the split of the 100-ms SOA into subblocks of trials, mainly in the intermediate-types, which may have resulted in a lack of sufficient statistical power. Given that the number of trials in each of the subblocks is rather low, modulatory effects of priming at that short interval could have been more difficult to observe, above all with extreme chronotypes. However, we consider important to include this analysis so that future studies may address this issue with more trials per condition and more appropriate tasks to tap automatic processing, as the present results with the shortest SOA suggest that the time course of automatic processing can differ significantly with time-on-task and time of testing in each chronotype.

Finally, given that hours sleeping in relation to automatic and controlled processing has given rise to a chronotype-related approach to studying these cognitive processes (Harrison & Horne, 1999; Horne, 1993), it would also be interesting to link future studies of the time course of both types of processes in relation to hours of sleep.

### ***Conclusions***

The present results are framed within the literature that has studied the modulation of cognitive processes by chronotype and time-of-day. Although it has usually been considered that only controlled processing is modulated by time-of-day, our study reflects a qualitative distinction between the two extreme chronotypes not only in controlled processing but also in automatic processing. Thus, while morning-types were able to apply control quickly and easily to reverse the automatic process, evening-types were more influenced by the time-of-day, so that they could reverse the automatic process only at the optimal time-of-day. This pattern of results points to the need to further study the differences between the extreme chronotypes and to consider them as distinct groups, given that their cognitive style of functioning seems to be apparently different. In addition, we highlight the need to understand circadian modulations based on the difficulty experienced by individuals in performing the task, which directly connects to their unique cognitive traits.

## Declarations

### *Ethics approval and consent to participate*

Written informed consent was obtained from all participants prior to their participation. Data obtained from participants have been fully anonymized under their acknowledgment. The study was approved by the Ethics Committee of the University of Murcia and was carried out in accordance with the ethical standards of the Declaration of Helsinki.

### *Data availability statement*

The dataset generated and analyzed during the current study as well as the R scripts used to preprocess the data are available in OSF repository (peer-review link): https://osf.io/kjhna/?view_only=16bc0c55949d4fa5ad800c60e0a692ec.

### *Disclosure statement*

The authors report no conflict of interest.

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### *Authors' contributions*

**LBP**: Conceptualization, Methodology, Investigation, Software, Formal analysis, Data curation, Writing - original draft, Visualization **MT**: Methodology, Investigation, **VMP**:

Methodology, Investigation **ASL**: Methodology, Investigation **GC**: Conceptualization, Methodology, Software, Supervision **LJF**: Conceptualization, Writing-Original draft preparation, Writing-Reviewing and Editing, Supervision, Visualization, Funding acquisition. All authors read and approved the final manuscript.

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## Study 4:

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**Abstract**:

We assessed whether self-related automatic and others-related controlled processes are modulated by chronotype and time-of-day. Here, we used a shape-label matching task in which three geometrical shapes (square, circle, triangle) were arbitrarily associated with labels referred to oneself (*you*) or others (*friend* and *stranger*). Participants with Morning-type or Evening-type chronotypes performed the shape-label matching task at the optimal and non-optimal times of day according to their chronotype. Morning-types showed no cost in performance at their non-optimal time of day, which suggests a better adaptation of these participants to non-optimal moments of the day. Contrary to our initial predictions, we found a modulation of self-related but not others-related processing by chronotype and time-of-day in Evening-type participants. We suggest that such modulation may be due to the dependence of the activation of the VMPFC cortex, an essential component of the self-attention network involved in self-related processing, on circadian rhythms

## Title

# Testing the Modulation of Self-related Automatic and Others-related Controlled Processing by Chronotype and Time-of-day

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## Abstract

We assessed whether self-related automatic and others-related controlled processes are modulated by chronotype and time-of-day. Here, we used a shape-label matching task in which three geometrical shapes (square, circle, triangle) were arbitrarily associated with labels referred to oneself (*you*) or others (*friend* and *stranger*). Participants with Morning-type or Evening-type chronotypes performed the shape-label matching task at the optimal and non-optimal times of day according to their chronotype. Morning-types showed no cost in performance at their non-optimal time of day, which suggests a better adaptation of these participants to non-optimal moments of the day. Contrary to our initial predictions, we found a modulation of self-related but not others-related processing by chronotype and time-of-day in Evening-type participants. We suggest that such modulation may be due to the dependence of the activation of the VMPFC cortex, an essential component of the self-attention network involved in self-related processing, on circadian rhythms.

## Keywords

Self-related automatic processing, others-related controlled processing, chronotype, time-of-day, self-prioritization effect, ventromedial prefrontal cortex

## 1. Introduction

There are numerous studies demonstrating processing advantage when information from the environment is related to oneself. Thus, when we perceive self-related cues, such as hearing our name or seeing our face, our attentional focus is automatically directed to those stimuli, while other information not associated with oneself is processed in a more controlled way. This effect is known as the Self-Prioritization Effect (SPE; for a review, see Cunningham & Turk, 2017). The SPE is purely adaptive in nature, as it leads individuals to not ignore cues that genuinely concern them (Cunningham, 2016; Cunningham et al., 2013; Klein, 2012). This prioritization scheme in processing information relevant to oneself has been robustly evidenced at both behavioral and neural levels.

At the behavioral level, the SPE is described as a performance advantage that is reflected in faster reaction times (RTs) and/or higher rates of accuracy in processing self-related information compared to information related to others. Specifically, this effect has been observed in a variety of cognitive tasks involving various cognitive domains such as attention (Arnell et al., 1999; Dalmaso et al., 2019; Liu et al., 2016; Shapiro et al., 1997; Sui et al., 2009; Sui & Han, 2007), memory (Conway & Pleydell-Pearce, 2000; Cunningham et al., 2008; Kesebir & Oishi, 2010; Rogers et al., 1977), working memory (Yin et al., 2019), and decision-making (Humphreys & Sui, 2015; Keyes & Dlugokencka, 2014; Ma & Han, 2010; Sui & Han, 2007). In addition, the SPE has been related to simpler cognitive operations, such as perceptual matching. In this regard, Sui et al., 2012, developed an unbiased approach to assess the SPE that begins with learning the association between three different geometric shapes (e.g., "circle", "square" and "triangle") with three labels related to the self (you), a close person (friend), or an unknown person (stranger). Subsequently, both the geometric shapes and the labels are presented in pairs to the participants, who have to judge whether or not the current pair is correct based on the associations they have previously learned. Results commonly observed using this shape-label matching task reflect an SPE in both shorter reaction times (RTs) and better accuracy rates for stimuli previously associated with the self (you), compared to friend- and stranger-related associations (Dalmaso et al., 2019; Desebrock et al., 2018; Liang et al., 2022; Martínez-Pérez, Campoy, et al., 2020). Moreover, this task is shielded from other variables potentially linked to SPE, such as the familiarity, concreteness, frequency, or length of the words used (Humphreys & Sui, 2015; Sui et al., 2012).

At the neural level, different studies have addressed the neural circuit related to the self. For example, in the meta-analysis conducted by Denny et al., (2012) the results highlighted the role of the medial prefrontal cortex (MPFC) in both self- and others-related judgments. Furthermore, these authors linked the ventromedial prefrontal cortex (VMPFC) to self-related judgments, while the dorsolateral prefrontal cortex (DLPFC) would be activated during judgments related to others. Sui et al., (2013) conducted a functional magnetic resonance imaging (fMRI) study using the aforementioned perceptual matching task and observed that self-associated stimuli involved the activation of the VMPFC, while those related to friend and stranger (others) involved activation of the DLPFC. These areas, together with the left posterior superior temporal sulcus (LpSTS) and the intraparietal sulcus (IPS), were assumed to be part of the self-attention network (SAN; Humphreys & Sui, 2015), forming the VMPFC and the LpSTS the ventral attentional part of the network, and the DLPFC and the IPS its frontoparietal attentional part. The ventral network would activate in self-related stimulus processing, automatically driving one’s attention to that type of stimuli. In contrast, the frontoparietal network is typically associated with goal-related task demands, and the network activation should increase with more complex tasks. Importantly, the two networks should be inversely related: as the processing of stimuli requires more control (self → friend → stranger), activation would move from the ventral to the frontoparietal network.

In the present study, we asked whether the processing of stimuli associated with oneself, which is assumed to draw attention in a rather automatic way, and processing of stimuli related to others, which is assumed to require cognitive control, can be differently modulated by individual differences in chronotype and the time of day in which the assessment takes places, that is, taking into consideration variations in circadian rhythm patterns.

Chronotype is related to circadian rhythms and is described as the difference in the preference individuals develop for performing their activities of daily living and rest times at one time of the day or another (Levandovski et al., 2013; Schmidt et al., 2007). The assessment of this trait allows individuals to be classified into different circadian profiles. Individuals who do not develop any circadian preference form the intermediate chronotype, while by extreme chronotypes we mean those individuals who show a clear preference for a time of day for their daily activities. Morning-types prefer to perform their activities in the early morning, while Evening-types prefer to perform their activities late in the day. Thus, it is possible to obtain indices of the cognitive performance of individuals at their optimal and non-optimal times depending on their chronotype. The effect commonly observed using this paradigm is called the *synchrony effect* and refers to the improvement in performance when it occurs at the time of day that coincides with the participants' preference, while deterioration arises when the time of day is the opposite of their chronotype. This synchrony effect has been demonstrated not only at the behavioral level but also at the level of cortical activation. For example, Salehinejad et al., (2021) reported cortical arousal/inhibition balances congruent with optimal and non-optimal times, respectively, for both Morning- and Evening-type chronotypes.

The relevance of looking at chronotype and time-of-day as potential modulators of stimuli associated with self or others is that automatic processes are less vulnerable than controlled processes to the low levels of arousal that characterize non-optimal times-of-day (Lara et al., 2014; Manly, 2002; May et al., 2005; although see Palmero et al., under review, for some evidence of chronotype and time of testing modulation of automatic processing). Here, we set out to determine whether automatic processing related to self and controlled processing related to others (friend and stranger), as commonly observed in the perceptual matching task (Sui et al., 2012), can be modulated by participants' chronotype (Morning- or Evening-types) and time-of-day (optimal or non-optimal). That is, we hypothesize that processing of self-related stimuli should resist when participants perform the task at the non-optimal compared to the optimal time of day. Conversely, concerning the processing of others-related stimuli, we hypothesize that as such processing requires more cognitive control (i.e., from friend- to stranger-related associations) a chronotype and time-of-day modulation is expected, causing larger costs in performance when processing stranger-related stimuli than when friend-related stimuli are processed at non-optimal time-of-day. It is important to highlight here that previous research has revealed that Morning-types performance is usually more constant and invariable throughout the day compared with Evening-types performance, perhaps because Morning-types are more sensitive than Evening-types to factors that increase their arousal levels even at their non-optimal time-of-day (Correa et al., 2014; Martínez-Pérez, Palmero, et al., 2020; Mongrain et al., 2008; Palmero et al., 2022, Palmero et al., under review). Consistent with this, in a previous study in which we evaluated the time course of the semantic priming effect (Palmero et al., under review), time-of-day was found to modulate automatic semantic processing only in Evening-types, whereas Morning-types showed no modulation. Therefore, it is likely that the expected modulatory effects described before may differ between the two extreme chronotypes.

We finally envisaged the possibility that chronotype and time-of-day do not produce differential modulatory effects according to the type of processing required (self-related or others-related processing), but rather participants are simply more effective at responding when they perform the task at their optimal moment compared to when they perform it at their non-optimal moment. If that were the case, we should observe an advantage when participants perform the perceptual matching task at their optimal time-of-day, irrespective of the kind of shape/label pair they are presented with.

## 2. Methods

### *2.1 Participants*

Our sample was composed of sixty-four healthy undergraduate volunteers (32 females, $M_{age}$ = 21.14; $SD_{age}$ = 5.45) who met the criteria of definite Morning- (scores between 17 and 25, M=18,50, SD=1.60) or Evening-types (scores between 4 and 11, M=9.25 SD=1.68) according to the reduced version of the Horne and Östberg's Morningness-Eveningness Questionnaire (rMEQ) standardized by Adan and Almirall (1991) for the Spanish population. The rMEQ consisted of five items to easily evaluate a participant's chronotype, with total scores ranging from 4 (definitively Evening-types) to 25 (definitively Morning-types). Participants received course credits as compensation for taking part in the study.

### *2.2 Apparatus, stimuli, and procedure*

Participants completed two sessions separated by approximately one week. The sessions were scheduled at 8:00 AM (morning session) and at 8:30 PM (evening session). The order of the sessions was balanced across participants within each chronotype group such that half of the Morning-types came to the lab in the first session at their optimal time of day and the other half half at their non-optimal time of day, and the same for the Evening-type participants. Participants were previously requested not to take any stimulant substance such as caffeine or theine in the 2 hours prior to the beginning of their sessions. Each experimental session had the same structure and duration (1 hour approximately). Participants were asked about the total hours of sleep the night before the experimental session and then began with the Psychomotor Vigilance Task (PVT, Lim & Dinges, 2008) and end up performing the shape-label matching task (Sui et al., 2012). In between, participants performed another cognitive task described in a separated study. In the PVT (10 min duration), each trial started with a blank screen during a random interval between 2 and 10 s. Then, a red circle of 50 pixels in diameter popped up at the center of the screen. Participants had to press the central button of the Chronos device with the index finger of their dominant hand as quickly as they detected the red circle. After responding, the screen became black, and a new trial began. The shape-label matching task (14 min approximately) was equivalent to that used by Sui et al., (2012) (Experiment 1) and (Martínez-Pérez et al., 2020) and was composed of two phases. Participants first were verbally asked to remember for 1 minute the associations between three geometric shapes (triangle, circle, and square) and three verbal labels (*you*, *friend*, or *stranger*). The shape-label associations were counterbalanced across participants and equally represented in each chronotype group. Also, it is important to note that participants completed the task in the same counterbalanced group in both sessions. In the second phase, participants performed a matching task in which they had to judge whether the different shape-label pairs matched or not the previously given associations. Each trial started with the presentation of a 500-ms central fixation cross. Suddenly, a shape and a label were simultaneously presented above and below the fixation cross for 100 ms, followed by a blank-1100 ms screen in which participants must give a response. In this line, participants had to indicate whether the shape-label pairs matched or mismatched by pressing as quickly and accurately as possible one of two response buttons (the rightmost button or the leftmost button of the response box, respectively). Then, a feedback message ("correct" or "incorrect") appeared during 500 ms and a new trial began. Each participant completed five blocks of 48 trials. Each block was composed of 8 trials for each shape-label combination (you-matched, you-nonmatched, friend-matched, friend-nonmatched, stranger-matched, and stranger-nonmatched) presented in random order. Participants completed a practice block of 48 trials with the same distribution as the experimental ones. With a view distance of 60 cm, the three geometrical shapes subtended visual angles of 4º × 4º approximately and were presented above a fixation cross (1º × 1º). The Spanish words *TU* (*you*), *AMIGO* (*friend*), and *EXTRAÑO* (*stranger*) were displayed below the fixation cross (1.7º high × 1.4º, 4º, or 4.2º width, respectively). The distances between the fixation cross and the center of the shape and the center of the label were 4º and 3º, respectively. The background color of the screen was gray, and stimuli were presented in white. The two experimental tasks were programed in E-Prime 3.0 software (Schneider et al., 2002) and presented on a 22-in. TFT monitor (resolution = 1920 × 1080 pixels). Responses were recorded by using a Chronos device (Psychology Software Tools).

## 3. Results

Data were processed using R software (R Core Team, 2017) and analyzed with JASP 0.9.2 (JASP Team, 2019). The PVT RTs and the perceptual matching task were transformed into natural logarithms to reduce skewness in the distribution.

The PVT data were fully reported in Palmero et al. (under review), as the shape-label matching task was part of the same experimental session and was performed by the same participants. Only the time-of-day (optimal, non-optimal) effect produced significant differences, indicating the suitability of this task for observing synchrony effects between extreme chronotypes.

As for the shape-label matching task, two different analyses were performed, one for RTs and the other for accuracy data. Practice trials and non-response trials (2.55%) were removed from both the TR and accuracy analyses. In addition, trials with incorrect response (10.76%) were also excluded from the RT analysis. Visual inspection of individual RT distributions for each participant in each condition did not reveal the presence of extreme outliers, and thus no trimming procedure was applied (note that there was a response window of 1100 ms, which limited the possibility of extreme RTs). RTs were partitioned into five rank-ordered RT bins to take into consideration the additional information that might arise from the RT distributions.

We adopted a statistical significance level of $\alpha = .05$ for all statistical analyses. In addition, we present effect size values for our contrasts using partial eta squared ($\eta_p^2$) in ANOVAs, and the mean difference (MD) and 95% CI for Student's t-tests. In addition, since different hypotheses are considered in this study and, in order to minimize the possibility of committing family-wise error rates (FWER), the Holm-Bonferroni correction is applied to all the post-hoc t-test analyses.

### *3.1 Accuracy*

Percentages of correct responses were introduced into a 2 × 3 × 2 × 2 mixed ANOVA with Match (matching, non-matching), Label (*you*, *friend*, *stranger*), and Time-of-day (optimal, non-optimal) as within-participants factors, and Chronotype (Morning-types, Evening-types) as the between-participants factor.

The main effect of Label was significant, $F(1,36) = 16.03$; $p < .001$; $\eta_p^2 = .30$, indicating that responses for the condition *you* were significantly more accurate (M = 92%) than those for conditions *friend* (M = 88%), and *stranger* (M = 87%). The difference between condition *friend* and condition *stranger* was not significant ($p_{\text{Holm}} = .28$). The Label × Match interaction was also significant, $F(2,76) = 11.60$; $p < .001$; $\eta_p^2 = .23$.

In the matching trials, conditions *you* and *friend*, *you* and *stranger*, and *friend* and *stranger* differed significantly, $t(39) = 3.5$, $p_{\text{Holm}} = .006$, MD = .05, 95% CI [.008, .91]; $t(39) = 6.97$, $p_{\text{Holm}} < .001$, MD = .09, 95% CI [.05, .14]; and $t(39) = 3.44$, $p_{\text{Holm}} = .008$, MD = .05, 95% CI [.006, .09], respectively. In the non-matching trials, none of the above three comparisons were statistically significant (all $ps_{Holm} >.12$). Neither the main effect of Time-of-day nor that of Chronotype reached statistical significace ($p$s > .18). The Label × Match × Time-of-day interaction did not reach statistical significance either, $F(2,76) = .50$; $p = .61$; $\eta_p^2 = .01$.

### *3.2 Reaction times (RTs)*

Mean ln-RTs were submitted to a 2 × 3 × 2 × 5 × 2 mixed ANOVA with Match (matching, non-matching), Label (*you*, *friend*, *stranger*), Time-of-day (optimal, non-optimal), and Bin (1-5) as within-participants factors, and Chronotype (Morning-types, Evening-types) as the between-participants factor.

The main effects of Match and Label were statistically significant, $F(1,38) = 393.86$; $p < .001$; $\eta_p^2 = .91$, and $F(2,76) = 68.96$; $p < .001$; $\eta_p^2 = .65$, respectively. RTs were faster in matching than in non-matching trials, and faster in the condition *you* than in conditions *friend* and *stranger* ($p$s$_{\text{holm}}$ <.001). The difference between conditions *friend* and *strange*r did not reach the statistical significance level ($p_{\text{holm}} = .26$). The lack of a significant Label × Match interaction indicates that the SPE was similar in matching and non-matching trials. Neither the main effect of Time-of-day nor that of Chronotype reached the statistical significance level (all $p$s >.14).

However, the main finding was the significant Label × Time-of-day × Chronotype × Bin interaction, $F(8,304) = 2.65$; $p= .008$; $\eta_p^2 = .06$. To further analyze the interaction, and in line with previous studies that have consistently shown different patterns of results between Morning- and Evening-types (Lara et al., 2014; Martínez-Pérez et al., 2020; Palmero et al., 2022), we conducted separate analysis for each chronotype.

### *3.2.1 Morning-types*

Mean ln-RTs were submitted to a 3 × 2 × 5 repeated-measures ANOVA with Label (*you*, *friend*, *stranger*), Time-of-day (optimal, non-optimal), and Bin (1-5) as within-participants factors. The main effect of Label was significant, $F(2,38) = 28.77$; $p < .001$; $\eta_p^2 = .60$, indicating faster RTs in the condition *you* than in conditions *friend* and *stranger*, but the difference between the conditions *friend* and *stranger* did not reach the statistical significance level ($p = .09$). The three-ways Label ×Time-of-day × Bin interaction was not statistically significant, $F(8,152) = .88$; $p = .53$; $\eta_p^2 = .04$. Neither was any other interaction involving the Time-of-day factor (all $p$s > .11). The results of these analyses are illustrated in Figure 1 (left panel).

**Figure 1.** Time-of-day modulation of performance of Morning-types (left panel), and Evening-types (right panel) in each label condition: *you*, *friend, stranger*, as a function of response speed.

Insert here Figure 1

### *3.2.2 Evening-types*

Mean ln-RTs were submitted to a 3 × 2 × 5 repeated-measures ANOVA with Label (*you*, *friend*, *stranger*), Time-of-day (optimal, non-optimal), and Bin (1- 5) as within-participants factors. The main effects of Label and Time-of-day were significant, $F(2,38) = 40.58$; $p < .001$; $\eta_p^2 = .68$, and $F(1,19) = 5.05$; $p = .04$; $\eta_p^2 = .21$, respectively. RTs were faster in the condition *you* than in conditions *friend* ($p_{\text{Holm}}$ <.001), and *stranger* ($p_{\text{Holm}}$ <.001)*,* but we did not find any significant difference between conditions *friend* and *stranger* ($p_{\text{Holm}} = .25$). RTs were also faster in the optimal than in the non-optimal time-of-day. Importantly, the Label × Time-of-day × Bin interaction was statistically significant, $F(4,76) = 3.25$; $p = .002$; $\eta_p^2 = .15$. According to our previous hypotheses, we were interested in assessing whether RTs differed between the optimal and non-optimal time-of-day in each label condition and if that potential time-of-day modulation of self- and others-related associations differed as a function of response speed.

In condition *you*, we found a significant effect of Time-of-day, $F(1,19) = 5.75$; $p = .02$; $\eta_p^2 = .23$. RTs were faster in the optimal than in the non-optimal time-of-day. However, the Time-of-day × Bin interaction did not reach the statistically significant level, $F(4,76) = 1.87$; $p = .12$; $\eta_p^2 = .09$. In condition *friend*, we found a significant effect of Time-of-day, $F(1,19) = 4.34$; $p = .05$; $\eta_p^2 = .19$. Again, RTs were faster in the optimal than in the non-optimal time-of-day. The Time-of-day × Bin interaction was also significant $F(4,76) = 4.37$; $p = .003$; $\eta_p^2 = .19$. The interaction analysis revealed that the advantage of the optimal time-of-day in performance occurred only in the first bin. In bin 1, $t(39) = 3.57$; $p_{\text{Holm}} = .01$; MD = .09, 95% CI [-.002, .19]; in bin 2, $t(39) = 1.80$; $p_{\text{Holm}} =.33$; MD = .04, 95% CI [.04, .14]; in bin 3, $t(39) = 1.57$; $p_{\text{Holm}} =.38$; MD = .05, 95% CI [.04, .14]; in bin 4, $t(39) = 1.39$; $p_{\text{Holm}} = .38$; MD = .04, 95% CI[ .03, .13]; and in bin 5, $t(39) = 1.18$; $p_{\text{Holm}} = .38$; MD = .03, 95% CI [.06, .13]. In condition *stranger*, neither the Time-of-day nor the Time-of-day × Bin interaction reached the statistically significant level, $F(1,19) = 1.99$; $p = .17$; $\eta_p^2 = .09$, and $F(4,76) = .27$; $p = .90$; $\eta_p^2 = .01$, respectively. The results of these analyses are illustrated in Figure 1 (right panel).

## 4. Discussion

In the present study, we aimed to link the SPE to a trait related to circadian rhythms known as chronotype and to the time of day at which the task is performed. The SPE was assessed using an unbiased task designed by Sui et al. (2012), in which participants associated three different geometric shapes with verbal labels related to themselves, a friend, or a stranger, and then judged whether the presented label-shape pairings were correct based on what they had previously learned. We found a significant SPE in both accuracy and RTs. Moreover, in RTs the SPE appeared specifically on both matching and non-matching trials, whereas in accuracy the SPE only appeared on matching trials. Our results replicate previous studies on the existence of the SPE in the perceptual domain using the shape-label matching task (Dalmaso et al., 2019; Desebrock et al., 2018; Martínez-Pérez, Campoy, et al., 2020; Sui et al., 2012).

In addition to studying the SPE, our main interest focused on the distinction between the processing styles of stimuli related to oneself (you) and those related to others (friend and stranger). In the former case the processing mode seems to occur rather automatically, whereas the latter requires a higher degree of cognitive control. In the present study and based on previous studies concerned with the modulation of automatic and controlled cognitive processing as a function of chronotype and time-of-day, we hypothesized that such circadian modulation would mainly affect controlled processes, that is, processing of friend-related and mainly stranger-related stimuli. However, contrary to our initial predictions, our results showed a significant modulation of chronotype and time-of-day on automatic processing, and it occurred only in the Evening-type participants. That is, we found differences between optimal and non-optimal time-of-day only when participants processed self-related stimuli, and partially when they processed friend-related stimuli. When participants processed stranger-related stimuli, we found no evidence of such modulation.

These results contradict those of previous studies that have observed an absence of chronotype and time-of-day modulation in automatic processing (Lara et al., 2014; Manly, 2002; May et al., 2005). However, in a recent study Palmero et al. (under review) observed a modulation of automatic semantic processing only in Evening-type participants. Participants performed a category semantic priming task at both their optimal and non-optimal time-of-day, and were able to suppress the automatic activation of the prime category only when the task was performed at their optimal time, producing a synchrony effect. Importantly, the synchrony effect was not observed neither in the Intermediate-type nor in the Morning-type groups of participants. These results reveal that under certain circumstances, automatic processes are prone to modulatory effects of both chronotype and moment of testing.

An explanation of the specific modulatory effect of time-of-day on self-related automatic processing observed in the current study may lie in the relationship between the main areas involved in the SAN and their close relationship with circadian rhythms. Humphreys and Sui (2015) pointed to the VMPFC and DLPFC regions as the core areas for the processing of self- and others-related stimuli, respectively. However, despite some studies have supported the SAN's proposal, contradictory results regarding the explicit role of each network-related area have also been reported.

Regarding the role of the VMPFC in self-related processing, Martínez-Pérez et al. (2020) attempted to modulate the SPE by applying excitatory and inhibitory high-definition transcranial direct current stimulation (HD-tDCS) over the VMPFC. The authors reported null results in terms of SPE modulation. Liang et al. (2022) conducted a study using transcranial magnetic stimulation (TMS) and showed that it was the LpSTS instead of the VMPFC which causally affected self-related processing. However, Liang et al. (2022) argued that because both areas are functionally connected, any damage to either the VMPFC or the LpSTS should decrease the SPE. Given that during the non-optimal time-of-day according to the chronotype, occurs a decrease in cortical excitability balance and an increase in inhibition levels (Salehinejad et al., 2021), our results suggest that VMPFC activation would be compromised when Evening-type participants performed the task at the non-optimal time-of-day, affecting negatively self-related automatic processing.

Regarding the role of the DLPFC, recent studies have shown its association with cognitive functions closely linked to circadian rhythms, such as vigilant attention (Martínez-Pérez et al., 2022; Sturm & Willmes, 2001), and that area is recruited in tasks requiring a high degree of executive control (Duncan & Owen, 2000; see Friedman & Robbins, 2022 for a recent review), although its activation is severely altered under conditions of sleep deprivation (Bratzke et al., 2012). However, both Liang et al. (2022) and Martínez-Pérez et al. (2020) failed to find any involvement of the DLPFC in the SPE. These results put into question any causal relationship of the DLPFC in others-related processing, probably due to the involvement of other attention-related brain regions such as the temporoparietal junction (TPJ), which is crucial in the distinction between self and others (Brass et al., 2009; Fuentes-Claramonte et al., 2020; Spengler et al., 2009). Thus, our failure to find any time-of-day modulation on the others-related processing suggests that the brain areas involved may not be so prone to changes in excitation/inhibition balance due to circadian rhythms.

An alternative explanation should also be taken into consideration. It is possible that performing the task at the non-optimal time-of-day affects participants' performance in those conditions where fast responding is promoted, as it happens in self-related processing. Fast responses have room to slow down under certain adverse circumstances, such as performing the task at a non-optimal time when the arousal level is low. In the case of slower responses, such as the processing of others-related stimuli, there might be less room for worsening performance, perhaps because performance is close to a ceiling effect. In favor of this explanation is the fact that the time-of-day modulation effect occurs in those conditions in which faster responses are observed, i.e., in condition *you* and in the range of faster responses (first bin) in condition *friend*. However, the fact that time-of-day modulation effects occur in both matching (faster responses) and non-matching trials (slower responses) in conditions *you* and *friend*, would argue against this explanation. Therefore, the findings of the present study lead us to reconsider the modulation of automatic processing by chronotype and time-of-day. In this sense, we cannot maintain the claim that there is no circadian influence on those processes that do not require cognitive control (i.e., automatic processing), but that there are other factors that are potentially influential in such modulation. One of those factors is the specific chronotype of the individuals, mainly Evening-types, who are particularly vulnerable to such influences, but also the type of task used as well as the brain areas involved. Thus, we conclude that the modulation we observed in self-related automatic processing as a function of time-of-day can be due to the association between activation levels of self-related brain areas involved in the SAN (e.g., the VMPFC) and circadian rhythms.

A final issue concerns the absence of time-of-day modulation effects in Morning-type participants. The pattern of results observed here in these participants is in line with the results of previous studies suggesting greater flexibility and adaptation of Morning-types when performing tasks at their non-optimal time-of-day (Adan et al., 2012; Lara et al., 2014; Martínez-Pérez et al., 2022; Mongrain et al., 2008; Palmero et al., 2022; Schmidt et al., 2015). At the behavioral level, the Evening-type chronotype has been linked to certain personality traits such as impulsivity, greater sensation seeking, less vigilance, and less conscientiousness (Finomore et al., 2009; Oginska et al., 2010). In addition, it has been suggested that changes in sleep dynamics could explain the differences between Morning- and Evening-types (Lara et al., 2014). Indeed, some research associates poor sleep habits and a greater need for sleep with Evening-types, while sleep hygiene would be more associated with Morning-types (Lehnkering & Siegmund, 2007; Taillard et al., 2002). At the physiological level, interactions have been described between the activation of certain hormones such as cortisol or other female sex hormones such as estrogen and progesterone and circadian rhythms, which would also explain the better performance of Morning-type women in sustained attention tasks (Palmero et al., 2022). However, more research is needed on linking specific physiological patterns in terms of cortical excitability, with specific chronotype profiles.

The present study has some limitations that should be taken into account. The interpretation of the results observed here would benefit from measuring cortical excitability levels of the brain areas involved (Salehinejad et al., 2021). In addition, more information on the sleep quality of our participants would have allowed for a more detailed explanation of the main differences observed between the two chronotypes. Addressing the proposed limitations would contribute to improving knowledge about the cognitive and physiological functioning of the Morning- and Evening- chronotypes and understanding the specific influences that certain circadian variables exert on automatic and controlled processes.

## 5. Conclusion

The results of the present study call for a reconsideration of the modulation of automatic processing by chronotype and time-of-day. In this sense, we cannot assert that there is no circadian influence on those processes that do not require any degree of cognitive control (i.e., automatic processing), but that there are other potentially influential variables in this modulation. One of the most important of these is the specific chronotype of individuals, especially the Evening-type chronotype, which has been found to be particularly vulnerable to such influences. Others refer to the type of tasks used, which may vary in the demands of cognitive control, as well as to the brain areas involved in them, specifically the VMPFC, involved in cognitive operations that seem to be especially influenced by circadian variations.

## 6. Ethical approval

The present study is part of a research project approved by the ethics committee of the University of Murcia and adjusted to the standards set forth in the Declaration of Helsinki in 1964.

## 7. Informed consent

Informed consent was obtained from all individual participants included in the study.

## Declaration of Competing Interest

The authors declare the lack of competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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**Data availability statement:** The R script and the original data of this study are available in OSF (view-only link for peer reviewing: https://osf.io/68aw9/?view_only=c8dc43a7b09a4b6eb3af5e9666d2f78a).

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## General Discussion and Conclusions

In the present doctoral thesis, we have approached the study of the circadian modulation of attentional processes through an experimental series of four studies with specific objectives. The circadian paradigm that has been transversal in this doctoral thesis (i.e., the chronotype), tightens the ties between biology and cognition in the scope of laboratory research not only narrower, all the more relevant is the social repercussion that derives from this sphere what renders the chronotype and time of day factors even more significant for being considered also in more organic contexts. In this vein, as the development of our society has prompted the abandonment of the individual's familiar 24-hour diurnal cycle, it is becoming increasingly common for certain activities to be necessarily relegated to time slots normally associated with rest, for instance, medical guards, the work of an air traffic controller at busy airports overnight, or simply the increase in night-time leisure activities. By using specific attentional paradigms, we attempted to reproduce particular settings where the cognitive demand is similar: sustained attentional functions over time, conflict resolution, or the application of control strategies in circumstances where operating in automatic modes may be highly maladaptive. In this regard, it is noteworthy that the study of modulatory variables on attentional processes that are linked to individual differences in the baseline of participants as the chronotype, not only expands the theoretical insights into the way these attentional processes operate but it also permits the exploration of promising interventions. In this respect, the chronotype paradigm has enabled us, on a basic level, to dissociate individuals' arousal states in order to subsequently assess their performance in higher-level attentional domains, thus leading us toward a more accurate understanding of their inner functioning. Thus, in **Study 1**, we analyzed how the synchrony effect occurs in Morning- and Evening-types in tasks of varying difficulty, as well as exploring in depth the interaction between alertness and executive control on the basis of Michael Posner's model of attentional networks. Also, in **Study 2**, we dissociated vigilance components in women with extreme chronotypes and observed that the time of day affected each of the cognitive processes assessed differently. Finally, **Studies 3**, and **4**, allowed us to delve deeper into the influence of chronotype on automatic and controlled processing, providing initial evidence of the circadian modulation of automatic processes.

On the other side, ascertaining the variation in performance according to the chronotype (especially at off-peak times of the day) allowed us to engage in the exploration of its potential restoration. In this regard, in Study 1 we analyzed the effect of triggering the phasic alerting process (i.e., by a warning signal presentation), which targeted Evening-types, as well as the novelty effect produced during the first experience that selectively benefited Morning-type participants. Moreover, in Study 2, we tested the effectiveness of progesterone in reverting the synchrony effect in participants of extreme chronotypes. Again, the results revealed a differentiated pattern as a function of chronotype and time of day.

The general conclusions obtained through this work are outlined below, and aim, on the one hand, to bring the attentional processes even closer to neuroscience, and, on the other, to transfer cognitive neuroscience into real-life scenarios so as to contribute to building the foundations for improving people's cognitive performance.

### *Study 1*

- In Morning-type participants, synchrony effects appeared in the PVT only when the first session occurred at its optimal time. This finding suggests that the novelty effect prevented performance decay at non-optimal times. Furthermore, in the ANTI, there was no synchrony effect at all, thereby indicating that the difficulty of the task itself maintained performance levels constant at either of the two extreme times of the day.

- Synchrony effects appeared in Evening-types in both tasks: neither benefited from task difficulty nor from the novelty effect.
- Evening-type participants benefited more noticeably from higher levels of phasic alertness at non-optimal times, raising the compensatory qualities of phasic alertness over tonic alertness levels.
- The presence of the warning signal produced a generalized acceleration in the RTs of Morning-types, thus suggesting the beyond-compensatory function of tonic alertness as rather being a trigger of the motor response.
- The negative effect of alertness on executive control was due solely to the transient state triggered by the warning tone. Moreover, the lack of compensation of this negative effect by the high level of tonic alertness (i.e., optimal times of day), hints that the interaction between these two networks fits better on the attentional spatial-diffusion hypothesis.

***Study 2***

- In the PVT, Morning-types exhibited a marginal improvement in performance in the mid-luteal phase, maximizing the synchrony effect. In contrast, Evening-types had a baseline (i.e., follicular phase) synchrony effect that disappeared at the mid-luteal phase by worsening performance during the optimal times.
- In the SART, Morning-types developed a generalized synchrony effect, although it was only statistically significant during the mid-luteal phase, indicating an increase in accuracy under conditions of elevated progesterone. Evening-types followed a similar pattern to the PVT but neither effect reached statistical

significance. In this case, the difficulty of the task might have prevented them from a progesterone-mediated impairment of performance.

- The seeming pattern of opposing results (i.e., improved optimal-time performance under conditions of high progesterone levels in Morning-types, and worsening in Evening-types), fits neatly into the theory of interaction between the adrenal and gonadal hormonal axes. Thus, beneath high cortisol levels, progesterone may elongate glucocorticoid secretion and circulatory capacity, boosting the general arousal of the individual. On the other hand, at low or no cortisol levels, progesterone might accelerate the inhibition of glucocorticoid secretion, plunging women into a state of arousal deficit that may lead to a significant drop in cognitive performance.

***Study 3***

- Morning-types displayed a strong trend toward the development of control-based responses from the shortest SOA (i.e., 100 ms) to the longest one (i.e., 850 ms). Moreover, this pattern was not affected in any case by the time of day. There might be several reasons behind the lack of modulation by the time-of-day factor. Firstly, the cognitive cost or perceived difficulty for Morning-types is not such and the levels are likely to be maintained during the two extreme points of the day. Secondly, similar to **Studies 1** and **2**, another plausible possibility might be that once faced with tasks that demand a high degree of cognitive control, Morning-types' alertness levels would be triggered, again proving their sensitivity to environmental variables that increase their arousal.
- Evening-types' performance was largely influenced by the time of day in relation to both automatic and controlled processing. These results suggest that circadian

influence on both processes does not occur in a linear fashion, but instead operates differently in individuals depending on their chronotype.

- In light of the pattern found, we raise the hypothesis that Morning- and Evening-types possess substantially different cognitive styles. Based on Braves' theory (2012), we argue for the possibility that the former is more proactive in the implementation of control strategies, while the latter appears to exhibit a more reactive profile.

***Study 4***

- Both automatic and controlled processing were shown to be robust to the effect of time of day in Morning-types, who again demonstrate their ability to adapt to the circumstances of being at their worst time of day.
- The most intriguing result stems from the Evening-type chronotype, which reflects a significant modulation of the time of day on automatic processing. These participants have proven to be far more sensitive to the effects of being off-optimal-time, as well as far less susceptible to performance enhancement through environmental and physiological variables.
- The specific chronotype trait (i.e., Evening-types) together with specific brain regions underlying particular cognitive processes in the processing of self-related stimuli, such as the VMPFC, are decisive factors in the circadian modulation of automatic processing.

## Resumen en español

### *Introducción*

El cronotipo y el momento del día en el que a los individuos se les demanda la realización de una actividad cognitiva concreta han sido propuestos como factores que, en todo caso, determinan el resultado final de la operación mental llevada a cabo. Estas variables, en la presente tesis doctoral englobadas dentro del ámbito de las diferencias individuales en la línea base o punto de partida de los individuos, son cruciales no solo para comprender con éxito el funcionamiento de sistemas cognitivos complejos, sino que también permiten la profundización en la investigación de técnicas que suplan las carencias que puedan, en alguna circunstancia, perjudicar el rendimiento cognitivo y todo lo que de él se deriva. En la presente tesis doctoral se plantea una serie experimental de cuatro trabajos donde combinamos el paradigma del cronotipo con el estudio de procesos cognitivos dependientes de la atención, que es el eje que los vertebra. Los dos primeros trabajos parten de la teoría de Posner y Petersen (1990), sobre redes atencionales. Concretamente, profundizamos en el funcionamiento de la red de alerta entendida como un componente dual (fásica y tónica), y en la interacción entre alerta y control ejecutivo (**Estudio 1**). En el **Estudio 2**, nos focalizamos en la función de vigilancia entendida como el sostenimiento del nivel de alerta en el tiempo, y disociamos a su vez el componente de arousal del ejecutivo (Luna et al., 2018), para estimar la afectación de ambos procesos por el cronotipo y el momento del día, así como la potencial influencia sobre la restauración del sistema por parte de la hormona progesterona, segregada durante la fase lútea media del ciclo menstrual de las mujeres.

Por otro lado, desde la teoría de Schneider y Shiffrin (1977), y Shiffrin y Schneider (1977), partimos del paradigma de disociación de sistemas de procesamiento cognitivo en función del nivel de control que demandan. El interés principal en este caso es analizar la modulación por parte del cronotipo y el momento del día sobre el procesamiento cognitivo automático y sobre el controlado. Así, partiendo de este planteamiento teórico utilizamos tanto el paradigma de categorización semántica (**Estudio 3**), como el del sesgo atencional a estímulos relacionados con uno mismo (en inglés SAN) (**Estudio 4**).

### *Objetivos e hipótesis*

El objetivo global de la presente tesis doctoral es el estudio de la modulación de ciertas diferencias individuales relacionadas con la biología; el cronotipo de forma transversal, y el ciclo menstrual específicamente en el **Estudio 2**, sobre los procesos cognitivos dependientes de la atención. Para conceptualizar los procesos atencionales nos centramos en dos modelos teóricos principalmente. En primer lugar, basándonos en la propuesta de Posner y Petersen (1990) sobre redes atencionales, en el **Estudio 1** pretendemos explorar más a fondo la interacción entre la red de alerta y la de control ejecutivo, además de analizar más concretamente los patrones de funcionamiento específicos en función del cronotipo junto con la hora del día. En relación con estos aspectos, en el presente trabajo nos planteamos cuatro objetivos específicos. En primer lugar, estudiamos cómo opera el efecto de sincronía en tareas de vigilancia de tipo arousal (PVT), y en tareas más desafiantes como la ANTI. En general, esperamos que el rendimiento mejorase de forma consistente en los momentos óptimos del día de acuerdo con el cronotipo de los individuos. Por otro lado, examinamos determinados mecanismos compensatorios del rendimiento en momentos no óptimos del día, como el efecto de novedad. Específicamente, lanzamos la posibilidad de que la primera experiencia con la tarea incremente los niveles de alerta endógena y compense el decaimiento en el rendimiento observado en momentos no óptimos siempre y cuando dicha primera sesión ocurriese en el peor momento del día para con el cronotipo de los participantes.

Además, abordamos la posible relación compensatoria entre los procesos de alerta fásica y tónica (es decir, la alerta exógena y endógena), sobre el rendimiento de tareas que exigen control ejecutivo. En este sentido, planteamos la posibilidad de que las señales auditivas compensen los bajos niveles de alerta tónica cuando los participantes están fuera de su momento óptimos de acuerdo con su cronotipo. Por último, examinamos qué tipo de proceso de alerta es responsable del efecto negativo típicamente observado sobre la red de control ejecutivo. Basándonos en dos propuestas que han explicado esta interacción negative, proponemos, desde la perspectiva del fenómeno del *vaciado de consciencia*, la posibilidad de que los elevados niveles de alerta endógena inherente al momento óptimo del día compensen el efecto de negativo de la alerta fásica sobre el control ejecutivo. Por otro lado, considerando la propuesta de la difusión del foco atencional, exploramos la posibilidad de que el efecto de interacción negativa ocurra de forma independiente al momento del día. El **Estudio 2** amplía los aspectos específicos de la función atencional de alerta basándose en la reciente disociación de los componentes de vigilancia (Luna et al., 2018). De esta manera, consideramos individualmente tanto el subtipo de vigilancia tipo arousal como el de vigilancia ejecutiva. Además de estudiar el efecto de sincronía en relación con ambos tipos de vigilancia (es decir, la PVT para el estudio de la vigilancia arousal, y el SART para abordar el componente ejecutivo de la vigilancia), en el presente estudio comprobamos la capacidad de las hormonas sexuales femeninas relacionadas con el ciclo menstrual para producir efectos de mejora del rendimiento en los dos procesos atencionales evaluados. En concreto, ahondamos en la interacción entre la progesterona durante la fase lútea media y el pico de cortisol de las participantes durante las horas de la mañana para potenciar el rendimiento en momentos no óptimos según su cronotipo. Así, hipotetizamos que la progesterona actuará como un potenciador de los niveles de alerta tónica, estabilizando los niveles de rendimiento cognitivo que se ven empeorados durante el momento no óptimo. Así, se produciría una mejora del rendimiento en la fase lútea media en comparación con la folicular. Por otro lado, consideramos también la posibilidad de que este efecto esté mediado por el cronotipo específico de los participantes, que determina el patrón de secreción del cortisol. Dependiendo de los niveles de cortisol existentes, la progesterona ejerce efectos de activación diferenciados, que afectarían específicamente al rendimiento cognitivo de los participantes.

Por otro lado, desde la propuesta teórica que diferencia los procesos cognitivos en función del grado de control demandado (Schneider y Shiffrin, 1977; Shiffrin y Schneider, 1977), investigamos la influencia del cronotipo y el momento del día a través de dos paradigmas diferentes que nos permiten profundizar en diversos aspectos de estos modos de procesamiento. En primer lugar, en el **Estudio 3**, utilizamos una tarea de categorización semántica para disociar el procesamiento automático del controlado. En este caso, no sólo pretendemos desentrañar si el cronotipo y el momento del día ejercen distintas influencias sobre cada tipo de procesamiento (es decir, si el efecto de sincronía se produce de forma desigual para con ambos procesos), sino que también abordamos el curso temporal desde el desarrollo de respuestas basadas en la automaticidad hasta la aplicación necesaria del control de forma sostenida para proporcionar una respuesta correcta. Así, hipotetizamos en primer lugar el desarrollo de efectos de priming facilitatorio en SOAs cortos, es decir, de 100 ms. De la misma manera, el priming inhibitorio o negative se observaría en SOAs largos. Sin embargo, estimamos además que el propio curso temporal de ambos procesos será variable dependiendo del cronotipo de los participantes y del momento del día en el que se realice la tarea. En este sentido, ahondamos en el rol que posee el rasgo del cronotipo junto con el momento del día en el cambio de estrategia promovido por la naturaleza de la propia tarea.

Finalmente, en el **Estudio 4**, incidimos en la disociación de los procesos atencionales automáticos y controlados, en este caso, a través de una tarea de emparejamiento perceptivo utilizada para evaluar el denominado SPE. En este sentido, buscamos determinar si el efecto de sincronía se produce de forma desigual en el procesamiento de estímulos relacionados con uno mismo (es decir, etiquetas relacionadas con uno mismo; procesamiento automático), y aquellos relacionados con otros, que requieren un procesamiento más controlado. En este tenor, pretendemos ampliar los resultados previos relativos a la modulación circadiana de los procesos automáticos y controlados mediante un enfoque novedoso en la disociación de ambos procesos. Basándonos en teorías relacionadas con la modulación circadiana de procesos cognitivos, hipotetizamos que el rendimiento en el procesamiento de etiquetas relacionadas con uno mismo no variará dependiendo del momento del día en el que se realice la tarea, mientras que aquellas relacionadas con otros, es decir, aquellas cuyo procesamiento requiere más control, serían sensibles a la observación de efectos de sincronía.

### *Discusión y conclusiones generales*

En la presente tesis doctoral hemos abordado el estudio de la modulación circadiana de procesos atencionales a través de una serie experimental de cuatro estudios con objetivos específicos. El paradigma circadiano que ha sido transversal en la presente tesis doctoral, es decir, el cronotipo, no solo contribuye a estrechar los lazos entre biología y cognición en el ámbito de la investigación, sino que su relevancia se extiende a ámbitos más orgánicos. Así, este rasgo hace depender de él mismo el potencial éxito o fracaso de tareas cognitivas a las que los seres humanos nos enfrentamos cada día. Por ejemplo, escenarios donde se requiere que sostengamos nuestro nivel de atención por períodos de tiempo prolongados, o situaciones predominantemente conducidas por procesos automáticos de cuya intervención humana depende la posibilidad de evitar un error que indudablemente cometeríamos. Utilizando paradigmas atencionales concretos, hemos tratado de reproducir en el laboratorio situaciones donde la demanda cognitiva es similar. En este sentido, es importante destacar que el auge en el estudio de variables moduladoras del rendimiento cognitivo se fundamenta no solo en el aumento del conocimiento acerca del funcionamiento de este último, sino que permite además la indagación en factores susceptibles de mejorar la ejecución en circunstancias específicas. El paradigma a través del cual se ha abordado el rendimiento cognitivo en la presente tesis doctoral ha sido el del cronotipo, englobado en el campo de estudio de los ritmos circadianos. A través de la unión de del cronotipo, entendido como una diferencia individual, con diferentes puntos temporales del día en el que los individuos son evaluados hemos disociado diferentes niveles de alerta para posteriormente estudiar su vinculación con el control ejecutivo (**Estudio 1**), los diferentes componentes de la vigilancia (**Estudio 2**), y operaciones cognitivas desarrolladas en procesamiento automático y controlado (**Estudios 3, y 4**). Además, también hemos profundizado en la optimización del rendimiento fuera del momento óptimo de los individuos según su cronotipo analizando el papel modulador de algunas variables como la alerta fásica, el efecto de novedad y la dificultad de las tareas en el **Estudio 1**, así como el ciclo menstrual en el **Estudio 2**.

Las conclusiones obtenidas en el presente trabajo doctoral se detallan a continuación más específicamente, y pretenden tanto estrechar los lazos entre la atención y la neurociencia como llevar la neurociencia cognitiva a entornos de la vida real.

**Estudio 1:**

- En participantes Matutinos, el efecto de novedad y la dificultad de la tarea previnieron el decaimiento del rendimiento en la PVT y el ANTI, respectivamente. Adicionalmente, la presencia del tono disminuyó los RTs, sugiriendo que en estos participantes la alerta fásica actuaría como acelerador de la respuesta motora más que como compensador del bajo nivel de alerta tónica.

- Los participantes Vespertinos no se beneficiaron del factor de la novedad ni de la dificultad, experimentando efectos de sincronía en ambas tareas. Sin embargo, sus niveles de rendimiento en momentos no óptimos mejoraron ante el incremento de los niveles de alerta fásica, indicando en este caso la capacidad compensatoria de ésta última sobre la tónica.
- La interacción negativa entre la alerta y el control ejecutivo se debió únicamente al efecto de la alerta fásica. Además, el déficit en la compensación de este efecto por el elevado nivel de alerta tónica en momentos óptimos del día de los participantes encuadra el hallazgo en la teoría de difusión espacial del foco atencional en condiciones de elevación de alerta fásica.

**Estudio 2:**

- El rendimiento en la fase lútea media mejoró marginalmente en participantes Matutinas en la PVT. Asimismo, en el SART, apareció un efecto de mejora del rendimiento que fue estadísticamente significativo durante la dase lútea media.
- Las participantes Vespertinas vieron su rendimiento en el momento óptimo perjudicado por acción de la progesterona en la fase lútea media en la PVT. En el SART, el patrón fue similar, aunque estadísticamente no resultó significativo. Una explicación a la observación de dicho efecto radicaría en la dificultad de la tarea, que podría haber prevenido el empeoramiento del rendimiento durante la fase lútea media.

- El patrón opuesto de resultados en cuanto al efecto de la progesterona durante la fase lútea media (mejora en matutinas; empeoramiento del rendimiento en vespertinas), se podría explicar por el resultado de la interacción entre los ejes hormonales adrenal y gonadal. En este sentido, en condiciones de cortisol elevado, la progesterona aumentaría la secreción y la capacidad circulatoria del cortisol en sangre, manteniendo los niveles de activación general elevados en los individuos. Sin embargo, ante los mínimos o nulos niveles de cortisol, la progesterona aceleraría la inhibición de la secreción del glucocorticoide, de manera que los niveles de activación general y rendimiento cognitivo disminuirían notablemente.

**Estudio 3:**

- Los participantes Matutinos desarrollaron respuestas basadas en el control desde el SOA más corto (100 ms), hasta el más largo (850 ms) sin afectación por el factor del momento del día. Las razones a la base de esta ausencia de modulación de procesamiento controlado pueden ser diversas. En primer lugar, que el coste o demanda cognitiva de la tarea para estos participantes no sea notoria, de manera que sean capaces de mantener el rendimiento pese a estar fuera de su momento óptimo. Por otro lado, similar a los **Estudios 1** y **2**, que la propia dificultad de la tarea actúe como activador de los niveles de alerta y sostenga la ejecución en matutinos a lo largo del día. Este hecho se relaciona con la sensibilidad demostrada por parte de estos participantes a variables ambientales que incrementarían sus niveles de arousal.
- El momento del día influyó significativamente a los participantes Vespertinos tanto en el desarrollo de respuestas automáticas como en las controladas. Este

patrón sugiere que la influencia circadiana de este tipo de procesos ocurre de forma distinta en función del cronotipo.

- Siguiendo la teoría de Braver (2012), sugerimos la posibilidad de que los estilos cognitivos de Matutinos y Vespertinos sean sustancialmente distintos. Mientras que los primeros podrían ser más proactivos, los de perfil más tardío podrían estar lidiando con el conflicto de forma más reactiva.

**Estudio 4:**

- Los participantes Matutinos no mostraron modulación por parte del momento del día ni en el procesamiento automático (etiquetas relacionadas con uno mismo) ni del controlado (etiquetas relacionadas con otros).
- El momento del día influyó significativamente en el rendimiento automático de participantes Vespertinos.
- El cronotipo Vespertino junto con las regiones cerebrales demandadas en ciertas tareas (VMPFC para procesar estímulos relacionados con uno mismo) son factores que influyen de forma directa en la modulación circadiana del procesamiento automático.

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